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Pathophysiology of bone and mineral metabolism

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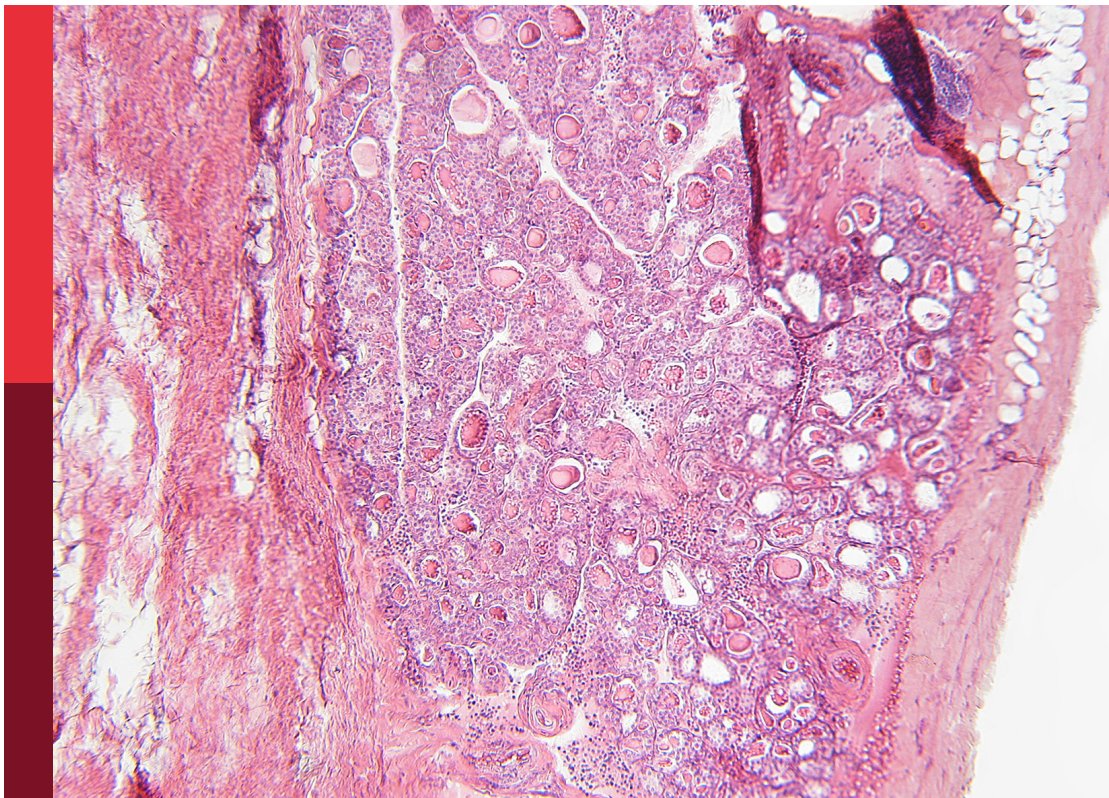
Published in
Frontiers in Endocrinology
Frontiers in Physiology
Frontiers in Medicine



ISSN 1664-8714
ISBN 978-2-8325-4554-6
DOI 10.3389/978-2-8325-4554-6

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Pathophysiology of bone and mineral metabolism

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Citation

Tocados, J. M. D., Naves-Diaz, M., Sato, A., Ambrogini, E., eds. (2024). *Pathophysiology of bone and mineral metabolism*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4554-6

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frontiers | Frontiers in Physiology

TYPE Editorial
PUBLISHED 23 February 2024
DOI 10.3389/fphys.2024.1383660

OPEN ACCESS

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RECEIVED 07 February 2024
ACCEPTED 09 February 2024
PUBLISHED 23 February 2024

CITATION
Ambrogini E, Sato AY, Naves-Diaz M and Díaz-Tocados JM (2024), Editorial: Pathophysiology of bone and mineral metabolism.
Front. Physiol. 15:1383660.
doi: 10.3389/fphys.2024.1383660

Editorial: Pathophysiology of bone and mineral metabolism

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KEYWORDS

osteoporosis, axial spondyloarthritis (AxSpA), renal osteodystrophy, diabetes and obesity, primary hyperparathyroidism, mendelian randomization, antiresorptive treatments, serum bone markers

Editorial on the Research Topic
Pathophysiology of bone and mineral metabolism

Bone plays a crucial role in maintaining overall systemic balance, but it is often affected by various chronic diseases and acute conditions, leading to bone mineral density loss. Aging, hormonal changes as well as factors like diet and physical activity also impact bone health. This vulnerability to multiple influences can reduce bone mineral density (BMD), increasing the risk of fractures, which are linked to higher mortality and cardiovascular disease. Treatments to prevent bone loss exist, including antiresorptive and osteoanabolic therapies. This Research Topic entitled "*Pathophysiology of Bone and Mineral Metabolism*" includes nine articles focusing on bone abnormalities in different diseases and treatments to prevent bone loss.

Wang et al. report the changes in serum calcium, vitamin D and parathyroid hormone (PTH) levels in women with pseudohypoparathyroidism (PHP), a rare disease affecting 1.2/100,000 people, during gestation and *postpartum*, when calcium metabolism is actually affected. The authors observed that high PTH levels could be predictors of a worsened calcium metabolism and treatment requirement and highlight the importance of controlling serum PTH concentration, calcium supplementation and calcitriol treatment during pregnancy to maintain normal calcium metabolism in PHP patients.

By Mendelian randomization analysis, Fu et al. studied the causal effects of blood homocysteine and serum levels of vitamins B6 and B12 on BMD at different bone sites and risk of fractures using public GWAS data from large-cohort studies. They found that the increased genetic prediction of homocysteine concentrations was causally associated with reduced heel BMD, and elevated genetic prediction of vitamin B12 had a causal impact on reduced total body BMD, especially at age over 45 years. However, there was no association of serum vitamins B6 and B12 or blood homocysteine concentrations with the risk of fracture. These observations encourage additional studies to clarify the existence of novel roles for B vitamins and homocysteine in the regulation of BMD and their use as potential treatments in the prevention of osteoporosis.

Another Mendelian randomization study conducted by Jiang et al. explores the causal association between BMD and osteoarthritis (OA) risk using data from a previous meta-analysis of total body BMD including 66,628 individuals and observed a causal association between higher genetic susceptibility to BMD and increased risk of OA, a link that can be of interest in clinical practices.

In a retrospective study, Wang et al. observed that patients with sepsis showed increased levels of serum markers of bone resorption as compared with non-sepsis controls. The authors also found higher concentrations piezo-type mechanosensitive ion channel component 1 (PIEZO1) in the serum of these patients, suggesting that circulating PIEZO1 could be associated with the inflammatory status in these patients. In addition, they suggest that serum levels of PIEZO1 together with concentration of markers of bone resorption may predict the occurrence of sepsis or sepsis sock, as well as increased mortality after 1 month.

In an interesting meta-analysis, Rajput et al. investigate the effects of two commonly used anti-resorptive therapies (bisphosphonates and denosumab), in the prevention of hypercalcemia and bone loss in primary hyperparathyroidism (PHPT). Their results showed that treatment with bisphosphonates and denosumab or denosumab alone results in higher BMD at the lumbar spine and femoral neck after 1 year, while bisphosphonate use only increased BMD at lumbar spine. All anti-resorptive treatments resulted in lower serum calcium concentration, increased serum PTH levels and lower serum concentration of markers of bone formation in PHPT patients, indicating that strong suppression of bone resorption by anti-resorptive therapy in PHPT may lead to the exacerbation of hyperparathyroidism despite of the improvement in BMD.

In a cross-sectional analysis including 10,564 elderly participants, Hou et al. studied the association between smoking status and serum levels of cotinine, a metabolite of nicotine, with bone loss (osteoporosis or osteopenia). They found that higher serum cotinine levels or being current smoker associated with higher prevalence of osteoporosis in three multivariate logistic regression models. Moreover, the authors demonstrated a positive correlation of serum cotinine levels and high prevalence of osteoporosis and osteopenia, suggesting possible mechanisms involving tobacco exposure in bone loss.

In an interesting study, Gómez-García et al. investigated the distinct bone and inflammatory profile of axial spondyloarthritis patients with or without radiographic features (r-axSpA or nr-axSpA respectively). They found that patients with r-axSpA have low plasma levels of bone formation markers as compared with controls, accompanied by low sclerostin concentration and higher levels of pro-inflammatory molecules. Notably, nr-axSpA patients had higher serum levels of the anti-inflammatory cytokine Interleukin 13 in comparison with r-axSpA patients and controls, which could exert protective effects on disease progression.

This Research Topic also includes two mini reviews, both focused on active areas of research in bone disease: On one hand, Aguilar et al. summarized current knowledge on the bone and mineral complications associated with chronic kidney disease (CKD-MBD), one of the most important factors increasing hip fracture occurrence together with aging. They address important Research Topic such as the need of performing a bone biopsy, factors that may affect diagnosis of bone disorders, the detrimental effects of secondary hyperparathyroidism and the mechanisms involved in bone alterations, as well as the use of current anti-osteoporotic treatments in CKD. On the other hand, Bathina and Armamento-Villareal revised the mechanisms involved in the imbalance of osteogenic-adipogenic differentiation in type 2 diabetes (T2DM) and obesity at cellular level, such as inflammation and oxidative stress. It is important to note that both, T2DM and obesity, are associated with an increased risk of fractures, highlighting the role of bone as an orchestrator of metabolic homeostasis. Furthermore, in this comprehensive review, the authors discuss the effectiveness of current treatments and drug repurposing on increasing bone formation in T2DM and obesity patients.

Author contributions

EA: Writing–review and editing. AYS: Writing–review and editing. MN-D: Writing–review and editing. JD-T: Writing–review and editing, Writing–original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. JMD-T was supported by a "Sara Borrell" postdoctoral contract (CD19/00055) funded by the Spanish Ministry of Science, Innovation and Universities, Carlos III Health Institute and co-funded by the European Regional Development Fund. AYS was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Numbers KL2TR003108 and UL1TR003107. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 16 November 2022
DOI 10.3389/fendo.2022.1050305

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

RECEIVED 21 September 2022
ACCEPTED 24 October 2022
PUBLISHED 16 November 2022

CITATION
Wang J-J, Yang Y, Wang Y-B, Song A, Jiang Y, Li M, Xia W-B, Liu Y-P, Wang O and Xing X-P (2022) Pseudohypoparathyroidism during pregnancy and the postpartum period: A case series of five patients. *Front. Endocrinol.* 13:1050305. doi: 10.3389/fendo.2022.1050305

Pseudohypoparathyroidism during pregnancy and the postpartum period: A case series of five patients

Jia-Jia Wang[1], Yi Yang[1], Ya-Bing Wang[1], An Song[1], Yan Jiang[1], Mei Li[1], Wei-Bo Xia[1], Yan-Ping Liu[2], Ou Wang[1*] and Xiao-Ping Xing[1*]

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Objectives: Pseudohypoparathyroidism (PHP) is a rare disease, especially when combined with pregnancy. We aimed to explore the changes in serum calcium/parathyroid hormone (PTH) level and medical treatment in a case series of PHP during pregnancy and the postpartum period.

Methods: A total of five PHP patients with six pregnancies were enrolled. The classification of PHP was based on (epi)genetic analysis. Clinical characteristics, biochemical indices, and treatment strategies before, during, and after pregnancy were retrospectively collected.

Results: All patients received calcium and vitamin D agents with nearly normal serum calcium before pregnancy except patient 2 who was found hypocalcemic during gestation. All patients chose Cesarean section, and one suffered preterm delivery due to oligoamnios. The neonatal birth weight ranged from 2,250 to 4,300 g, and all neonates were free of hypocalcemia-related symptoms. The change in calcium metabolism was inconsistent including stable, improved, or worsened during pregnancy. Serum PTH level remained low in the first two trimesters in patients with stable and improved conditions while increased in the last two trimesters in patients with a worsened condition. Serum calcium changed inconsistently while PTH increased consistently during lactation. For patients who did not breastfeed, calcium homeostasis improved after delivery.

Conclusion: Calcium homeostasis and medicine dosage changed differently in PHP patients during pregnancy and lactation. However, most patients had good pregnancy outcomes. Serum PTH levels might predict changes in calcium metabolism during pregnancy.

KEYWORDS

pseudohypoparathyroidism, pregnancy, lactation, serum calcium, serum PTH, treatment

1 Introduction

Pseudohypoparathyroidism (PHP) is a rare endocrine disease characterized by resistance to the action of the parathyroid hormone (PTH), which is divided into PHP1 caused by (epi)genetic defects of *GNAS* gene which encodes stimulatory guanine nucleotide-binding protein (Gsα). PHP1a and PHP1b are the main subtypes of PHP1. PHP1a is caused by heterozygous loss-of-function mutations in maternally inherited *GNAS* exons 1–13, while PHP1b is caused by abnormal methylation at differentially methylated regions (DMRs) of the *GNAS* gene. Aside from PTH resistance, PHP1a patients also develop resistance to other hormones, such as thyroid-stimulating hormone (TSH), calcitonin, and growth hormone-releasing hormone (GHRH). In addition, patients with PHP1a can display Albright hereditary osteodystrophy (AHO) features characterized by short stature, brachydactyly, obesity with a round face, and heterotopic ossifications. PHP1b patients mainly manifest PTH resistance and are used to be considered free of the AHO phenotype. Recent studies showed that some PHP1b patients also develop TSH resistance and some AHO features, indicating an overlap between clinical features of both subtypes of PHP1 (1).

Since PTH is one of the most essential peptide hormones for calcium and phosphorus homeostasis, patients with PHP suffer from hypocalcemia, hyperphosphatemia, and high serum PTH level. At present, the treatment for PHP includes calcium and active vitamin D to maintain a normal serum calcium level and reduce the PTH level to normal range as much as possible (1).

Pregnancy and lactation are two periods in which calcium homeostasis changes greatly. During pregnancy, intestinal calcium absorption increases to ensure adequate fetal skeleton mineralization, with approximately 80% of the mineral accruing in the fetus in the third trimester (2). Many calcium-regulated hormones change markedly during this period, including PTH, PTH-related peptide (PTHrP), and calcitriol. According to previous studies, serum PTH typically declines to low levels at the early stage and then return to the mid-normal range by term when the calcium intake is sufficient. This suppression may not occur (or even secondary hyperparathyroidism may develop) in women whose calcium intake is insufficient (2). The PTHrP level progressively increases to reach peak levels in the third trimester, which is most likely secreted by the placenta and breasts. The calcitriol level begins to increase in the first trimester and may reach triple or more of the preconception level by the third trimester. However, the ionized or albumin-corrected serum calcium level is stable. Different from the pregnancy period, which relies mainly on increased intestinal calcium absorption to meet fetal calcium needs, increased skeletal resorption contributes to the majority of calcium requirements during lactation. This is consistent with the decline of serum calcitriol level to normal concentration. The PTH level is still typically suppressed toward the lower end of the normal range during lactation, while some studies have reported increased PTH in women from regions of Africa and Asia in which low calcium and vitamin D intakes are more prevalent. The PTHrP level remains high and the ionized or albumin-adjusted calcium level is normal to slightly increased in breastfeeding women (2).

For patients with PHP, the change in calcium metabolism during gestation and lactation is different from that of unaffected women. It is important to adjust medicine dosage in time to maintain calcium homeostasis for both maternal and fetal health. However, according to the limited previous case reports, the change in serum calcium level in PHP patients during pregnancy is inconsistent and the requirements of medication can change dramatically (3). Some patients had improved outcomes during pregnancy with abated hypocalcemic symptoms, normocalcemia, decreased to near-normal PTH level, and discontinuation of supplemental vitamin D agents (4), while other reports showed that the dose of calcium and/or active vitamin D had to be increased in PHP women during pregnancy (5–7). The data regarding mineral homeostasis in PHP patients during lactation are extremely rare. Only one case report published in 2017 was found (8).

Due to the rarity of the disease, the mechanism of the different reactions of PHP patients to pregnancy is still unknown. The aim of this study was to report a case series of pregnant women affected by PHP to help evaluate the clinical and biochemical course and pharmacological management during pregnancy and the postpartum period.

2 Subjects and methods

2.1 Subjects

PHP patients who were followed at the Endocrinology Clinic of the Peking Union Medical College Hospital (PUMCH) and pregnant during 2010 and 2021 were included in this study. The diagnostic criteria of PHP were PTH resistance and/or ectopic ossifications, and/or multiple hormone resistance including TSH resistance, and/or AHO (1). The further classification relied on (epi)genetic analysis of the *GNAS* gene, which was conducted and published previously (9). A total of five patients, including six pregnancies, were finally enrolled in the study. Three of them were diagnosed as PHP1b, and one was PHP1a. Patient 5 did not undergo gene detection, so her PHP type was not confirmed.

2.2 Data collection

2.2.1 Clinical investigation

All of the clinical information was retrospectively obtained from the Hospital Information System of PUMCH. The PHP-related information included onset age, onset symptoms (e.g., muscle cramps, paresthesia, and seizures), treatment (dose of vitamin D agents and calcium), and complications of PHP (e.g.,

cataract screened by a slit lamp, renal stones/calcification screened by abdominal ultrasound, and intracranial calcification evaluated by cranial computed tomography). Pregnancy- and postpartum-related data included age of pregnancy, duration of the disease, maternal comorbidities and complications during pregnancy, pregnancy outcomes, delivery mode, birth weight, neonatal symptoms, and duration of lactation.

2.2.2 Laboratory examinations

Serum biochemical indices, including total calcium (Ca), phosphorus (P), alkaline phosphatase, creatinine, and alanine aminotransferase, were measured using a Beckman automatic biochemical analyzer (AU5800; Beckman Coulter, CA, USA). The serum PTH level and total 25-hydroxyvitamin D (25OHD) level were measured using an electrochemiluminescence immunoassay (e601; Roche Cobas, Germany). Calcium homeostasis can be divided into three conditions based on the changes in serum calcium and drug dosage, including improved, worsened, and stable. Given that the serum total calcium level can be slightly decreased due to the physiological reduction of the serum albumin level, patients whose serum calcium level declined obviously (defined as a decline of >0.20 mmol/L) with stable medicine dosage were described as worsened. Patients who had increased serum calcium level with stable/reduced medicine requirement were described as improved. Patients with stable serum calcium levels (including patients with a decline in the serum total calcium level <0.2 mmol/L) and unchanged drug dosage were described as stable (10).

2.2.3 Molecular analysis

2.2.3.1 DNA extraction

Genomic DNA was extracted from peripheral leukocytes using the QIAGEN DNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.2.3.2 Detection of *GNAS* mutations

The primers for *GNAS* were previously described (11). Sequencing of *STX16 and GNAS* was performed using the chain-termination method on an automatic sequencer (Applied Biosystems 3730 Genetic Analyzer, Foster City, CA, USA). Mutation taster (http://mutationtaster.org/) was used to predict the pathogenicity of the mutations.

2.2.3.3 Analysis of *GNAS*-A/B:TSS-DMR, *GNAS*-NESP : TSS-DMR, *GNAS*-AS1:TSS-DMR, *GNAS*-XL : Ex1-DMR, and *STX16*

Methylation-specific multiple ligation-dependent probe amplification (MS-MLPA; MRC-Holland, Amsterdam, Netherlands) was performed to ascertain the methylation status of *GNAS* DMRs. Sporadic PHP1b was defined as broad alterations in methylation involving two or more DMRs, while familial PHP1b was defined as isolated loss of methylation (LOM) at *GNAS*-A/B:TSS-DMR, which was mostly due to deletion in the *STX16* gene.

3 Results

3.1 Case histories of the five patients

3.1.1 Patient 1

Patient 1 was found hypocalcemic with unknown PTH level when she was 28 years old. When she was 31 years old, she came to our clinic due to the unconscious twitching of her fingers. Physical examination showed her height of 155 cm, weight of 61 kg, round face, and short neck without brachydactyly. Both Chvostek sign and Trousseau sign were positive. Blood testing showed severe hypocalcemia and elevated PTH level (Table 1). After (epi)genetic analysis, she was diagnosed with sporadic PHP1b (Table 1) and treated with calcium and vitamin D agents.

She was pregnant at 32 years old. The detailed changes in serum calcium, 24-h urinary calcium excretion, and serum PTH level, as well as the adjustment of medication, were shown in Tables 2 and 3 and Figure 1A, which revealed stable calcium metabolism. The serum 25OHD level was 46–62 ng/ml during her pregnancy period. Serum creatinine was within normal range all the time. A Cesarean section was carried out at week 35 due to gestational hypertension. The birth weight of the neonate was 2,250 g (-0.75 SD) with normal serum calcium level. She did not breastfeed after delivery (Table 1).

Before this pregnancy, the patient experienced her first pregnancy at 28 years old and was found hypocalcemic during the pregnancy period. However, detailed information about that pregnancy was missing. Cesarean section was carried out, and a healthy male infant was delivered with a birth weight of 2,900 g.

3.1.2 Patient 2

Patient 2 suffered from generalized convulsions from 9 years old and was treated with calcium supplements all the time. She first came to our clinic at 29 years old while at week 9 of her first pregnancy. Physical examination showed AHO phenotype, including a round face, short neck, and short metacarpals/metatarsals. Blood testing showed severe hypocalcemia with a high PTH level (Table 1) as well as vitamin D deficiency (serum 25OHD level was 12.9 ng/ml). Genetic testing supported a diagnosis of PHP1a (Table 1). As shown in Tables 2 and 3 and Figure 1B, her calcium metabolism worsened during pregnancy with decreased serum calcium and increased medicine dosage. The serum 25OHD level rose to 30.1 ng/ml at week 33 after vitamin D supplementation. She did not come to our clinic after week 36, so the clinical data after that time were unavailable. She underwent Cesarean section at approximately week 37 due to fetal intrauterine hypoxia. She underwent surgery at the local hospital, and the neonatal Apgar score was not available. The

10.3389/fendo.2022.1050305

TABLE 1 General characteristics of the five patients.

Variables	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5-1	Pt 5-2
PHP type	1b	1a	1b	1b	/	/
(epi)genetic data*	GOM at *GNAS-NESP* LOM at *GNAS-XL*, *GNAS-AS1*, *GNAS-A/B*	*GNAS*: Exon1: c.1247G>A	GOM at *GNAS-NESP* LOM at *GNAS-XL*, *GNAS-AS1*, *GNAS-A/B*	GOM at *GNAS-NESP* LOM at *GNAS-XL*, *GNAS-AS1*, *GNAS-A/B*	/	/
Onset age (y)	28	9	9	3	12	12
Onset symptom	none	convulsion	convulsion	convulsion	muscle cramp	muscle cramp
Biochemical indices at the first visit[$]						
Serum Ca (mmol/L)	1.45	1.62	1.65	1.93	1.80	1.80
Ionized Ca (mmol/L)	0.74	/	/	0.86	0.68	0.68
Serum P (mmol/L)	1.77	2.04	1.72	1.88	2.10	2.10
Serum PTH (pg/ml)	334.6	188.2	314	731	518	518
24hUCa (mmol)	0.44	/	1.28	/	1.55	1.55
Complications						
Cataract	–	/	–	+	/	/
Renal stones	–	/	–	–	–	–
Intracranial calcification	+	/	+	+	+	+
Subclinical hypothyroidism	+	+	+	+	+	+
Levothyroxine (μg/day)[#]	50	82.14	107.14	67.5	50	0
Pregnant age (y)	32	29	24	27	22	27
Pregnancy outcome						
Full-term delivery[^]	N	Y	Y	Y	Y	Y
C-section	Y	Y	Y	Y	Y	Y
Birth weight (g/SD)	2250/ -0.75	3000/ 0.21	3600/ 1.09	3500/ 0.82	3600/ 0.64	4300/ 2.97
Neonatal serum Ca	normal	/	/	normal	normal	normal
Lactation period (m)	0	/	2	0	12	9

Pt, patient; PHP, pseudohypoparathyroidism; GOM, gain of methylation; LOM, loss of methylation; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; UCa, urine calcium excretion; C-section, Cesarean section; N, no; Y, yes; y, year; m, month.
/, data unavailable; -, negative; +, positive.
*Patients 1–4 did not have heterozygous deletions at the *STX16* gene.
[$]Biochemical indices at the first visit time in our clinic.
[#]Levothyroxine dosage at the last visit time during the third trimester.
[^]Full-term delivery was defined as gestation period equal to or more than 37 weeks.
Normal reference ranges for indices: serum Ca, 2.13–2.70 mmol/L; ionized Ca, 1.08–1.28 mmol/L; serum P, 0.81–1.45 mmol/L; serum PTH, 13–65 pg/ml; 24hUCa, <7.5 mmol.

neonatal birth weight was about 3,000 g (0.21 SD). Unfortunately, the baby died within several months due to an intestinal fistula.

3.1.3 Patient 3

Patient 3 suffered convulsion and unconsciousness at the age of 9 years. In the beginning, she was diagnosed with hypoparathyroidism (HP) and treated with calcium and calcitriol. When she was 21 years old, she came to our clinic and was diagnosed with sporadic PHP1b on the basis of hypocalcemia, high PTH levels, and (epi)genetic testing (Table 1). She was pregnant at 24 years old. The serum 25OHD level was detected at week 4, and the result was 30.8 ng/ml. As shown in Tables 2 and 3 and Figure 1C, her calcium homeostasis improved during pregnancy with a decreased medicine dosage and a stable serum calcium. A Cesarean

10.3389/fendo.2022.1050305

TABLE 2 Changes in serum calcium, 24-h urinary calcium, and serum PTH level during pregnancy and after delivery.

	Prepregnancy*			During pregnancy#									Postdelivery$(9 m)		
				First trimester			Second trimester			Third trimester					
Indices	SCa	24hUCa	PTH	SCa	24hUCa	PTH	SCa	24hUCa	PTH	SCa	24hUCa	PTH	SCa	24hUCa	PTH
Pt 1	2.25	2.49	97.5	2.14	/	82.7	2.21	3.79	59.4	/	4.71	/	2.34	4.10	130.4
Pt 2	/	/	/	1.28	/	202	1.98	10.66	57.9	1.97	7.09	75.7	/	/	/
Pt 3	2.47	3.95	19	2.36	4.50	12.75	2.31	2.90	18.9	2.32	4.41	/	2.21	2.35	93.8
Pt 4	2.19	3.71	281	2.23	5.67	34.9	2.23	4.35	35.3	2.30	6.91	25.6	2.31	8.51	88.8
Pt 5-1	2.12	2.32	/	/	/	/	2.06	2.07	/	2.09	2.37	90.3	2.16	1.82	156
Pt 5-2	2.32	5.47	70.4	2.17	3.48	50.8	2.07	6.90	77.8	2.12	4.00	66.5	2.27	4.37	236.8

Pt, patient; SCa, serum calcium (mmol/L); PTH, parathyroid hormone (pg/ml); 24hUCa, 24-h urinary calcium excretion (mmol); m, month.
/, data unavailable.
*Serum calcium, 24-h urinary calcium excretion, and serum PTH level at the last visit time before pregnancy.
#Average value of serum calcium, 24-h urinary calcium excretion, and serum PTH level during the three trimesters: the first trimester was before week 13; the second trimester was week 13 to week 27; the third trimester was week 28 to delivery.
$Average value of serum calcium, 24-h urinary calcium excretion, and serum PTH level within 9 months after delivery.
Normal reference ranges for indices: serum Ca, 2.13–2.70 mmol/L; serum PTH, 13–65 pg/ml; 24hUCa, <7.5 mmol.

section was carried out at week 39 due to oligoamnios, and the neonatal birth weight was 3,600 g (1.09 SD). The neonate was free of hypocalcemia-related symptoms, while the serum calcium level was not detected. She breastfed for about 2 months due to little milk quantity. After delivery, the drug dosage needed to be increased.

3.1.4 Patient 4

Patient 4 suffered from intermittent convulsion from 3 years old and had seizure episodes with loss of consciousness and urinary incontinence from 10 years old. She came to our clinic at 20 years old due to intermittent convulsion. Sporadic PHP1b was diagnosed based on biochemical testing and (epi)genetic analysis (Table 1). She was pregnant at 27 years old. However, calcium metabolism was difficult to evaluate during pregnancy due to the change in vitamin D agents (Table 3; Figure 1D). Her serum 25OHD level was tested at week 4, and the value was 195 ng/ml. She delivered at week 39 by Cesarean section due to the cord around the neck. The neonatal birth weight was 3,500 g (0.82 SD), and the serum calcium was normal. The patient did not breastfeed due to little milk quantity, and her treatment needs decreased after delivery.

3.1.5 Patient 5

Patient 5 suffered from muscle cramps at 12 years old. Blood testing showed hypocalcemia, high PTH level, and normal kidney/liver function (Table 1). However, the type of PHP was unclear due to the lack of (epi)genetic analysis. A family history of parathyroid disease was denied. Her first pregnancy was at 22 years old. During this pregnancy period, calcium metabolism worsened with

TABLE 3 Changes in medicine dosage during pregnancy and after delivery.

	Prepregnancy*			During pregnancy#									Postdelivery$ (9 months)		
				First trimester			Second trimester			Third trimester					
Indices	Ca (mg/day)	Active D(μg/day)	Plain D(IU/day)	Ca (mg/day)	Active D(μg/day)	Plain D(IU/day)	Ca (mg/day)	Active D(μg/day)	Plain D(IU/day)	Ca (mg/day)	Active D(μg/day)	Plain D(IU/day)	Ca (mg/day)	Active D(μg/day)	Plain D(IU/day)
Pt 1	800	0.875	0	800	0.875	0	800	0.875	0	/	/	/	800	0.875	0
Pt 2	/	/	/	600	0	200	1,800	0.75	1,000	1,800	1.00	1,667	/	/	/
Pt 3	1,200	1.036	0	1,200	0.75	0	1,200	0.625	0	1,200	0.5	0	1,200	0.625	0
Pt 4	1,200	0.25	4,2800	1,200	0.375	4,2800	1,200	0.688	2,1400	1,200	1	0	1,200	0.25	0
Pt 5-1	1,200	0.25	3,7500	/	/	/	1,200	0.25	3,0000	1,200	0.5	3,0000	1,200	0.5	3,4300
Pt 5-2	1,200	1.25	0	1,200	1.25	0	1,200	1.25	0	1,200	1.5	0	1,200	0.92	0

Pt, patient; Ca, elemental calcium dosage; Active D, calcitriol dosage; Plain D, calciferol/cholecalciferol dosage.
*Drug dosage at the last visit time before pregnancy.
#Average drug dosage during the three trimesters: the first trimester was before week 13; the second trimester was week 13 to week 27; the third trimester was week 28 to delivery.
$Average drug dosage within 9 months after delivery.

10.3389/fendo.2022.1050305

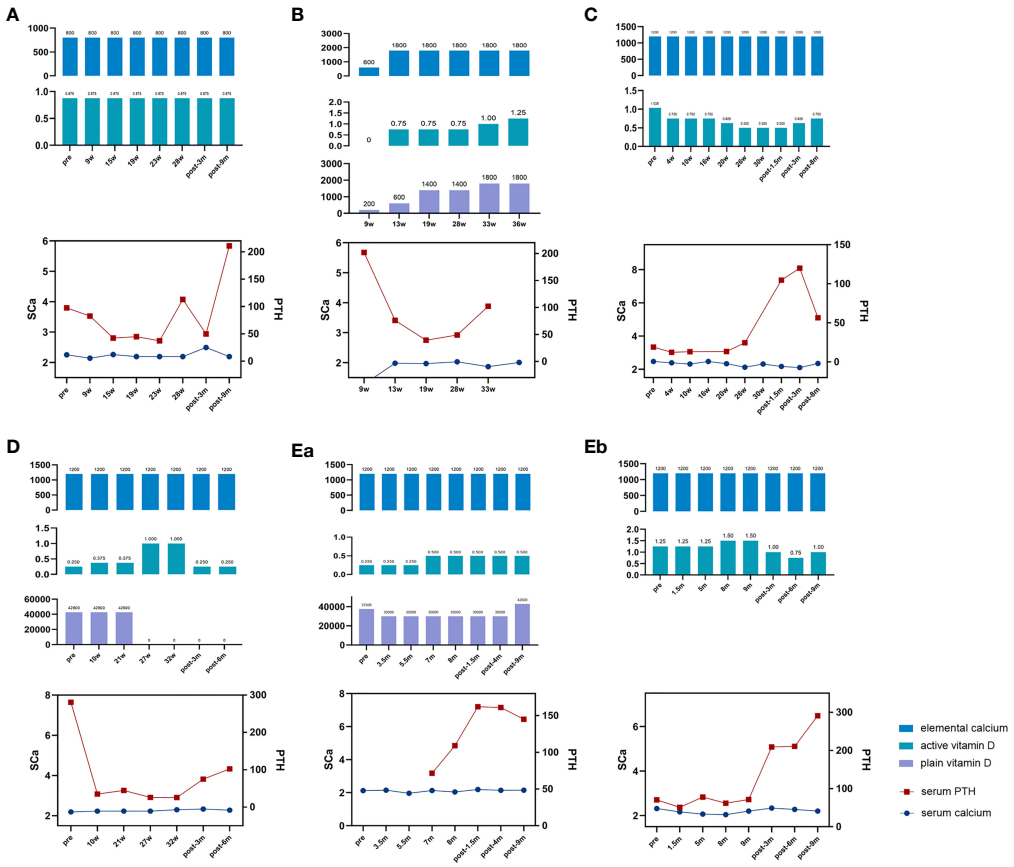

FIGURE 1
Changes in biochemical indices and medicine dosage of the patients. Elemental calcium (mg); active vitamin D, calcitriol (μg); plain vitamin D, calciferol/cholecalciferol (IU); serum PTH, serum parathyroid hormone level (ng/ml); SCa, serum calcium level (mmol/L); pre, prepregnancy; w, gestation week; post, postpregnancy; m, month after delivery. **(A)** Drug dosage, serum calcium, and PTH level of patient 1. **(B)** Drug dosage, serum calcium, and PTH level of patient 2. **(C)** Drug dosage, serum calcium, and PTH level of patient 3. **(D)** Drug dosage, serum calcium, and PTH level of patient 4. **(Ea)** Drug dosage, serum calcium, and PTH level of patient 5's first pregnancy. **(Eb)** Drug dosage, serum calcium, and PTH level of patient 5's third pregnancy.

obviously declined serum calcium and increased drug dosage (Tables 2, 3; Figure 1Ea). The serum 25OHD level was not tested during this pregnancy period. A female neonate was delivered by Cesarean section at about week 41 due to decreased range of motion of the hips. The infant weighed 3,600 g (0.64 SD) with a normal serum calcium level. She breastfed for about 1 year with a small amount of milk. During lactation, the treatment needs increased because the PTH level obviously elevated.

She was pregnant again at 26 years old but suffered arrested fetal development in month 3 with an unknown reason. When she was 27 years old, she was pregnant for the third time. Serum 25OHD level was detected before this pregnancy, and it was 60.4 ng/ml. It was tested again at the eighth month during pregnancy, and the result was 46.5 ng/ml. Calcium metabolism also worsened during this pregnancy period (Tables 2, 3; Figure 1Eb). She delivered at week 39 by Cesarean section. The infant weighed 4,300 g (2.97 SD), and the serum calcium level was at the lower part of the normal range. She breastfed for more than 1 year, and the amount of milk was more than that of her first lactation. The medicine dosage was reduced, and PTH level increased compared to that during her pregnancy.

3.2 General characteristics of the five patients

As shown in Table 1, a total of five patients (Pts) with six pregnancies were included in this study. The average onset age for the five patients was 12.2 years, and the mean disease course from onset to pregnancy was 14.7 years. Four of them had intracranial calcification except for Pt 2 with missing data. Cataract was found in one patient. In addition, all patients were found suffering from subclinical hypothyroidism (TSH resistance) before or during pregnancy and were suggested to

have levothyroxine treatment (Pt 5 did not follow medical advice during her second pregnancy period).

3.3 Changes in biochemical indices and treatment needs during pregnancy and after delivery

As shown in Figure 1, during pregnancy, calcium homeostasis was described as stable in Pt 1 due to stable serum calcium level and drug dosage (Figure 1A), improved in Pt 3 due to increased serum calcium and reduced drug dosage (Figure 1C), and worsened in Pt 5 because of the obviously decreased serum calcium level and increased drug dosage during the two pregnancies (Figure 1E). For patients 2 and 4, the changes in PHP condition were difficult to evaluate due to the lack of prepregnancy data and the change in vitamin D agent type (Figures 1B, D).

All patients had normal 24-h urinary calcium level before pregnancy except patient 2 with missing data. Also, the 24-h urinary calcium level slightly increased during pregnancy compared to prepregnancy among all patients. Except patient 2, who did not accept standardized and regular treatments until week 9 of gestation, all patients had normal 24-h urinary calcium level during pregnancy and patient 4 suffered from hypercalciuria after delivery. Renal function remained normal in all patients.

The serum PTH level was obviously decreased in the first trimester. For patient 1, whose condition was stable during the whole gestation period, the PTH level further decreased in the second trimester. For patient 3, whose condition improved during pregnancy, the PTH level remained very low in the second trimester. And for the third pregnancy of patient 5, whose condition has worsened, the PTH level was slightly increased in the last two trimesters compared to that of the first trimester (Table 2; Figure 1).

Only two patients breastfed after three deliveries. Pt 3 breastfed for 2 months. At 1.5 months after delivery, a slight decrease in serum calcium level (from 2.32 to 2.17 mmol/L) and a marked increase in PTH level (from 24.5 to 104.7 pg/ml) were observed. Pt 5 breastfed for 12 months after her first pregnancy and more than 1 year after her third pregnancy. Increased serum calcium and PTH levels were found during these two lactation periods compared to those during pregnancy (Table 2; Figure 1E).

For patients who did not breastfeed (Pts 1 and 4), it seemed that the calcium condition changed consistently. The serum calcium level increased from 2.19 (week 28) to 2.49 mmol/L (3 months postpartum) with stable medicine and declined PTH level (from 113 to 49.9 pg/ml) in patient 1 (Figure 1A). Calcitriol was reduced from 1 μg/day (week 32) to 0.25 μg/day (3 months postpartum) with stable serum calcium and increased PTH level (from 25.6 to 75.1 pg/ml) in Pt 4 (Figure 1D).

3.4 Pregnancy outcomes

All patients chose Cesarean section due to variable reasons, and one suffered preterm delivery due to oligoamnios (Pt 1). The neonatal birth weight ranged from 2,250 to 4,300 g, and all neonates were free of hypocalcemia-related symptoms. Serum calcium levels were detected in four neonates, and all were in the normal range. Thyroid hormone was tested in all neonates using heel blood at birth. In addition, their thyroid functions were all in the normal range.

4 Discussion

PHP is a rare disease with a reported prevalence of about 1.2/100,000 (12). It can be challenging to manage especially during pregnancy. Information about calcium metabolism change and medicine dosage adjustment in such condition is scarce. Previously, only 12 cases with 14 pregnancies were reported. Our study reported five PHP patients with six pregnancies to show the changes in serum calcium/PTH level and medicine dosage during pregnancy and the postpartum period, as well as their pregnancy outcome, providing more information on this rare but important clinical entity.

The results showed that PHP patients can have different conditions in calcium homeostasis and treatment needs during pregnancy: improved, stable, or worsened, which was similar with previous case reports. According to previous studies, patients with improved outcome had some common characteristics: firstly, their calcium supplementation was high (usually more than 1 g/day); secondly, the PTH level was continuously low during the whole pregnancy period; thirdly, the serum calcitriol level increased 2–4-fold compared to prepregnancy during the entire gestation period (4). On the contrary, as for patients with worsened outcome, calcium supplementation was very low (<0.5 g/day) (5–7). In addition, the fall in albumin-adjusted calcium level in the latter half of the pregnancy period was accompanied by a declined calcitriol level and a risen PTH level (6). In normal women, the physiology of pregnancy means that the recommended dietary intake of calcium (1.25 g/day), combined with doubling of efficiency of intestinal calcium absorption, should be more than sufficient to meet the combined needs of the mother and fetus during the third trimester (13). So, as mentioned before, when the calcium intake is insufficient, pregnant women can develop secondary hyperparathyroidism to provide additional minerals. Since PHP patients are PTH resistant, it is not surprising that the PHP-related condition will worsen if calcium intake is inadequate during pregnancy. Another important factor that may affect the PHP condition during pregnancy is the calcitriol level, since the worsened patients had declined calcitriol levels. However, factors that influence the calcitriol level during pregnancy remain to be elucidated. According to this study, patient 5 had worsened

outcome with elemental calcium supplementation of 1,200 mg/day. Thus, her worsened status during her two pregnancies might be due to the reduced serum calcitriol level. Regretfully, this index was not detected during her pregnancy. In terms of PTH level, our study showed that the decreased serum calcium level was accompanied by an elevated PTH level, which was consistent with previous studies. The PTH response to pregnancy in PHP patients was similar to that of a normal person, characterized by declining in the first trimester then possibly elevating in the third trimester due to further increased calcium demands. The low calcium intake, declined calcitriol level, and elevated PTH level might indicate the need to increase medicine dosage for PHP pregnant women.

As to the urinary calcium excretion, firstly, our results showed that the 24-h urinary calcium level was slightly increased during pregnancy compared to prepregnancy, which was similar to unaffected women and might be due to the increased intestinal calcium absorption. Secondly, different from patients with HP, who easily developed hypercalciuria under active vitamin D and calcium treatment, the 24-h urinary calcium excretion was within normal range in most patients with PHP. This indicated that the renal resistance to PTH in patients with PHP1 was limited to the proximal tubular cells and did not involve the distal tubular cells.

In the aspect of pregnancy and neonate outcomes, our study showed that all patients underwent Cesarean section due to variable reasons and most of them reached full-term delivery. In addition, all available serum calcium levels of neonates were within normal range. However, one neonate (the second child of patient 5) in the present study had macrosomia. Up to now, all of the PHP pregnant women reported in previous literature chose Cesarean section at full term while delivering neonates with normal birth weight (2,593–3,460 g) and mostly normal serum calcium level. The reasons for Cesarean section were the reduced pelvic size and decreased range of motion of the hips due to local ossifications. However, high birth weight had been reported in some previous cases with PHP1b caused by paternal uniparental disomy of chromosome 20 (14) and *STX16* microdeletion (15), which means that both sporadic and familial PHP1b patients could manifest as macrosomia themselves. In 2015, Bréhin et al. (16) reported 114 PHP1b patients (61 familial and 53 sporadic) and 12 PHP1a patients with their own birth conditions. Their results showed that PHP1a patients were free of intrauterine overgrowth, while PHP1b patients themselves had heavier birth weight than the normal population (Z-score of +0.73 ± 1.2, $P < 0.0001$). Moreover, familial PHP1b patients tended to have slightly heavier birth weight than sporadic PHP1b patients. In addition, familial PHP1b patients had heavier birth weight than their healthy siblings. These indicated that biallelic A/B expression might contribute to enhanced intrauterine growth through an unknown mechanism. Regretfully, Pt 5 and her second child did not undergo (epi)genetic analysis, so their PHP types were unknown. However, since she had PHP and her second child had macrosomia while the first child had normal birth weight, it was suspected that she and her second child had familial PHP1b. Thus, an (epi)genetic analysis for them is helpful, and the serum calcium and PTH levels of the second child should be monitored.

In the present study, two patients breastfed after delivery with different responses to breastfeeding. Patient 3 showed worsened response with a slight decrease in serum calcium level and marked increase in PTH level. Patient 5 showed an improved condition characterized by increased serum calcium level compared to the pregnancy period during both lactations. We had retrieved only one case report describing calcium homeostasis in PHP patients during the lactation period, which showed a stable serum calcium level and medicine dosage in a PHP1a patient with calcitriol 0.5 μg/day and calcium carbonate 2,500 mg/day (1,000 mg elemental calcium). The PTH level was not described (8). Since the elemental calcium intake was similar among the two patients in the present study and the patient in a previous case report, there should be other factors that influence calcium metabolism. Calcium homeostasis in lactation relies mainly on bone resorption mediated by PTHrP, which comes mainly from the mammary gland. In the present study, patient 3 had worsened calcium homeostasis during lactation with little milk amount and short breastfeeding duration. Whereas patient 5 had improved calcium homeostasis with more milk amount and a longer lactation duration than those in patient 3. So, the different change in calcium homeostasis between patient 3 and patient 5 in their lactation period might be related to the different amount of milk and PTHrP level. Hence, there might be some relationship between milk amount and PTHrP level. However, there was no convincing method to evaluate that the amount of milk and PTHrP level were not detected in both of these two studies. So, this assumption needs further exploration. Different from serum calcium level, the change in PTH level was consistent in the two patients' three lactation periods. This phenomenon was also found in normal women who suffered from insufficient calcium intake when breastfeeding. So, it may be an indicator of the insufficient calcium intake.

The calcium metabolism for those PHP patients who did not breastfeed (patient 1 and patient 4) seemed to improve with elevated serum calcium level (patient 1) or declined medicine dosage (patient 4). This was inconsistent with a previous study that showed reduced serum calcium level and increased drug dosage after delivery (4). The underlying mechanism of the different response was unknown and needed further exploration. Genetic or ethnic differences may have some effect.

Our center has reported a case series about the change in calcium metabolism of HP patients during pregnancy and the lactation period (10). The results showed that HP patients could suffer from marked serum calcium fluctuations during these two periods and the risk of adverse pregnant outcomes was higher than that of a normal person. During pregnancy, hypercalcemia

can occur and HP patients who had improved calcium metabolism might need to discontinue active vitamin D. While for HP patients who had worsened calcium homeostasis during pregnancy, a marked decrease in serum calcium level can be found and might lead to abortion/stillbirth. During lactation, most patients had improved calcium metabolism with increased serum calcium level. Some patients even developed hypercalcemia. There were some similarities and differences between PHP and HP during pregnancy and lactation periods. During the pregnancy period, on the one hand, both PHP and HP patients could experience opposite changes in calcium homeostasis as objectively improved or objectively worsened status. On the other hand, the serum calcium fluctuation and pregnant outcomes were different. Compared to HP, PHP patients seemed to have a much milder serum calcium fluctuation and better pregnancy outcomes. During the lactation period, the fluctuation of serum calcium level also seemed milder in PHP patients than that in HP patients. These indicated that PTH may still have some effect on calcium metabolism in PHP pregnant patients.

This study had some limitations. First, because of the retrospective nature, some data were missing and the follow-up time points could not be controlled. Second, neither the ionized calcium nor serum albumin level was routinely detected. Since the serum albumin level was reduced during pregnancy due to dilution, the total serum calcium level without albumin adjustment might underestimate true calcium conditions and influence the drug dosage adjustment. Third, some indices were not detected in the study, such as the maternal 25OHD, $1,25(OH)_2D$, and PTHrP levels, which restricted further exploration. Fourth, due to the rarity of PHP, especially combined with pregnancy, the sample size of the study was small, which cannot fully represent the changes in calcium metabolism in PHP patients during pregnancy and lactation. However, this study was still a relatively large single-center study that focused on PHP conditions among pregnant women, providing useful information on these clinical entities.

5 Conclusion

In conclusion, the present study described the changes in serum calcium level, PTH level, and treatment needs in PHP patients during pregnancy and after delivery. It confirmed that the calcium condition and drug dosage changed differently in PHP patients during pregnancy and lactation but were more stable than those in HP patients. Most patients had good outcome with full-term delivery and normal birth weight. During pregnancy, an elevated PTH level might predict a worsened calcium metabolism and increase of medicine requirement. It reminds clinicians to focus on PTH level and adjust medicine dosage in time to maintain normal calcium metabolism in PHP pregnant patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by The Institutional Review Board of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Study design: J-JW, OW, and X-PX. (Epi)genetic analysis: YY and Y-BW. Data collection: AS, YJ, ML, Y-PL, and W-BX. Data analysis: J-JW and OW. Drafting manuscript: J-JW and OW. Revising manuscript content: OW. Approving final version of manuscript: J-JW, YY, Y-BW, AS, YJ, ML, W-BX, Y-PL, OW, and X-PX. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by grants from the National Natural Science Foundation of China (No. 81873641 and No. 82070817) and the Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (CIFMS) (2017-I2M-1-001).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Mantovani G, Bastepe M, Monk D, de Sanctis L, Thiele S, Ahmed SF, et al. Recommendations for diagnosis and treatment of pseudohypoparathyroidism and related disorders: An updated practical tool for physicians and patients. *Horm Res Paediatr* (2020) 93(3):182–96. doi: 10.1159/000508985

2. Kovacs CS. Maternal mineral and bone metabolism during pregnancy, lactation, and post-weaning recovery. *Physiol Rev* (2016) 96(2):449–547. doi: 10.1152/physrev.00027.2015

3. Marcucci G, Altieri P, Benvenga S, Bondanelli M, Camozzi V, Cetani F, et al. Hypoparathyroidism and pseudohypoparathyroidism in pregnancy: an Italian retrospective observational study. *Orphanet J Rare Dis* (2021) 16(1):421. doi: 10.1186/s13023-021-02053-3

4. Breslau NA, Zerwekh JE. Relationship of estrogen and pregnancy to calcium homeostasis in pseudohypoparathyroidism. *J Clin Endocrinol Metab* (1986) 62 (1):45–51. doi: 10.1210/jcem-62-1-45

5. O'Donnell D, Costa J, Meyers AM. Management of pseudohypoparathyroidism in pregnancy. case report. *Br J Obstet Gynaecol* (1985) 92(6):639–41. doi: 10.1111/j.1471-0528.1985.tb01405.x

6. Seki K, Osada H, Yasuda T, Sekiya S. Pseudohypoparathyroidism type 1b in pregnancy. *Gynecol Obstet Invest* (1999) 47(4):278–80. doi: 10.1159/000010122

7. Ochiai D, Uchino H, Ikeda T, Yakubo K, Fukuiya T. Pseudohypoparathyroidism type 1a in pregnancy. *J Obstet Gynaecol* (2013) 33(8):900. doi: 10.3109/01443615.2013.834302

8. Guedes Ramallo P, Boix Carreño E. Management of pseudohypoparathyroidism in pregnancy: a case report. *J Obstet Gynaecol* (2017) 37(4):519–20. doi: 10.1080/01443615.2016.1250733

9. Chu X, Zhu Y, Wang O, Nie M, Quan T, Xue Y, et al. Clinical and genetic characteristics of pseudohypoparathyroidism in the Chinese population. *Clin Endocrinol (Oxf)* (2018) 88(2):285–94. doi: 10.1111/cen.13516

10. Wang J-J, Wang O, Wang Y-B, Yang J, Song A, Jiang Y, et al. Changes in serum calcium and treatment of hypoparathyroidism during pregnancy and lactation: A single-center case series. *J Clin Endocrinol Metab* (2021) 106(12): e5054–63. doi: 10.1210/clinem/dgab530

11. Ahrens W, Hiort O, Staedt P, Kirschner T, Marschke C, Kruse K. Analysis of the GNAS1 gene in albright's hereditary osteodystrophy. *J Clin Endocrinol Metab* (2001) 86(10):4630–4. doi: 10.1210/jcem.86.10.7946

12. Takatani R, Kubota T, Minagawa M, Inoue D, Fukumoto S, Ozono K, et al. Prevalence of pseudohypoparathyroidism and nonsurgical hypoparathyroidism in Japan in 2017: A nationwide survey. *J Epidemiol* (2022). doi: 10.2188/jea.JE20220152

13. Ross AC, Taylor CL, Yaktine AL, Del Valle HB eds. Institute of medicine committee to review dietary reference intakes for vitamin d, calcium. the national academies collection: Reports funded by national institutes of health. In: *Dietary reference intakes for calcium and vitamin d*. Washington (DC): National Academies Press (US: National Academy of Sciences.

14. Fernández-Rebollo E, Lecumberri B, Garin I, Arroyo J, Bernal-Chico A, Goñi F, et al. New mechanisms involved in paternal 20q disomy associated with pseudohypoparathyroidism. *Eur J Endocrinol* (2010) 163(6):953–62. doi: 10.1530/EJE-10-0435

15. de Lange IM, Verrijn Stuart AA, van der Luijt RB, Ploos van Amstel HK, van Haelst MM. Macrosomia, obesity, and macrocephaly as first clinical presentation of PHP1b caused by STX16 deletion. *Am J Med Genet A* (2016) 170(9):2431–5. doi: 10.1002/ajmg.a.37818

16. Bréhin A-C, Colson C, Maupetit-Méhouas S, Grybek V, Richard N, Linglart A, et al. Loss of methylation at GNAS exon A/B is associated with increased intrauterine growth. *J Clin Endocrinol Metab* (2015) 100(4):E623–31. doi: 10.1210/jc.2014-4047

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 28 November 2022
DOI 10.3389/fendo.2022.1037546

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

RECEIVED 11 September 2022
ACCEPTED 07 November 2022
PUBLISHED 28 November 2022

CITATION
Fu L, Wang Y and Hu Y-Q (2022) Inferring causal effects of homocysteine and B-vitamin concentrations on bone mineral density and fractures: Mendelian randomization analyses. *Front. Endocrinol.* 13:1037546. doi: 10.3389/fendo.2022.1037546

Inferring causal effects of homocysteine and B-vitamin concentrations on bone mineral density and fractures: Mendelian randomization analyses

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Objectives: In the progress of bone metabolism, homocysteine (Hcy) and B vitamins play substantial roles. However, the causal associations of homocysteine, B-vitamin concentrations with bone mineral density (BMD), and fractures remain unclear. Therefore, we employed a two-sample Mendelian randomization (MR) design to infer the causal effects of Hcy and B vitamins on BMD and fractures.

Methods: We selected instrumental variables from large genome-wide association studies (GWASs). Specifically, the exposures mainly included Hcy (sample size: 44,147), vitamin B12 (sample size: 45,576), folate (sample size: 37,465), and vitamin B6 (sample size: 1,864). The outcome variables included total body BMD (sample size: 66,628), heel BMD (sample size: 142,487), femoral neck BMD (sample size: 32,735), lumbar spine BMD (sample size: 28,498), and forearm BMD (sample size: 8143). Additionally, the total body BMD in several age strata was also included. Furthermore, the fractures of the forearm, femoral neck, lumbar spine, heel corresponding with the BMD regions, and femoral neck and lumbar spine BMD in men and women, separately, were added as additional outcomes. Two-sample MR approaches were utilized in this study. Inverse variance weighting (IVW) was adopted as the main analysis. MR-PRESSO, MR-Egger, the weighted median estimate, and multivariable MR were performed as sensitivity methods.

Results: In the main analysis, Hcy concentrations have an inverse association with heel BMD (Beta = 0.046, 95% confidence interval (CI) -0.073 to -0.019, P = 9.59E-04) per SD unit. In addition, for one SD increase of vitamin B12, the total body BMD decreased 0.083 unit (95%CI -0.126 to -0.040, P = 1.65E-04). The trend was more obvious in age over 45 years (Beta = -0.135, 95%CI -0.203–0.067, P = 9.86E-05 for age 45-60; Beta = -0.074, 95%CI -0.141 to -0.007, P = 0.031 for age over 60 years). No association of B vitamins and Hcy levels with the risk of fractures and femoral neck and lumbar spine BMD in men and

women was found in this study. Other sensitivity MR methods elucidated consistent results.

Conclusions: Our findings indicated that there exist the inversely causal effects of Hcy and vitamin B12 on BMD in certain body sites and age strata. These give novel clues for intervening bone-related diseases in public health and nutrition.

KEYWORDS

bone mineral density, homocysteine, B vitamins, fractures, Mendelian randomization

Introduction

The reduction of bone mineral density (BMD) and the deterioration of bone microarchitecture have been recognized as the primary characteristics of osteoporosis (1, 2). Osteoporosis is a chronic skeletal disorder, which contributes to the elevated risk of bone fragility and is susceptible to occurrence of osteoporotic fracture (1, 2). Osteoporosis is a common disease and has an increased global prevalence, becoming a main worldwide public health issue (3, 4). It was reported that over 30% of women and 20% of men beyond the age of 50 were affected by osteoporosis (5, 6). To date, utilizing the dual X-ray absorptiometry for gauging BMD becomes the current gold standard to diagnose osteoporosis (7). However, applying quantitative ultrasound for measuring BMD can provide additional information including bone size, geometry, and microarchitecture, which are poorly captured by dual X-ray absorptiometry (8). Notably, researchers proved that BMD is generally impacted by various risk factors, including gender (9), age (10), smoking (11), and alcohol consumption (12).

In the general population, the lack of folate and vitamins B12 and B6 would result in elevated concentrations of homocysteine (Hcy) as these vitamins serve as cofactors for the varieties of enzymes involved in Hcy metabolism (Figure 1) (13, 14). The published studies indicated that milder elevation in Hcy levels was associated with a two- to fourfold elevated risk of hip and other fractures (15, 16). Some studies also revealed that increased Hcy concentrations might be associated with high osteoclast activity (17), elevated rates of bone turnover (17), and decreased BMD (18); some others have found no association of Hcy with BMD (19–21) and the markers of bone metabolism (22). A clinical trial utilizing randomized and placebo-controlled approaches implicated that folate and vitamin B12 concentrations over a 2-year period did not observe the changed levels of BMD or decreased fractures in elderly subjects with enhanced Hcy concentrations (23, 24). Thus, whether Hcy, together with B vitamins, directly influenced BMD and bone mass or is an "innocent bystander" is still kept unclear (25). It is necessary to take action to further investigate the relationship between BMD and the Hcy and B-vitamin concentrations.

The traditional observational studies have methodological limitations with the bias of undetected confounding factors and reverse causality, resulting in the deficiency of testing the causal associations between exposures and outcomes (26). Nowadays, the genetic variants, regarded as instrumental variables and being associated with exposures, were fully used by the Mendelian randomization (MR) design to act as the proxies of the risk factor for outcomes for inferring the causal effects of exposure and the outcome (27). Because of the genetic variants of offspring inherited randomly from their parents, in general, confounding factors are less likely to have an impact on these genetic instruments, and, hence, the studies with the MR design scarcely suffer from reverse causality and confounding factors (28).

Given the abovementioned evidence that Hcy concentrations could probably lead to the changes of both bone mass and bone quality, together with the dependence of Hcy metabolism and B vitamins (29), as well as the controversial findings about the associations of B vitamins and Hcy with BMD in the observational studies (15, 19–21, 30–32), it is urgent to infer the causal associations of B vitamins and Hcy levels with BMD and then provide accurate causal effects. In this study, two-sample MR study approaches were carried out to explore the associations of the genetic prediction of B vitamins (folate and vitamins B12 and B6) and Hcy concentrations with total body BMD. Concurrently, different regions and age-specific associations between the concentrations of B vitamins, Hcy, and BMD were assessed for further exploration. The schematic overview of this MR study design is presented in Figure 2.

Material and methods

We conducted a two-sample MR study using public GWAS data. Generally, MR should be satisfied with three conditions

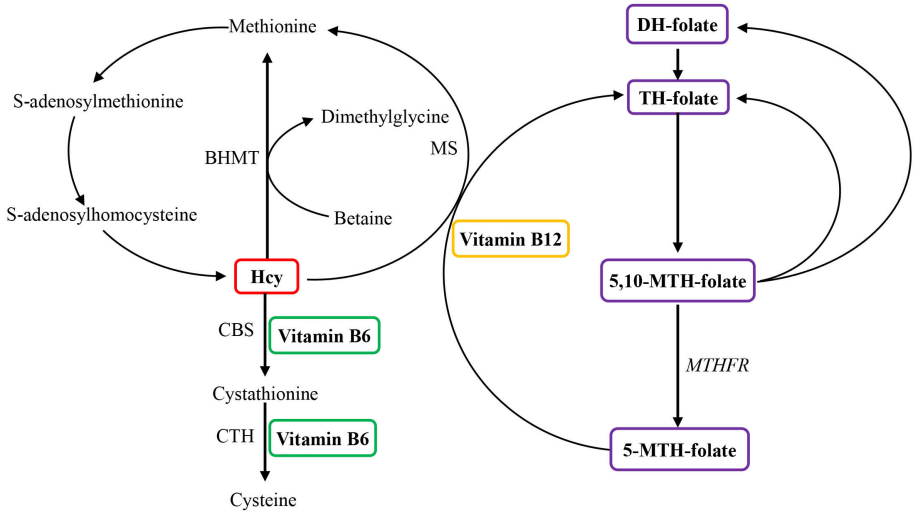

FIGURE 1
Overview of the role of vitamin B6, vitamin B12, and folate in homocysteine metabolism. Homocysteine is reconverted to methionine by acquiring a methyl group from 5-methyltetrahydrofolate, the positive form of betaine, or folate in the remethylation pathway. Irreversible removal of homocysteine happens across the transsulfuration pathway in which homocysteine condenses with serine to form cystathionine. BHMT, betaine homocysteine methyltransferase; CBS, cystathionine-β-synthase; CTH, cystathionine-gamma-ligase; DH, dihydo; Hcy, homocysteine; MS, methionine synthase (encoded by the MTR gene); MTH, methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; TH, tetrahydro.

(Figure 2). First, as genetic instruments, the genetic variants have to be strongly associated with the exposures, which are B vitamins and Hcy in this study. We adopted the genome- wide significant level (P < 5E-08) together with r^2 < 0.01 (this corresponds to linkage disequilibrium measure to define "independent genetic instrument") as the inclusion criteria for genetic instruments. Second, no confounder exists in the relationships between the genetic instruments and the

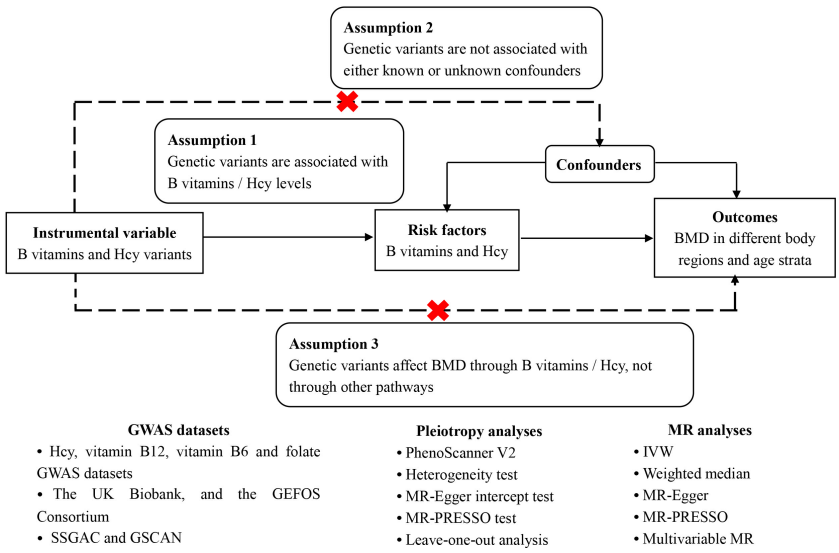

FIGURE 2
Schematic overview of the Mendelian randomization study design.

outcomes. Third is the exclusion restriction assumption (33, 34), namely, the genetic instruments affect the outcomes only through exposures. Horizontal pleiotropy testing could verify this assumption where the genetic instruments directly influence the outcomes or not (35).

Ethical approval

Published or public data were used, and no original data were involved in this MR study. The original publications or included consortia described ethical approval and informed consent from every individual for the corresponding studies in the research.

Selection of genetic instruments

Based on the literature of exposures on serum vitamin B12 (sample size: 45,576) (36), blood Hcy (sample size: 44,147) (37), serum folate (sample size: 37,465) (36) and serum vitamin B6 (sample size: 1864) (38), SNPs that are associated with the corresponding exposures at the genome-wide significant level (P < 5E-08) were chosen as candidate genetic instruments, respectively. Then, we estimated the linkage disequilibrium among these SNPs for each exposure with the PLINK clumping method on the basis of 1000 Genome European reference panel as the GWASs are mostly from European origin. We also excluded palindromic variants with equivocal strands. Consequently, diverse SNPs without linkage disequilibrium (r^2 < 0.01) were utilized as the genetic instruments (Supplementary Table 1). Specifically, there were 14 independent SNPs for Hcy, 14 independent SNPs for vitamin B12, 2 independent SNPs for folate, and 1 SNP for vitamin B6 (Supplementary Table 1). In total, the SNPs accounted for 6.0% of variance for Hcy (37) and vitamin B12 (36), respectively, and 1.3% of variance for vitamin B6 (38), as well as 1.0% of variance for folate (36). In case these SNPs for B vitamins and Hcy are associated with other correlated phenotypes, we used the internet resource (PhenoScanner V2) (39) and found that three SNPs (rs548987, rs42648, and rs838133) for Hcy and two SNPs (rs56077122 and rs34324219) for vitamin B12 were associated with other phenotypes (the search was conducted in April 2022), which might exert pleiotropic effects. Therefore, these five SNPs were removed. The flow chart of this MR study is shown in Figure 3. As the exposures, B vitamins and Hcy were transformed to one standard deviation (SD) unit. For testing the strength of genetic instruments, we calculated the F-statistics for different exposures (F = 256.1 for Hcy, F = 242.4 for vitamin B12, F = 189.2 for folate, and F = 24.5 for vitamin B6). All F-statistics were greater than 10, indicating the supportive evidence for the strength of genetic instruments.

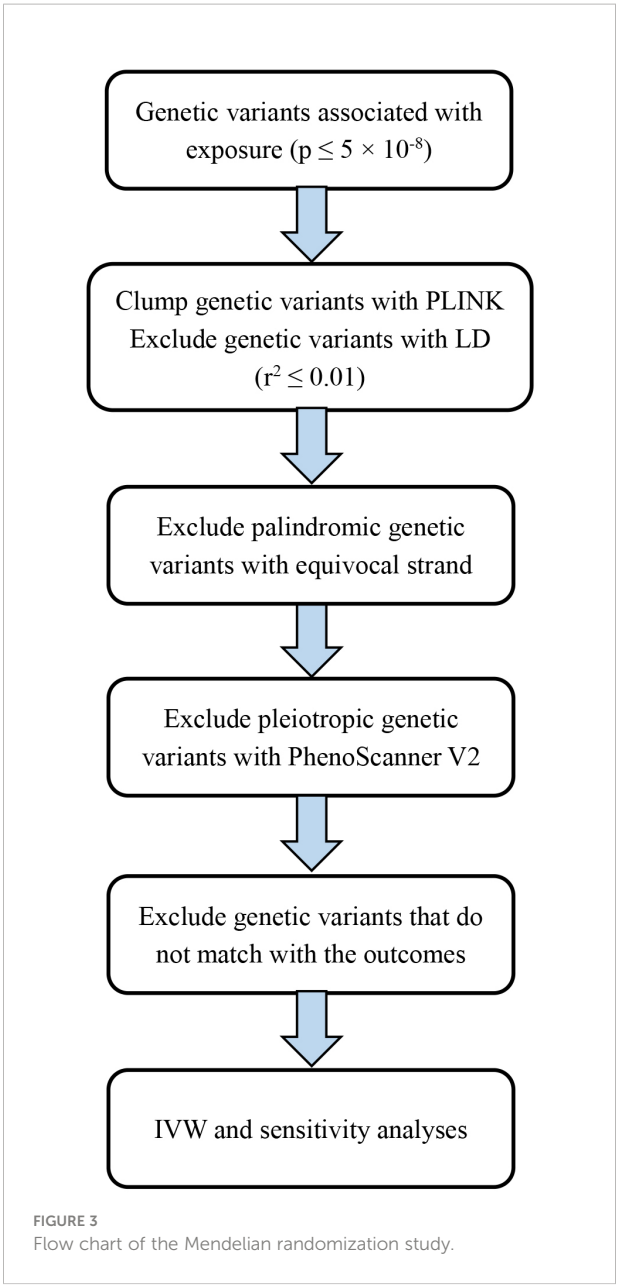

FIGURE 3
Flow chart of the Mendelian randomization study.

Datasets used for genetic association with outcomes

The forearm, femoral neck, lumbar spine, and heel are the prevalent sites of osteoporosis. The loss of BMD in these regions elevated the risk of osteoporosis and fractures compared to other body regions (40, 41). Thus, the BMD in these four body regions was firstly considered as outcomes. The summary statistics for BMD in the forearm, femoral neck, and lumbar spine were extracted from the GEnetic Factors for Osteoporosis (GEFOS) consortium (42), including 8,143, 32,735, and 28,498 subjects, respectively. For heel BMD, the summary statistics were

provided by a GWAS dataset from UK Biobank (43), comprising 142,487 subjects. For BMD measured and validated across the study participants, heel BMD was done by quantitative ultrasound while other sites were done by dual X-ray absorptiometry.

We also explored the associations of B vitamins and Hcy with total body BMD (measured by dual X-ray absorptiometry) and BMD in different age strata (age strata referred to the total body BMD GWAS), which encompassed five age groups, namely, younger than 15 years old, 15–30, 30–45, 45–60, and older than 60 years old, as age has been deemed as a risk factor for becoming osteoporosis. According to a large GWAS meta-analysis (8), we acquired summary statistics for the total body BMD and BMD in these five age strata, which contained 66,628 subjects in total.

Before analyses in this MR study, all the outcomes of BMD were SD-transformed. A detailed information of data sources was displayed in Table 1.

Regarding the fact that fractures arising from fragile bones were clinically supposed to be a result of osteoporosis (44), the fractures of the forearm, femoral neck, lumbar spine, and heel corresponding with the BMD regions were added as additional outcomes. We extracted the summarized data for fractures from the Neale Lab (http://www.nealelab.is/uk-biobank), which encompassed 361,194 subjects, with 8,438 cases. Additionally, we explored the causal associations of Hcy and vitamin B12 with femoral neck and lumbar spine BMD in men and women, separately, for the consideration of the different effects because of sex. The data were from the GEFOS Consortium (45).

TABLE 1 Descriptions of the genome-wide association studies (GWASs) employed in this Mendelian randomization study.

Exposure	Data source (PMID)	Sample size	%European	Covariates adjusted in research
Homocysteine (SD of log transformed)	PMID: 23824729	44,147	100	Age and sex and principal components in individual studies where applicable
Vitamin B12 (SD of quantile transformed)	PMID: 23754956	45,576	100	Age, year of birth, sex and the first principal component
Folate (SD of quantile transformed)		37,465		
Vitamin B6 (SD)	PMID: 19303062	1864	100	Not reported
Outcomes	**Data source (PMID)**	**Sample size**	**%European**	**Covariates adjusted in GWAS**
Forearm BMD (SD)	The GEFOS Consortium (26367794)	8,143	100	Age, age^2, sex, and weight
Femoral neck BMD (SD)		32,735		
Lumbar spine BMD (SD)		28,498		
Heel BMD (SD)	The UK Biobank study (UKB, 28869591)	142,487	100	Genotyping array, age, sex, and the first four ancestry principal components
Total body BMD (SD)	The GEFOS Consortium (29304378)	66,628	86	Age, weight, height, and genomic principal components, as well as any additional study-specific covariates
Total body BMD of age 0–15 (SD)		11,807		
Total body BMD of age 15–30 (SD)		4,180		
Total body BMD of age 30–45 (SD)		10,062		
Total body BMD of age 45–60 (SD)		18,805		
Total body BMD of age over 60 (SD)		22,504		
Confounders	**Data source (PMID)**	**Sample size**	**%European**	**Covariates adjusted in research**
Years education attained (SD)	SSGAC (30038396)	766,345	100	Sex, birth year, their interaction, and 10 principal components of the genetic relatedness matrix
Smoking Heaviness (SD of cigarettes per day)	GSCAN (30643251)	337,334	100	Age, sex, age × sex interaction, and the first 10 genetic principle components
Alcohol (SD of log transformed drinks per week)		941,280		

BMD, bone mineral density; GEFOS, GEnetic Factors for OSteoporosis Consortium; GSCAN, GWAS and Sequencing Consortium of Alcohol and Nicotine use; SSGAC, Social Science Genetic Association Consortium; the summary-level data utilized in this study can be downloaded from the GWAS Catalog (https://www.ebi.ac.uk/gwas/) and Neale Lab (http://www.nealelab.is/uk-biobank).

Exploring possible sources of horizontal pleiotropy

Under the Instrument Strength is Independent of Direct Effect (InSIDE) assumption, the risk factors predicted by the genetic instruments should not influence both the exposures and outcomes simultaneously. In order to comply with this assumption, we assessed the possible relationship between the genetic instruments and risk factors, encompassing the degree of education (46), smoking, and alcohol using the rate (47). The descriptions of these variables are shown in Table 1.

Harmonizing allele

In light of harmonizing the effect of alleles and their corresponding directions, we combined the genetic statistics from the GWAS of exposures and outcomes. The effect allele frequency was also utilized to make sure that palindromic genetic instruments were aligned properly. For SNPs that did not exist in the outcome datasets, we applied the substitute SNPs with $r^2 > 0.8$ for the exposure-associated SNPs. Missing SNPs without matching substitutes for outcome GWAS summary statistics were ruled out from the subsequent analyses.

Statistical analysis

Inverse variance weighting (IVW) with multiplicative random effects was employed as the main analysis (48). Additionally, four MR methods, including the weighted median estimate (49), MR-Egger (50), MR-PRESSO (51), and multivariable MR (52), were performed as sensitivity approaches for vitamin B12 and Hcy. IVW stands for the weighted regression slope of the effect of SNP-outcome on SNP-exposure assuming that the intercept is restricted to zero (48). When 50% SNPs are invalid instruments, the weighted median estimate could provide an unbiased evaluation (49). Under the InSIDE assumption, the horizontal pleiotropy can be estimated by the MR-Egger approach across the *P*-value for its intercept, as well as an assessment was given after the pleiotropic effects were adjusted. However, the MR-Egger approach may probably obtain wider confidence intervals (CIs) because of a lost statistical power (50). MR-PRESSO is another statistical method testing biases in the case of pleiotropy (the global test). It gives a corrected estimate *via* removing the outliers and supplies a distortion test, which evaluates whether the calculations with or without outliers obtain similar assessments under the InSIDE assumption (51). We also evaluated Cochran's Q statistic to investigate the magnitude of heterogeneity (53) among the employed SNPs in every analysis. Subsequently, we utilized an online approach to calculate power (Supplementary Table 4) in addition to providing a supporting evidence of the strength of genetic instruments like *F*-statistics (54). Additionally, effects (betas) with the corresponding 95%CI were transformed to one SD increase in the genetic prediction of B vitamins and Hcy.

Linkage disequilibrium score regression (LDSC) was performed to assess sample overlap with LD hub (http://ldsc.broadinstitute.org/) (55) in case overlapping samples between two datasets bias the estimated causal effects. Considering the possibility that genetic instruments might have collider or ascertainment bias by conditioning on the possible confounders, we performed multivariable MR (52), which admits the direct effects of multiple variables on an outcome to be evaluated jointly. The effects of Hcy and vitamin B12 (because of insufficient instruments for folate and vitamin B6) on total body BMD and BMD in different body regions and age strata were assessed with adjusting for potential confounders, including educational attainments, smoking, and alcohol usage. We also conducted leave-one-out analysis to test the robustness of the significant main findings.

In terms of multiple testing adjustment, we employed a conservative *P*-value threshold of 1.25E-03 by Bonferroni correction, considering four exposures and 10 outcomes (0.05/40) for BMD. The *P*-value between the Bonferroni-adjusted significance level and the traditional significance level ($P < 0.05$) was regarded as suggestive significance. All analyses were performed by R Version 4.1.0 utilizing R packages ("TwoSampleMR") (56), ("MRPRESSO") (51), ("MendelianRandomization") (57).

Results

LDSC was employed to estimate overlapping samples between the exposure GWAS and the outcome GWAS. Results showed an approximately zero intercept of genetic covariance less than 10^{-3} in the pairs of the exposure–outcome GWAS ($P > 0.1$ by z-test in all pairs, data not shown), indicating approximately no sample overlap in the pairs of two datasets.

For the causal effects of B vitamins and Hcy on BMD in different body regions, we observed a significant association of the genetically predicted elevated concentrations of Hcy with decreased BMD in the heel region (Figure 4). For 1-SD increase in the genetic prediction of Hcy levels, the estimated beta was -0.046 (95CI, -0.073 to -0.019, $P = 9.59E-04$) for the 1-SD BMD of the heel. Results remained directionally consistent by the weighted median estimate and the MR-Egger and MR-PRESSO methods (Supplementary Tables 5, 7). We noticed that there is no heterogeneity on the basis of Cochran's Q statistic and no pleiotropy across the MR-Egger and MR-PRESSO methods for vitamin B12 and Hcy in the analyses of BMD in different body regions (Supplementary Tables 5–7). Leave-one-out analysis showed that the significant association between Hcy levels and

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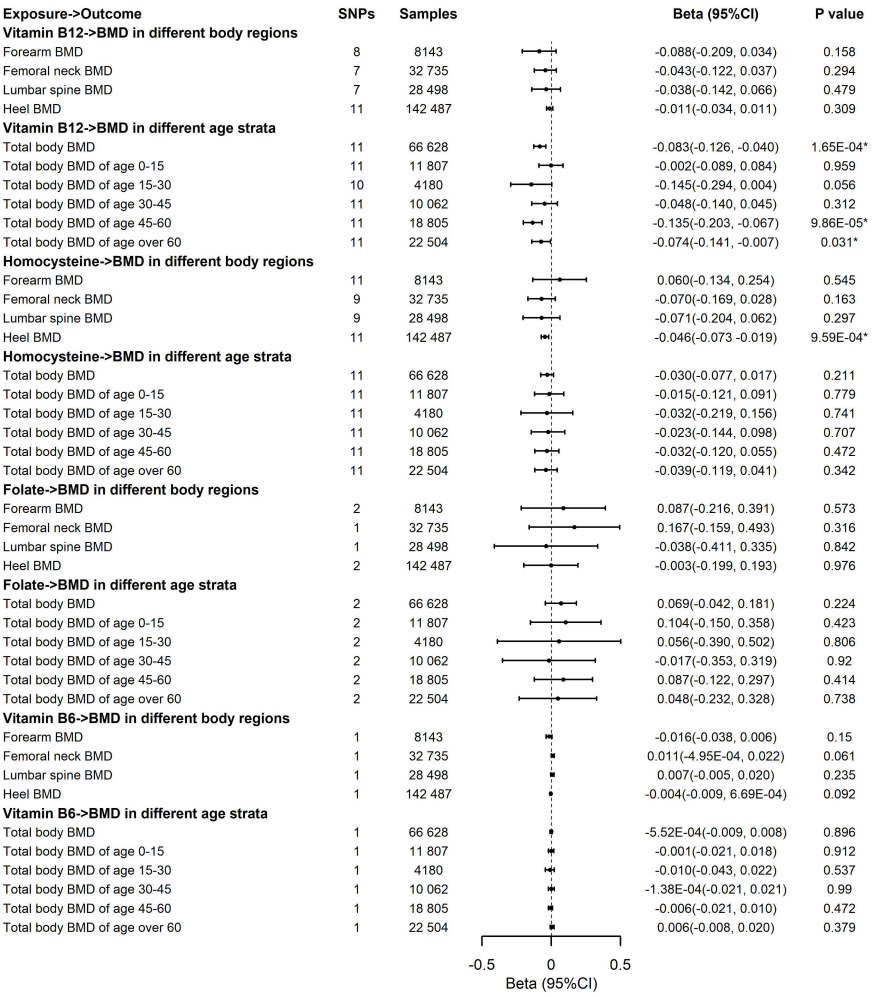

FIGURE 4
Effects of the genetic prediction of circulating vitamin B12, homocysteine, folate, and vitamin B6 on bone mineral density in different body regions and different age strata with the inverse variance weighting method. BMD, body mineral density; CI, confidence interval; GEFOS, GEnetic Factors for OSteoporosis Consortium; IVW, inverse variance weighting; The summary statistics data utilized in this study can be downloaded from the GWAS Catalog (https://www.ebi.ac.uk/gwas/) and Neale Lab (http://www.nealelab.is/uk-biobank); *The P-value reached the significant level; SNP, single-nucleotide polymorphism.

heel BMD was not affected by single SNPs associated with Hcy concentrations (Supplementary Figure 2). The results were fairly robust from the outputs from various approaches. The genetic prediction of B-vitamin concentrations had no effect on BMD in different body regions (Figure 4). Multivariable MR analyses showed that the genetic prediction of increased Hcy levels was significantly associated with lower heel BMD (Beta, -0.041; 95%CI, -0.070 to -0.012; P = 6.28E-03) after adjusting for education attainment, smoking, and alcohol usage (Supplementary Figure 1). Thus, according to all sensitivity analyses, it is confident that the significance of the elevated concentrations of Hcy contributing to decreased heel BMD was credible in this study. In addition, we observed no association of B vitamins and Hcy levels with the risk of fractures of the four regions (Supplementary Tables 8–10) and no association of vitamin B12 and Hcy levels with femoral neck and lumbar spine BMD in men and women, separately (Supplementary Tables 11, 12–14).

The associations of the genetic prediction of B vitamins and Hcy concentrations with total body BMD and BMD in different age strata are displayed in Figure 4. We observed the significant associations of genetically predicted elevated vitamin B12 with lower total body BMD (Beta, -0.083; 95%CI, -0.126 to -0.040; P = 1.65E-04), especially decreased total body BMD of age 45–60 (Beta, -0.135; 95%CI, -0.203 to -0.067; P = 9.86E-05) and age over 60 years (Beta, -0.074; 95%CI, -0.141 to -0.007; P = 0.031). No significant association was revealed in Hcy and other B vitamins with total body BMD in different age strata (Figure 4,

Supplementary Tables 5, 7). The significant findings of associations between vitamin B12 and total body BMD, as well as the BMD of age 45–60 and age over 60 kept consistent significantly by the weighted median estimate and the MR-PRESSO approach (Supplementary Tables 6, 7). Additionally, Cochran's Q statistic and the MR-Egger and MR-PRESSO approaches indicated no heterogeneity and no pleiotropy existing in these analyses (Supplementary Tables 6, 7). In the multivariable MR analysis, the genetic prediction of increased vitamin B12 was more significantly associated with lower total body BMD (Beta, -0.085; 95%CI, -0.133, -0.038; P = 4.39E-04), total body BMD of age 45–60 (Beta, -0.128; 95%CI, -0.198, -0.058; P = 3.55E-04), and age over 60 years (Beta, -0.076; 95% CI, -0.145, -0.008; P = 0.029) after adjusting for education attainment, smoking, and alcohol usage (Supplementary Figure 1). Leave-one-out analysis showed that a single SNP was unable to influence the significant associations, confirming these significant results (Supplementary Figure 2).

Considering the confounding factors including education, smoking, and alcohol usage, we noticed that the effect alleles for the B vitamins and Hcy instruments (harmonized to increased concentrations of B vitamins and Hcy) were not associated with education, smoking, and alcohol usage (Supplementary Table 3), which suggested that the relationships between exposures and outcomes were unlikely to be influenced by these confounders.

Discussion

In the present study, the genetic variants of exposures (SNPs-B vitamins and SNPs-Hcy) and outcomes (SNPs-BMD in different body regions and age strata) were collected from various large-scale GWAS data sources, and then, we conducted MR analyses to evaluate the causal effects of B vitamins and Hcy on the changes of total body BMD and BMD in distinct body sites and age strata. The findings of our study elucidated that the increased genetic prediction of Hcy concentrations was causally associated with the reduced heel BMD, and the elevated genetic prediction of vitamin B12 had a causal impact on decreased total body BMD, especially in the strata of age 45–60 and age over 60 years. Moreover, there was no association of B vitamins and Hcy concentrations with the risk of fracture of any of the bones and femoral neck and lumbar spine BMD in men and women.

Evidence showed that Hcy concentrations and B-vitamin levels had an effect on the change of BMD (15, 30–32), One study focused on elderly people disclosed the potential effect of Hcy on hip fracture, and its results therein indicated that the subjects with higher concentrations of Hcy had an increased risk of hip fracture than those with reduced Hcy levels, revealing that Hcy might be a vital factor for hip fracture in older persons (15). Therefore, it should be focused on age and fracture sites. The effects of Hcy levels on bone health may be different in distinct body regions and age strata. Another cross-sectional study has suggested the higher Hcy concentrations were inversely associated with the BMD in femoral neck and lumbar spine regions (58), which gave the support that different associations between Hcy levels and body regions for our motivation. Our findings declared that elevated Hcy levels had a significant influence on decreased heel BMD through the MR study design, revealing a novel clue for reducing heel BMD loss as heel BMD has been reported to be a vital risk factor for incident disability and mortality (59). Thus, this finding has great significance for public health.

With regard to the age-specific causal associations of B vitamins and Hcy with BMD, previous studies have demonstrated enhanced Hcy concentrations with decreased BMD in different ages (30, 60). A remarkable increase in Hcy concentrations with reduced BMD was found in postmenopausal women (60). Moreover, several scholars have explored the associations between Hcy and BMD in different ages, in which the concentrations of Hcy were negatively associated with the BMD of femoral neck, lumbar spine and hip regions in women aged under 50 (30). These indicated that age might be a risk for BMD loss. It would be better to test the effects of B vitamins and Hcy levels on BMD in different age strata. Our finding indicated that increased vitamin B12 significantly reduced BMD, especially in age over 45. Several observational studies unraveled that the elevated concentrations of vitamin B12 and folate have no effect on BMD (18, 20, 61–63). These negative results can be attributed to the multifactorial etiology of the disease and the different populations analyzed in these studies. The findings of the current study give relatively strong support for the significant causal association between vitamin B12 levels and total body BMD. Recently, an MR study by Wang et al. employed six SNPs associated with Hcy as instruments elucidated that genetically reduced Hcy was only associated with the increase of forearm BMD (64). However, too-few instruments would result in insufficient statistical power in this study by Wang et al. (64). In addition, some flaws existed in this study (64) as it elucidated that genetically reduced Hcy was associated with the increase of forearm BMD, while genetically determined Hcy elevation was not correlated with BMD. These are contradictory findings. The reasons of the inconsistent results were the effect alleles had to be unified before MR analysis was performed, and artificially dividing genetic instruments into two groups would reduce statistical power and lead to inaccurate results. Previously, we have developed genetic statistics to detect genetic variation in complex diseases and used MR approaches to investigate the causal relationships between complex diseases, including the casual association of B vitamins and Hcy with musculoskeletal diseases (28, 65–71). In the present study, we performed this MR using the latest instruments for Hcy and B-vitamin levels, offering both F-statistics and power calculations, to provide sufficient statistical power for inferring the causal effects of B vitamins and Hcy concentrations on total body BMD and BMD in different body regions and age strata. Compared to the study by Wang et al.

(64), our study not only gave sufficient statistical power for inferring (effect alleles were unified before MR analysis) but also expanded the types of exposures (both Hcy and B vitamins) and outcomes (BMD in different body regions, sex and age strata, and fractures in different body regions). Furthermore, pleiotropy tests and sensitivity analyses (including multivariable MR) were also performed to ensure the robustness of our findings. Furthermore, Hcy and B vitamins were able to influence bone tissue formation by means of disrupting the development of collagen cross-links and reducing bone blood flow (72, 73). These support our findings that higher levels of Hcy and vitamin B12 mainly lead to bone catabolism.

Several mechanisms have been found to explain the relationships between BMD and B vitamins and Hcy levels. *In vitro* studies have demonstrated that Hcy and B vitamins could improve the activity and differentiation of osteoclasts and then induce the apoptosis of human bone marrow stromal cells *via* elevating reactive oxygen species (64). Furthermore, they also showed the ability of reducing bone blood flow, which was probably related to the mechanical bone properties (73). Specifically, Hcy and B vitamins produced apoptosis in bone marrow cells by means of the action of Nuclear Factor (NF)-kappa B and reactive oxygen species (74). The intracellular reactive oxygen species induced by them motivated osteoclast formation (75). Because the antioxidant N-acetyl cysteine could disrupt such negative impacts on bone cells (75), an enhancement in reactive oxygen species generated by them was able to have a substantial influence on the elevation in bone resorption in hyperhomocysteinemia. In addition, Hcy produced apoptosis in osteoblastic MC3T3-E1 cells by the virtue of inducing intracellular reactive oxygen species in a Hcy dose-dependent way (76). Age is a non-negligible risk factor for BMD. The average BMD may reduce considerably with age (77), particularly in subjects over 50 years. Dual X-ray absorptiometry and thoracic quantitative computed tomography suggested that bone mass reduces with age (78). Taken together, the above evidence revealed a possible pathogenic function of Hcy and B vitamins in the reduction of BMD, and age should be considered for further study.

The strengths of the present study came from the usage of MR, which could reduce residual confounding and diminish the possibility of false negatives by large GWASs. In addition, utilizing a series of non-overlapping data sources and applying more SNPs of B vitamins and Hcy were capable of enlarging sample size and explaining more phenotypic variance, respectively, as well as insuring statistical power. Notice that some participants present in the GWAS for BMD in distinct age strata were not restricted to European ancestry. This situation impeded the generalization of our results because the allele frequencies among populations were distinguished. Considering the likelihood of pleiotropy as an important issue in this study, we got directionally consistent results with the same significance in most sensitivity approaches (the weighted median estimate, MR-Egger, MR-PRESSO, and multivariable MR) after removing the obvious instruments with pleiotropy (Supplementary Table 2). The confounding factors encompassing education, smoking, and alcohol use were also tested in the case of confusing the relationships between exposures and outcomes since these confounders could influence BMD (11, 12). Moreover, no pleiotropic effect was observed in the findings of MR-Egger and MR-PRESSO, which suggested that unobserved pleiotropy and confounding did not bias our results. For the concern that vitamin B12 and Hcy may not be independent variables for causal inferring, actually, no matter vitamin B12 or Hcy, the three conditions (Figure 2) of conducting the MR study are satisfied. For avoiding the horizontal pleiotropy because of the interlink between vitamin B12 and Hcy, we used the strict screening criteria for selecting SNPs as instrumental variables. In other words, these instruments could absolutely infer the causal association between vitamin B12 and BMD and Hcy and BMD, respectively. However, our negative findings for vitamin B6 may reflect inadequate statistical power. Moreover, the present study provided little evidence on the association of B vitamins and Hcy levels with the risk of fracture. As one of the assumptions in MR is that the genetic variants affect outcomes only through exposure, the selected genetic variants for B vitamins and Hcy may not influence fractures *via* the Hcy metabolism. We only evaluate BMD in the forearm, femoral neck, lumbar spine and heel, which are common sites. Some BMD in other sites, such as BMD in spine, finger, rib, and scapula, would affect the total BMD. That is the reason why vitamin B12 showed association with total body BMD but not at any one of our evaluated sites. Moreover, dietary intake was not taken into account and the variation in each of the exposures that was explained by SNPs was small. Furthermore, both quantitative ultrasound and dual X-ray absorptiometry have their own advantages for evaluating BMD (7, 8). Due to the complex relationship between B vitamins and Hcy, as well as different measures for gauging BMD, further studies need to take the correlation between them into account to better assess their effects on BMD. Although we took into account the effects of age and sex on BMD, further studies should consider to explore data in separated populations according to the sex and menopausal status within the different gender groups (i.e., women >55 years old) since the hormonal status is the most important issue to consider.

Conclusions

In conclusion, the present study demonstrated that the causalities of genetic prediction of Hcy increase with the reduced heel BMD, as well as genetic prediction of vitamin B12 elevation in relation to the total body BMD, especially in the strata of age 45–60 and age over 60, indicating that the concentrations of B vitamins and Hcy might play a crucial role

in the development of bone health in distinct body regions and age strata. Future studies are, however, needed to explore the potential mechanisms by which B vitamins and Hcy regulate BMD. To this end, the levels of Hcy and vitamin B12 may be utilized in the early treatment of osteoporosis.

Data availability statement

Publicly available datasets were analyzed in this study. The authors thank the UK Biobank study, and the summary statistics data of UKB can be download from Neale lab (http://www.nealelab.is/uk-biobank). The authors thank the GEFOS consortium, and the summary statistics data in GEFOS Consortium can be downloaded from the website (https://www.gefos.org/). The authors thank SSGAC for supplying the summary statistics of years of education attainment, which have been downloaded from https://www.thessgac.org/data. The authors thank GSCAN for supplying the summary statistics of smoking and alcohol use phenotypes, which have been downloaded from https://conservancy.umn.edu/handle/11299/201564.

Author contributions

Study concept and design: LF and Y-QH; Acquisition of data: LF, Y-QH, and YW; Analysis and interpretation of data: LF; Drafting of the manuscript: LF and Y-QH; Critical revision of the manuscript for important intellectual content: LF, YW, and Y-QH; All authors have read and approved the final version of the manuscript.

Funding

This study was supported by grants to LF and Y-QH from the National Natural Science Foundation of China (grants no. 82204063, 11971117, 11571082).

Acknowledgments

The authors thank the UK Biobank study, and the summary statistics data of UKB can be downloaded from Neale lab (http://www.nealelab.is/uk-biobank). The authors thank the GEFOS consortium, and the summary statistics data in the GEFOS Consortium can be downloaded from the website (https://www.gefos.org/). The authors thank SSGAC for supplying the summary statistics of the years of educational attainment, which have been downloaded from https://www.thessgac.org/home. The authors thank GSCAN for supplying the summary statistics of smoking and alcohol use phenotypes, which have been downloaded from https://conservancy.umn.edu/handle/11299/201564. The authors thank all the reviewers and editors for their useful suggestions for improving this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1037546/full#supplementary-material

References

1. NIH Consensus Development Panel on Osteoporosis Prevention D, and Therapy Osteoporosis prevention, diagnosis, and therapy. *JAMA* (2001) 285:785–95 doi: 10.1001/jama.285.6.785

2. Akkawi I, Zmerly H. Osteoporosis: Current concepts. *Joints* (2018) 6:122–27. doi: 10.1055/s-0038-1660790

3. Cawthon PM, Ewing SK, Mackey DC, Fink HA, Cummings SR, Ensrud KE, et al. Change in hip bone mineral density and risk of subsequent fractures in older men. *J Bone Miner Res* (2012) 27:2179–88. doi: 10.1002/jbmr.1671

4. Black DM, Rosen CJ, Solomon CGPostmenopausal osteoporosis. *N Engl J Med* (2016) 374:1797. doi: 10.1056/NEJMx160008

5. Cooper C. Epidemiology of osteoporosis. *Osteop Int* (1999) 9 Suppl 2:S2–8. doi: 10.1007/pl00004156

6. Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European union: medical management, epidemiology and economic burden. A report prepared in collaboration with the international osteoporosis foundation (IOF) and the European federation of pharmaceutical industry associations (EFPIA). *Arch Osteop* (2013) 8:136. doi: 10.1007/s11657-013-0136-1

7. Peacock M, Turner CH, Econs MJ, Foroud T. Genetics of osteoporosis. *Endocr Rev* (2002) 23:303–26. doi: 10.1210/edrv.23.3.0464

8. Medina-Gomez C, Kemp JP, Trajanoska K, Luan J, Chesi A, Ahluwalia TS, et al. Life-course genome-wide association study meta-analysis of total body BMD and assessment of age-specific effects. *Am J Hum Genet* (2018) 102:88–102. doi: 10.1016/j.ajhg.2017.12.005

9. Alswat KA. Gender disparities in osteoporosis. *J Clin Med Res* (2017) 9:382–87. doi: 10.14740/jocmr2970w

10. Qu Z, Yang F, Hong J, Wang W, Yan S. Parathyroid hormone and bone mineral density: A mendelian randomization study. *J Clin Endocrinol Metab* (2020) 105:dgaa579. doi: 10.1210/clinem/dgaa579

11. Bijelic R, Milicevic S, Balaban J. Risk factors for osteoporosis in postmenopausal women. *Med Arch* (2017) 71:25–8. doi: 10.5455/medarh.2017.71.25-28

12. Cheraghi Z, Doosti-Irani A, Almasi-Hashiani A, Baigi V, Mansournia N, Etminan M, et al. The effect of alcohol on osteoporosis: A systematic review and meta-analysis. *Drug Alcohol Depend* (2019) 197:197–202. doi: 10.1016/j.drugalcdep.2019.01.025

13. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* (1993) 270:2693–8. doi: 10.1001/jama.1993.03510220049033

14. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the framingham offspring cohort. *Am J Clin Nutr* (2001) 73:613–21. doi: 10.1093/ajcn/73.3.613

15. McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, et al. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med* (2004) 350:2042–9. doi: 10.1056/NEJMoa032739

16. van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, van der Klift M, de Jonge R, Lindemans J, et al. Homocysteine levels and the risk of osteoporotic fracture. *N Engl J Med* (2004) 350:2033–41. doi: 10.1056/NEJMoa032546

17. Herrmann M, Kraenzlin M, Pape G, Sand-Hill M, Herrmann W. Relation between homocysteine and biochemical bone turnover markers and bone mineral density in peri- and post-menopausal women. *Clin Chem Lab Med* (2005) 43:1118–23. doi: 10.1515/cclm.2005.195

18. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Gjessing HK, et al. Plasma total homocysteine level and bone mineral density: The hordaland homocysteine study. *Arch Intern Med* (2006) 166:88–94. doi: 10.1001/archinte.166.1.88

19. Browner WS, Malinow MR. Homocyst(e)inaemia and bone density in elderly women. *Lancet* (1991) 338:1470. doi: 10.1016/0140-6736(91)92782-w

20. Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A. Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women. *Bone* (2003) 33:956–9. doi: 10.1016/j.bone.2003.07.001

21. Golbahar J, Hamidi A, Aminzadeh MA, Omrani GR. Association of plasma folate, plasma total homocysteine, but not methylenetetrahydrofolate reductase C667T polymorphism, with bone mineral density in postmenopausal Iranian women: a cross-sectional study. *Bone* (2004) 35:760–5. doi: 10.1016/j.bone.2004.04.018

22. Nilsson K, Gustafson L, Isaksson A, Hultberg B. Plasma homocysteine and markers of bone metabolism in psychogeriatric patients. *Scand J Clin Lab Invest* (2005) 65:671–80. doi: 10.1080/00365510500348153

23. van Wijngaarden JP, Swart KM, Enneman AW, Dhonukshe-Rutten RA, van Dijk SC, Ham AC, et al. Effect of daily vitamin b-12 and folic acid supplementation on fracture incidence in elderly individuals with an elevated plasma homocysteine concentration: B-PROOF, a randomized controlled trial. *Am J Clin Nutr* (2014) 100:1578–86. doi: 10.3945/ajcn.114.090043

24. Enneman AW, Swart KM, van Wijngaarden JP, van Dijk SC, Ham AC, Brouwer-Brolsma EM, et al. Effect of vitamin B12 and folic acid supplementation on bone mineral density and quantitative ultrasound parameters in older people with an elevated plasma homocysteine level: B-PROOF, a randomized controlled trial. *Calcif Tissue Int* (2015) 96:401–9. doi: 10.1007/s00223-015-9968-6

25. Raisz LG. Homocysteine and osteoporotic fractures–culprit or bystander? *N Engl J Med* (2004) 350:2089–90. doi: 10.1056/NEJMe048030

26. Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent developments in mendelian randomization studies. *Curr Epidemiol Rep* (2017) 4:330–45. doi: 10.1007/s40471-017-0128-6

27. Paternoster L, Tilling K, Davey Smith G. Genetic epidemiology and mendelian randomization for informing disease therapeutics: Conceptual and methodological challenges. *PloS Genet* (2017) 13:e1006944. doi: 10.1371/journal.pgen.1006944

28. Fu L, Li YN, Luo D, Deng S, Hu YQ. Plausible relationship between homocysteine and obesity risk *via* MTHFR gene: A meta-analysis of 38,317 individuals implementing mendelian randomization. *Diabetes Metab Syndr Obes* (2019) 12:1201–12. doi: 10.2147/dmso.s205379

29. Saito M, Marumo K. The effects of homocysteine on the skeleton. *Curr Osteop Rep* (2018) 16:554–60. doi: 10.1007/s11914-018-0469-1

30. Kim JI, Moon JH, Chung HW, Kong MH, Kim HJ. Association between homocysteine and bone mineral density according to age and sex in healthy adults. *J Bone Metab* (2016) 23:129–34. doi: 10.11005/jbm.2016.23.3.129

31. Saoji R, Das RS, Desai M, Pasi A, Sachdeva G, Das TK, et al. Association of high-density lipoprotein, triglycerides, and homocysteine with bone mineral density in young Indian tribal women. *Arch Osteop* (2018) 13:108. doi: 10.1007/s11657-018-0525-6

32. Liu C-T, Karasik D, Xu H, Zhou Y, Broe K, Cupples LA, et al. Genetic variants modify the associations of concentrations of methylmalonic acid, vitamin b-12, vitamin b-6, and folate with bone mineral density. *Am J Clin Nutr* (2021) 114:578–87. doi: 10.1093/ajcn/nqab093

33. Lawlor DA. Commentary: Two-sample mendelian randomization: opportunities and challenges. *Int J Epidemiol* (2016) 45:908–15. doi: 10.1093/ije/dyw127

34. Davies NM, Holmes MV, Davey Smith G. Reading mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ* (2018) 362:k601. doi: 10.1136/bmj.k601

35. Yang Q, Sanderson E, Tilling K, Borges MC, Lawlor DA. Exploring and mitigating potential bias when genetic instrumental variables are associated with multiple non-exposure traits in mendelian randomization. *Eur J Epidemiol* (2022) 37:683–700. doi: 10.1007/s10654-022-00874-5

36. Grarup N, Sulem P, Sandholt CH, Thorleifsson G, Ahluwalia TS, Steinthorsdottir V, et al. Genetic architecture of vitamin B12 and folate levels uncovered applying deeply sequenced large datasets. *PloS Genet* (2013) 9:e1003530. doi: 10.1371/journal.pgen.1003530

37. van Meurs JB, Pare G, Schwartz SM, Hazra A, Tanaka T, Vermeulen SH, et al. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. *Am J Clin Nutr* (2013) 98:668–76. doi: 10.3945/ajcn.112.044545

38. Tanaka T, Scheet P, Giusti B, Bandinelli S, Piras MG, Usala G, et al. Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. *Am J Hum Genet* (2009) 84:477–82. doi: 10.1016/j.ajhg.2009.02.011

39. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, et al. PhenoScanner V2: An expanded tool for searching human genotype-phenotype associations. *Bioinformatics* (2019) 35:4851–53. doi: 10.1093/bioinformatics/btz469

40. Seeley DG, Browner WS, Nevitt MC, Genant HK, Scott JC, Cummings SR. Which fractures are associated with low appendicular bone mass in elderly women? the study of osteoporotic fractures research group. *Ann Intern Med* (1991) 115:837–42. doi: 10.7326/0003-4819-115-11-837

41. Stone KL, Seeley DG, Lui LY, Cauley JA, Ensrud K, Browner WS, et al. BMD at multiple sites and risk of fracture of multiple types: long-term results from the study of osteoporotic fractures. *J Bone Miner Res* (2003) 18:1947–54. doi: 10.1359/jbmr.2003.18.11.1947

42. Zheng HF, Forgetta V, Hsu YH, Estrada K, Rosello-Diez A, Leo PJ, et al. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature* (2015) 526:112–7. doi: 10.1038/nature14878

43. Kemp JP, Morris JA, Medina-Gomez C, Forgetta V, Warrington NM, Youlten SE, et al. Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis. *Nat Genet* (2017) 49:1468–75. doi: 10.1038/ng.3949

44. Ensrud KE, Crandall CJ. Osteoporosis. *Ann Intern Med* (2017) 167:ITC17–32. doi: 10.7326/aitc201708010

45. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet* (2012) 44:491–501. doi: 10.1038/ng.2249

46. Lee JJ, Wedow R, Okbay A, Kong E, Maghzian O, Zacher M, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nat Genet* (2018) 50:1112–21. doi: 10.1038/s41588-018-0147-3

47. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet* (2019) 51:237–44. doi: 10.1038/s41588-018-0307-5

48. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data mendelian randomization. *Stat Med* (2017) 36:1783–802. doi: 10.1002/sim.7221

49. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* (2016) 40:304–14. doi: 10.1002/gepi.21965

50. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int J Epidemiol* (2015) 44:512–25. doi: 10.1093/ije/dyv080

51. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat Genet* (2018) 50:693–98. doi: 10.1038/s41588-018-0099-7

52. Burgess S, Thompson SG. Multivariable mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol* (2015) 181:251–60. doi: 10.1093/aje/kwu283

53. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* (2002) 21:1539–58. doi: 10.1002/sim.1186

54. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in mendelian randomization studies. *Int J Epidemiol* (2013) 42:1497–501. doi: 10.1093/ije/dyt179

55. Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, et al. LD hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics* (2017) 33:272–79. doi: 10.1093/bioinformatics/btw613

56. Hemani G, Zheng J, Elsworth B, Wade K, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. *Elife* (2018) 7:e34408. doi: 10.7554/elife.34408

57. Yavorska OO, Burgess S. MendelianRandomization: an r package for performing mendelian randomization analyses using summarized data. *Int J Epidemiol* (2017) 46:1734–39. doi: 10.1093/ije/dyx034

58. Bahtiri E, Islami H, Rexhepi S, Qorraj-Bytyqi H, Thaçi K, Thaçi S, et al. Relationship of homocysteine levels with lumbar spine and femur neck BMD in postmenopausal women. *Acta Reum Port* (2015) 40:355–62.

59. Ross RD, Shah RC, Leurgans SE, Buchman AS, Bennett DA. Association of heel bone mineral density with incident disability and mortality in community-dwelling older adults. *JBMR Plus* (2020) 4:e10390. doi: 10.1002/jbm4.10390

60. De Martinis M, Sirufo MM, Nocelli C, Fontanella L, Ginaldi L. Hyperhomocysteinemia is associated with inflammation, bone resorption, vitamin B12 and folate deficiency and MTHFR C677T polymorphism in postmenopausal women with decreased bone mineral density. *Int J Environ Res Public Health* (2020) 17:4260. doi: 10.3390/ijerph17124260

61. Morris MS, Jacques PF, Selhub J. Relation between homocysteine and b-vitamin status indicators and bone mineral density in older americans. *Bone* (2005) 37:234–42. doi: 10.1016/j.bone.2005.04.017

62. Tucker KL, Hannan MT, Qiao N, Jacques PF, Selhub J, Cupples LA, et al. Low plasma vitamin B12 is associated with lower BMD: the framingham osteoporosis study. *J Bone Miner Res* (2005) 20:152–8. doi: 10.1359/jbmr.041018

63. Baines M, Kredan MB, Usher J, Davison A, Higgins G, Taylor W, et al. The association of homocysteine and its determinants MTHFR genotype, folate, vitamin B12 and vitamin B6 with bone mineral density in postmenopausal British women. *Bone* (2007) 40:730–6. doi: 10.1016/j.bone.2006.10.008

64. Wang P, Liu L, Lei SF. Causal effects of homocysteine levels on the changes of bone mineral density and risk for bone fracture: A two-sample mendelian randomization study. *Clin Nutr* (2021) 40:1588–95. doi: 10.1016/j.clnu.2021.02.045

65. Fu L, Zhang M, Hu YQ, Zhao X, Cheng H, Hou D, et al. Gene-gene interactions and associations of six hypertension related single nucleotide polymorphisms with obesity risk in a Chinese children population. *Gene* (2018) 679:320–27. doi: 10.1016/j.gene.2018.09.019

66. Fu L, Li YN, Luo D, Deng S, Wu B, Hu YQ. Evidence on the causal link between homocysteine and hypertension from a meta-analysis of 40 173 individuals implementing mendelian randomization. *J Clin Hypertens* (2019) 21:1879–94. doi: 10.1111/jch.13737

67. Fu L, Wang Y, Li T, Hu YQ. A novel approach integrating hierarchical clustering and weighted combination for association study of multiple phenotypes and a genetic variant. *Front Genet* (2021) 12:654804. doi: 10.3389/fgene.2021.654804

68. Fu L, Cheng H, Zhao X, Hou D, Xie X, Mi J. Distinct causal effects of body fat distribution on cardiometabolic traits among children: Findings from the BCAMS study. *Nutr Metab Cardiovasc Dis* (2022) 32:1753–65. doi: 10.1016/j.numecd.2022.03.030

69. Fu L, Wang Y, Hu YQ. Causal effects of B vitamins, homocysteine on fat and musculoskeletal diseases: A Mendelian randomization study. *Front Nutr* (2022) 9:1048122. doi: 10.3389/fnut.2022.1048122

70. Fu L, Wang Y, Hu YQ. Association between homocysteine and nonalcoholic fatty liver disease: Mendelian randomisation study. *Eur J Clin Invest* (2022) 00:e13895. doi: 10.1111/eci.13895

71. Fu L, Wang Y, Li T, Yang S, Hu YQ. A novel hierarchical clustering approach for joint analysis of multiple phenotypes uncovers obesity variants based on ARIC. *Front Genet* (2022) 13:791920. doi: 10.3389/fgene.2022.791920

72. Lubec B, Fang-Kircher S, Lubec T, Blom HJ, Boers GH. Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. *Biochim Biophys Acta* (1996) 1315:159–62. doi: 10.1016/0925-4439(95)00119-0

73. Tyagi N, Kandel M, Munjal C, Qipshidze N, Vacek JC, Pushpakumar SB, et al. Homocysteine mediated decrease in bone blood flow and remodeling: role of folic acid. *J Orthop Res* (2011) 29:1511–6. doi: 10.1002/jor.21415

74. Kim DJ, Koh JM, Lee O, Kim NJ, Lee YS, Kim YS, et al. Homocysteine enhances apoptosis in human bone marrow stromal cells. *Bone* (2006) 39:582–90. doi: 10.1016/j.bone.2006.03.004

75. Koh JM, Lee YS, Kim YS, Kim DJ, Kim HH, Park JY, et al. Homocysteine enhances bone resorption by stimulation of osteoclast formation and activity through increased intracellular ROS generation. *J Bone Miner Res* (2006) 21:1003–11. doi: 10.1359/jbmr.060406

76. Kanazawa I, Tomita T, Miyazaki S, Ozawa E, Yamamoto LA, Sugimoto T. Bazedoxifene ameliorates homocysteine-induced apoptosis and accumulation of advanced glycation end products by reducing oxidative stress in MC3T3-E1 cells. *Calcif Tissue Int* (2017) 100:286–97. doi: 10.1007/s00223-016-0211-x

77. Luo Y. Bone mineral density averaged over a region of interest on femur is affected by age-related change of bone geometry. *Osteop Int* (2018) 29:1419–25. doi: 10.1007/s00198-018-4461-5

78. Mao SS, Li D, Syed YS, Gao Y, Luo Y, Flores F, et al. Thoracic quantitative computed tomography (QCT) can sensitively monitor bone mineral metabolism: Comparison of thoracic QCT vs lumbar QCT and dual-energy X-ray absorptiometry in detection of age-relative change in bone mineral density. *Acad Radiol* (2017) 24:1582–87. doi: 10.1016/j.acra.2017.06.013

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 01 December 2022
DOI 10.3389/fmed.2022.1017411

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Intensive Care Medicine and Anesthesiology, a section of the journal Frontiers in Medicine

RECEIVED 12 August 2022
ACCEPTED 16 November 2022
PUBLISHED 01 December 2022

CITATION
Wang D, Wang J, Zheng X, Diao S, Li W and Ma W (2022) Bone homeostasis disorders increased the mortality of sepsis patients: A preliminary retrospective cohort study. *Front. Med.* 9:1017411. doi: 10.3389/fmed.2022.1017411

Bone homeostasis disorders increased the mortality of sepsis patients: A preliminary retrospective cohort study

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Introduction: Sepsis is a common clinical syndrome and nearly 20% of all deaths are related to sepsis. As an important part of the body, bone homeostasis disorders are closely related to inflammatory response, but the correlation between bone homeostasis and sepsis, sepsis shock was unknown. The objective of this study was to explore the relation of bone homeostasis on sepsis and sepsis shock.

Methods: In this retrospective cohort study, patients were enrolled between April 2018 and May 2022 from Beijing Chaoyang hospital. Primary outcomes were serum indicators reflected bone homeostasis, such as cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I), tartrate-resistant acid phosphatase 5b (TRACP-5b) and piezo-type mechanosensitive ion channel component 1 (PIEZO1).

Results: The data were analyzed retrospectively. among 88 evaluable patients, 45 were sepsis (19 were sepsis shock) and 43 were non-sepsis. There was no significant difference in age, gender, BMI, combination diseases, operation time, intraoperative blood loss, and hospital stay. Patients with sepsis or sepsis shock had higher serum CTX-I, TRACP-5b, PIEZO1 ($p < 0.05$). Spearman's rank correlation test showed that CTX-I, TRACP-5b, PIEZO1 and the three together (CTX-I + TRACP-5b + PIEZO1) had strong correlation with sepsis or sepsis shock ($p < 0.05$). The receiver operating characteristic curve (ROC) and precision-recall curve (PRC) showed that these indicators could predict the occurrence of sepsis or sepsis shock ($p < 0.05$). Besides, decision curve analysis (DCA) and interventions avoided curve (IAC) displayed a high net benefit of bone homeostasis disorders indicators on sepsis or sepsis shock.

Kaplan–Meier survival curves revealed that sepsis or shock patients with high value indicators (>0.47227) had a higher mortality ($p < 0.05$).

Conclusion: Bone homeostasis disorders could increase the mortality of sepsis and sepsis shock patients.

KEYWORDS

bone homeostasis disorders, CTX-I, TRACP-5b, PIEZO1, sepsis, sepsis shock, mortality, retrospective cohort study

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and nearly 20% of all deaths are related to sepsis (1–3). According to sepsis 3.0 diagnostic criteria, septic shock is a subtype of sepsis with more unstable circulation and higher mortality (4–8). The severity of sepsis is closely related to the body's response to inflammation. Sepsis patients have multiple system organs regulation disorders and even function suppression (9, 10).

Bone is a tissue rich in nerve and blood vessels (11–13). Current studies have found that bone tissue is closely related to the inflammation regulation of patients (14–16). The concept of immune-skeletal interface has been proposed that immune cells directly regulate the bone microenvironment homeostasis (14, 17). Besides, osteoblast and osteoclast also play a very important role in immune cells functions and differentiation (16, 17). Although there are many studies on bone homeostasis and inflammation reaction, there is no report on the relationship between bone homeostasis and sepsis, especially sepsis shock.

We hypothesized that bone homeostasis disorders were closely related to sepsis and sepsis shock. Besides, bone homeostasis disorders would aggravate sepsis and even increase the mortality of patients. Therefore, a preliminary retrospective cohort study was conducted to investigate the relationship between bone homeostasis disorders and sepsis.

Materials and methods

Study design

This study was a retrospective cohort study observed the relation of bone homeostasis, sepsis and patients' mortality. Primary outcome was serum indicators reflected bone homeostasis, such as cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I), tartrate-resistant acid phosphatase 5b (TRACP-5b) and piezo-type mechanosensitive ion channel component 1 (PIEZO1). In additions, the secondary outcomes were acute physiology and chronic health evaluation-II (APACHE II) scores, sequential organ failure assessment (SOFA) scores, Combination diseases, such as hypertension, diabetes mellitus, coronary heart disease, chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), cerebrovascular disease (CVD), acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), hepatic failure, disseminated intravascular coagulation (DIC), operation received, operation time, intraoperative blood loss (IBL), intraoperative fluid balance, mechanical ventilation time, ICU stay time, hospital stay time and 30-day all caused mortality.

Besides, some blood indicators were also measured, such as blood PH value, PaCO2, HCO3- concentration, blood lactic acid concentration, white blood cell number, hemoglobin, serum creatinine, serum alanine aminotransferase (ALT), aspartate transaminase (AST), B-type natriuretic peptide (BNP), albumin, blood calcium, and blood phosphorus.

The study was performed at Beijing Chao-Yang hospital affiliated to Capital Medical University between April 2018 and May 2022. It was approved by the ethics committee of Beijing Chao-Yang Hospital Affiliated to Capital Medical University (2020-ke-236) and the data were analyzed retrospectively.

Patients

The inclusion criteria for patients was as follows: (1) age $\geq$ 18 years; and (2) admitted to ICU. The exclusion criteria were: (1) pregnant and lying-in woman; (2) patients with nerve, brain or bone injury; (3) patients with metabolic and immune diseases, such as systemic lupus erythematosus, polymyositis and Sjogren's syndrome; (4) patients treated with long-term glucocorticoid therapy; (5) patients receiving bone injury surgery, such as craniotomy, sternotomy, thoracoplasty; (6) patients with bone tumors, such as osteosarcoma, osteochondroma, bone metastasis; (7) patients with osteoarthritis, rheumatoid arthritis, bone non-union or fracture healing stage; (8) patients with insufficient serum samples and unable to complete the detection of serum bone

homeostasis indicators; (9) patients loss of visit; (10) patients who did not meet the inclusion criteria.

Two clinical observers (XZ and JW) with clinical research experience enrolled the patients strictly according to the inclusion and exclusion criteria. The baseline information of patients was recorded, such as patient's name, gender, age, and BMI.

Sample size

Stata/MP 16.0 software was used to calculate the sample size. There is no relevant clinical study on the relationship of bone homeostasis and sepsis. Therefore, the relevant pre-experiment was carried out and two patients were enrolled. The serum PIEZO1 concentration of sepsis patients were 15.43, 18.17 ng/ml and non-sepsis patients were 13.46, 10.41 ng/ml. CTX-I concentration of sepsis patients were 5.67, 4.71 ng/ml and non-sepsis patients were 3.47, 2.51 ng/ml. TRACP-5b of sepsis patients were 4.24, 5.64 mIU/ml and non-sepsis patients were 2.58, 2.25 mIU/ml. Alpha was set as 0.01 (two-sided) and power was set as 0.95. The ratio of sepsis group and non-sepsis group was 1. The calculated minimum sample size of each group was 9.

Definition and outcomes

Sepsis is defined by sepsis 3.0 as life-threatening organ dysfunction caused by a dysregulated host response to infection and sepsis shock defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (4). Clinical parameters to identify patients with sepsis are increasing in SOFA score $\geq$ 2 from baseline or qSOFA $\geq$ 2 and suspected infection (4, 5). The septic shock clinical parameters are: vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dl) in the absence of hypovolemia (4, 5).

The main collagen component in bone tissue is type I collagen, accounting for more than 90% of the bone matrix. CTX-I is a degradation product of type I collagen, which can sensitively and specifically reflect human bone destruction (18). Besides, TRACP-5b in human serum is mainly secreted by osteoclasts, and is also a sensitive and specific indicator of bone destruction (19). PIEZO1 is a mechanically sensitive ion channel protein and a key force sensor for osteoblast differentiation (20). The serum level of PIEZO1 indicates bone formation (20). In this study, bone homeostasis disorders were assessed using serum CTX-I, TRACP-5b and PIEZO1 concentration. The serum CTX-I, TRACP-5b and PIEZO1 were detected by ELISA.

APACHE II scores, SOFA scores, and the combination diseases were recorded at the time of patients' serum collection (serum was to detect bone homeostasis indicators). The operation time, IBL, intraoperative fluid balance, mechanical ventilation time, ICU stay time, hospital stay time and 30-day all caused mortality were acquired by patient records checked and discharge 30-days follow up.

Besides, blood PH value, PaCO2, HCO3- concentration, blood lactic acid concentration, white blood cell number, hemoglobin, serum creatinine, serum ALT, AST, BNP, calcium, phosphorus and albumin were all measured at the same time of patients' serum collection (serum was to detect bone homeostasis indicators).

Laboratory methods

Human CTX-I ELISA kit (CSB-E11224h, Cusabio, China), human TRACP-5b ELISA kit (CSB-E08490h, Cusabio, China) and human PIEZO1 ELISA kit (EH15116, FineTest, China) were used. 2 ml blood collected from patients was centrifuged at 3500 rm/r, 15 min. Then, CTX-I, TRACP-5b, and PIEZO1 of supernatant were detected according to the instructions of ELISA kit.

Statistical analysis

SPSS statistics 25.0 (IBM, Chicago, IL, USA), MedCalc v.20.014 (MedCalc Software Ltd., Ostend, Belgium[1]; 2021) and Stata/MP 16.0 (College Station, TX77845, USA) were used to analyze the data. Shapiro-Wilk test was used to evaluate the normality of the measurement data. If the measurement data followed normal distribution, it was described as means $\pm$ standard and compared by independent-sample *t*-test between two groups or one-way ANOVA for more than two groups. If the measurement data obeyed the skewed distribution, it was represented by median (quartile range) and compared by Mann–Whitney *U* test between two group and Jonckheere-Terpstra test for more than two groups. The enumeration data was represented by occurrence rate and compared by Chi-square test or Fischer's exact test.

Correlation coefficients were obtained by applying Spearman's rank correlation or Pearson correlation analysis. A receiver operating characteristic (ROC) curve and precision-recall curve (PRC) were used to evaluate the correlation intensity. The optimal cutoff value was determined by the Youden index.

Logistic regression analysis was performed to find out the relationship of CTX-I, TRACP-5b, and PIEZO1 with sepsis. Clinical parameters (not included CTX-I, TRACP-5b,

1 https://www.medcalc.org

and PIEZO1) with $p < 0.10$ in univariate analyses were included in the multivariate logistic regression model. The multivariate logistic regression model was used to constructed the clinic prediction model. Decision curve analysis (DCA) and interventions avoided curve (IAC) were used for exploring the value of bone homeostasis disorders in the treatment of sepsis and sepsis shock. Furthermore, Kaplan–Meier curves are provided for exploring the role of bone homeostasis disorders on the mortality of sepsis and sepsis shock patients. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics of the patients

During the study period, 88 patients were screened (Figure 1). Sepsis group were 45 patients, in additions, 29 were male and 16 were female. The age was 68 (17.5) years. In these patients, 19 were diagnosed sepsis shock. Sepsis shock patients had higher APACHE II scores, SOFA scores and longer mechanical ventilation treatment time, and higher 30-day all caused mortality. There was no significant difference in age and gender proportion among the groups, and the groups were comparable (Table 1).

Outcomes

The results of peripheral blood indicators in three groups were showed in Table 2. Sepsis patients had lower blood PH value, higher Lactic acid level, higher ALT, AST and BNP in serum ($p < 0.05$). Besides, sepsis patients had higher serum CTX-I, TRACP-5b, PIEZO1 level ($p < 0.05$), which meant that sepsis patients had bone homeostasis disorders.

The relation of sepsis and bone homeostasis disorders

Correlation test, ROC curve, DCA and IAC curve were used to evaluate the relation of sepsis and bone homeostasis disorders. Correlation test showed that CTX-I, TRACP-5b, PIEZO1 and the three together (CTX-I + TRACP-5b + PIEZO1)

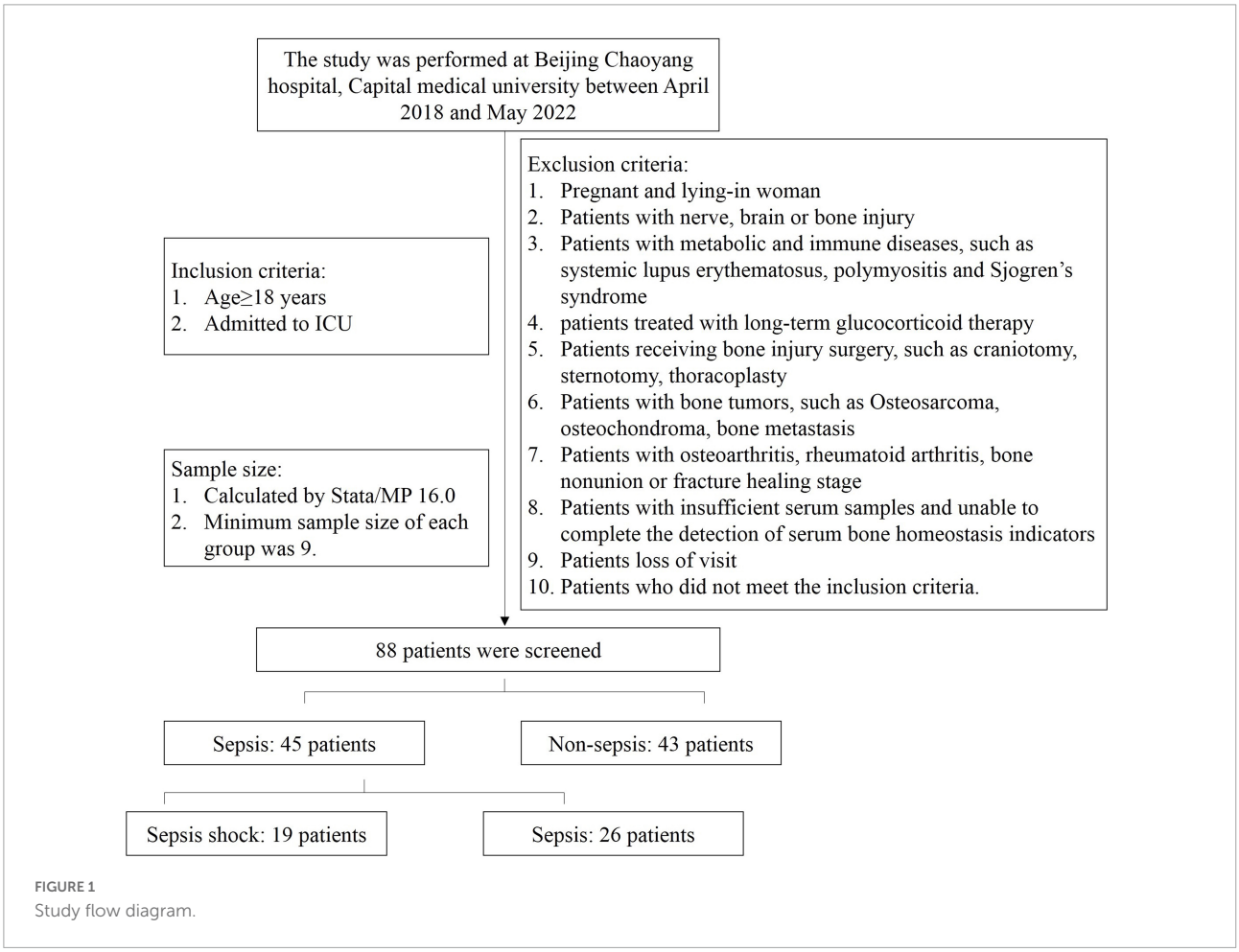

FIGURE 1
Study flow diagram.

TABLE 1 Demographics and baseline characteristics.

	Sepsis shock (n = 19)	Sepsis (n = 26)	Non-sepsis (n = 43)	P-value
Age (years)	67 (20)	68 (16)	70 (14)	0.43[a]
Sex				
Female	7	9	22	0.33[b]
Male	12	17	21	0.33[b]
BMI (kg/m^2)	23.72 ± 5.11	22.82 ± 3.72	24.49 ± 3.58	0.25[c]
APACHE II score	16 (10)	11.5 (5.25)	10 (4)	<0.01[a]
SOFA score	7 (3)	3 (3)	1 (2)	<0.01[a]
Combination diseases				
Hypertension	11	11	22	0.57[b]
DM	3	6	11	0.70[b]
CHD	3	5	8	0.95[b]
COPD	0	3	5	0.30[b]
CLD	0	0	1	0.59[b]
CKD	2	0	1	0.14[b]
CVD	0	6	5	0.07[b]
ARDS	2	2	0	0.12[b]
AKI	3	2	0	0.04[b]
Hepatic failure	3	2	0	0.04[b]
DIC	2	1	0	0.11[b]
Postoperative patient	18	25	43	0.36[b]
Operation time (h)	3.09 (2.19)	2.25 (4.87)	3.67 (1.92)	0.22[a]
IBL	175 (275)	50 (350)	100 (150)	0.32[a]
Intraoperative fluid balance	2,450 (1,005)	1,925 (1,727.5)	1,750 (760)	0.02[a]
Mechanical ventilation (h)	26.5 (24.75)	13 (6.25)	3.33 (12)	<0.01[a]
ICU stay (h)	97 (89.25)	48.5 (59.75)	24 (21)	<0.01[a]
Hospital stay (h)	421 (667.5)	387.5 (588.25)	432 (268)	0.78[a]
30-day mortality (%)	21.05	3.85	0	<0.01[b]

[a]Jonckheere-Terpstra test.
[b]Chi-square test.
[c]One-way ANOVA (unpaired, two-tailed).
BMI, body mass index; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; DM, diabetes mellitus; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; CLD, chronic liver disease; CKD, chronic kidney disease; CVD, cerebrovascular disease; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; DIC, disseminated intravascular coagulation; IBL, intraoperative blood loss.

had strong correlation with sepsis (p = 0.03, p < 0.01, p = 0.03, p < 0.01, respectively). Besides, TRACP-5b, PIEZO1 and CTX-I + TRACP-5b + PIEZO1 had also strong correlation with SOFA scores (p = 0.03, p < 0.01, p < 0.01, respectively; Figure 2).

ROC curve results showed that these indicators could predict the occurrence of sepsis (p = 0.02, p < 0.01, p = 0.02, p < 0.01, respectively; Figure 3). Area under the ROC curve (AUC) were 0.636, 0.665, 0.637, and 0.722, which further confirmed the correlation test results. The PRC results further confirmed the ROC results (details in Supplementary material).

DCA curve showed the value of bone homeostasis disorders in the treatment of sepsis (Figure 4). The X-axis indicates the threshold probability for sepsis development and the Y-axis indicates the net benefit. A high net benefit was provided by those indicators models that are far away from the slanted dash blue line and the horizontal red line (Figure 4). IAC results showed the treatment value of bone homeostasis disorders in sepsis. The X-axis also indicates the threshold probability for sepsis development and the Y-axis indicates the net reduction in intervention per 100 patients. The result showed that sepsis at 60% of the probability threshold, bone homeostasis indicators could reduce nearly 25% patients using the existing diagnosis means in clinic, including invasive operation inspection (Figure 5).

The relation of sepsis shock and bone homeostasis disorders

The correlation between sepsis shock and bone homeostasis disorders was also assessed. Correlation test showed that CTX-I, TRACP-5b, PIEZO1 and the three together (CTX-I + TRACP-5b + PIEZO1) had strong correlation with sepsis shock (p < 0.01, p < 0.01, p = 0.02, p < 0.01, respectively)

TABLE 2 Study outcomes.

	Sepsis shock (n = 19)	Sepsis (n = 26)	Non-sepsis (n = 43)	P-value
PH	7.42 (0.10)	7.425 (0.10)	7.45 (0.12)	0.03[a]
PaCO2 (mmHg)	37 (9.0)	36.5 (7.5)	36 (9)	0.29[a]
HCO3- (mmol/L)	25.4 (6.3)	23.75 (3.45)	25.20 (2.70)	0.23[a]
Lactic acid (mmol/L)	2.50 (2.60)	1.20 (1.57)	1.00 (0.70)	<0.01[a]
White blood cell ($\times 10^9$/L)	9.80 (10.50)	10.10 (8.40)	8.09 (5.10)	0.04[a]
Hemoglobin (g/L)	99.58 ± 24.11	102.88 ± 15.38	109.21 ± 15.40	0.11[b]
Creatinine (μmol/L)	65.60 (34.40)	66.20 (30.90)	55.80 (23.30)	0.21[a]
ALT (U/L)	30.00 (63.00)	30.50 (42.50)	14.00 (16.00)	<0.01[a]
AST (U/L)	48.00 (105.00)	54.50 (63.25)	21.00 (14.00)	<0.01[a]
BNP (pg/ml)	103.00 (148.00)	75.50 (71.75)	51.00 (85.00)	0.02[a]
Albumin (g/L)	25.30 (9.60)	28.40 (6.30)	31.80 (6.20)	<0.01[a]
CTX-I (ng/ml)	5.85 (7.02)	4.11 (5.29)	3.84 (4.54)	<0.01[a]
TRACP-5b (mIU/ml)	2.56 (3.00)	2.39 (1.88)	1.75 (1.45)	<0.01[a]
PIEZO1 (ng/ml)	9.37 (29.09)	5.60 (14.52)	4.60 (6.12)	0.02[a]

[a]Jonckheere-Terpstra test.
[b]One-way ANOVA (unpaired, two-tailed).
ALT, alanine aminotransferase; AST, aspartate transaminase; BNP, B-type natriuretic peptide; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; TRACP-5b, tartrate-resistant acid phosphatase 5b; PIEZO1, piezo-type mechanosensitive ion channel component 1.

and SOFA ($p = 0.03$, $p < 0.01$, $p < 0.01$, $p < 0.01$, respectively; Figure 2).

ROC curve results further confirmed the correlation test results, which showed that these indicators could predict the sepsis shock occurrence ($p < 0.01$, $p < 0.01$, $p = 0.02$, $p < 0.01$, respectively; Figure 3). The AUC were 0.747, 0.733, 0.680, and 0.775. The PRC results further confirmed the ROC results (details in Supplementary material).

DCA showed that the high net benefit was provided by those indicators models that are far away from the slanted dash blue line and the horizontal red line (Figure 4). IAC results showed that the bone homeostasis indicators could reduce almost 40% patients using the existing diagnosis means in clinic, including invasive operation inspection, when sepsis shock at 40% of the probability threshold (Figure 5).

Bone homeostasis disorders and the mortality of sepsis shock

Kaplan–Meier curves are explored the relationship between bone homeostasis disorders and the mortality of sepsis, sepsis shock. The Youden index was used to calculate the optimal cutoff value. The cutoff value of the three indicators together (CTX-I + TRACP-5b + PIEZO1) on sepsis prediction was 0.47227 and on sepsis shock prediction was 0.27256. Then, sepsis or sepsis shock patients were divided into two groups based on the value of the three indicators together. Kaplan–Meier curves showed the mortality difference of the two groups. Sepsis patients with high value (>the cutoff value of the three indicators together) had the higher mortality ($p < 0.01$), besides, the curve showed a higher mortality of sepsis shock patients with high value (>the cutoff value of the three indicators together) ($p = 0.02$; Figure 6).

Discussion

Bone tissue is rich in nerve and blood vessels. In recent years, it has been found that bone tissue has a regulatory effect on various organs of the body, such as brain, kidney, and liver (21–23). Besides, bone played a very important role on immune functions (14–16). Bone homeostasis reflects immune system reaction abilities. Sepsis causes organ dysfunction after infection, which is closely related to body immune system. So far, there is no specific effective drugs on sepsis treatment, and nearly 20% of all deaths are sepsis related (3). To find out the influence factors of sepsis occurrence and development, so as to provide a research basis for finding drugs for the treatment of sepsis or early diagnosis. At present, there is no research on the relationship between bone homeostasis and sepsis. This study was used retrospective cohort study method to preliminarily explore the relationship between bone homeostasis and sepsis.

CTX-I, TRACP-5b, and PIEZO1 were used as indicators to evaluate bone homeostasis disorders. First, comparing sepsis group with non-sepsis group, it was found that CTX-I, TRACP-5b, and PIEZO1 in sepsis group all were significantly higher than that in non-sepsis group, which indicated that sepsis could lead to bone homeostasis disorders. At the same time, the CTX-I, TRACP-5b, and PIEZO1 of sepsis shock group were also higher

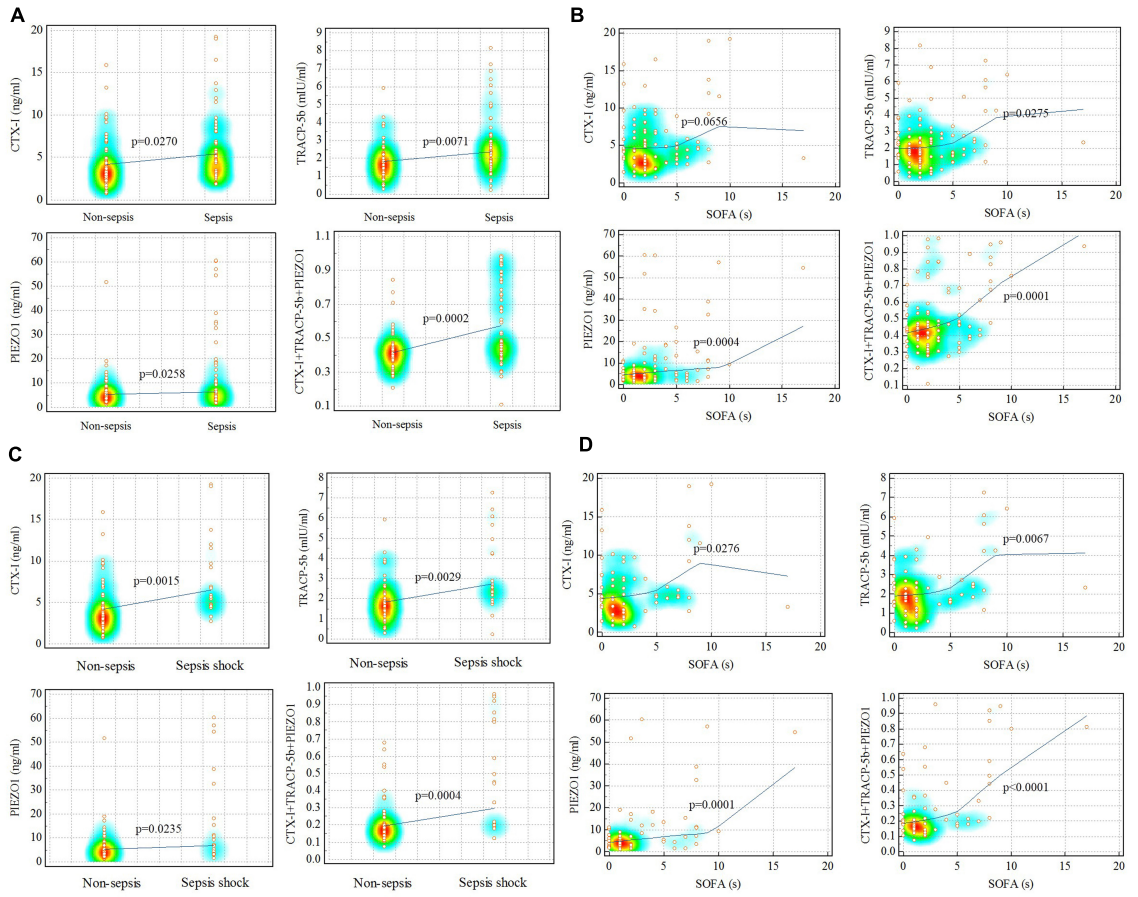

FIGURE 2
Correlation analysis for sepsis and non-sepsis. **(A,B)** Sepsis vs. non-sepsis; **(C,D)** sepsis shock vs. non-sepsis. Red represented the data concentration area.

than that of sepsis group, indicating that the disorders of bone homeostasis increased as the aggravation of sepsis.

CTX-I is the degradation product of type I collagen. Collagen in bone tissue is mainly type I collagen. This study found that sepsis is associated with CTX-I, indicating that sepsis may be closely related to type I collagen metabolism. At present, there are few literatures on the correlation between collagen metabolism and sepsis. Gäddnäs et al. (24) found that collagen degradation was associated with sepsis in 2009. However, type I and type II were not clear (25). TRACP-5b is a cytokine mainly secreted by osteoclasts, which can sensitively and specifically reflect the activity of osteoclasts in the body. Our study found that elevated TRACP-5b could predict sepsis occurrence and was closely related to sepsis mortality. At present, only a few studies have found a possible relationship between osteoclast function and sepsis through transcriptomics (26, 27). This study provided a basis for exploring the relationship between osteoclasts and sepsis. PIEZO1 is a mechanically sensitive protein and is closely related to osteoblast differentiation. At present, there are few studies on PIEZO1 and sepsis. Aykut et al. (28) proposed that PIEZO1 might be related to sepsis. Inhibition of PIEZO1 had a positive effect on the treatment of sepsis. However, the specific mechanism was needed the further study.

Since there were differences in bone homeostasis indicators among sepsis, sepsis shock and non-sepsis groups, correlation was used to evaluate whether there was a relationship between bone homeostasis and sepsis, sepsis shock. The results showed that there was a strong correlation between bone homeostasis disorders and sepsis, sepsis shock. Z.A. Puthucheary et al. (29) investigated the relationship between sepsis and bone. They used rat sepsis model induced by cecal ligation and puncture (CLP) and found that rat femoral trabecular bone strength was reduced within 24 h and was associated with collagen reduction. J. Bayer et al. (30) also used animal CLP induced sepsis model and proposed that the bone was the major source of high circulating intact fibroblast growth factor-23 in asepsis animal. A. Terashima (31) reviewed the role of bone cells in immune regulation during the course of infection and summarized that bone homeostasis played a crucial role in infection. However, few clinical studies explored the relationship

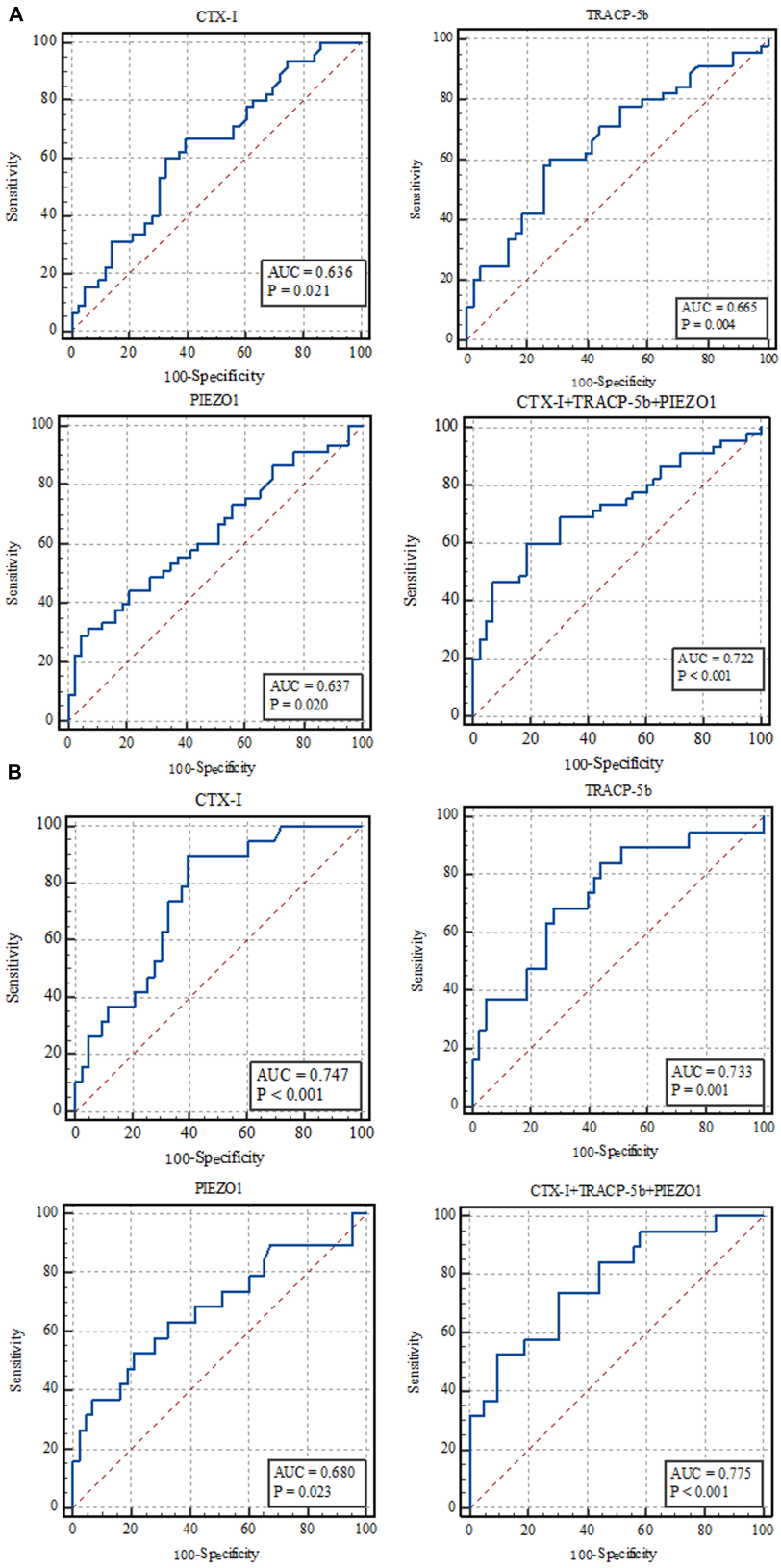

FIGURE 3
ROC analysis results. **(A)** Sepsis vs. non-sepsis; **(B)** sepsis shock vs. non-sepsis.

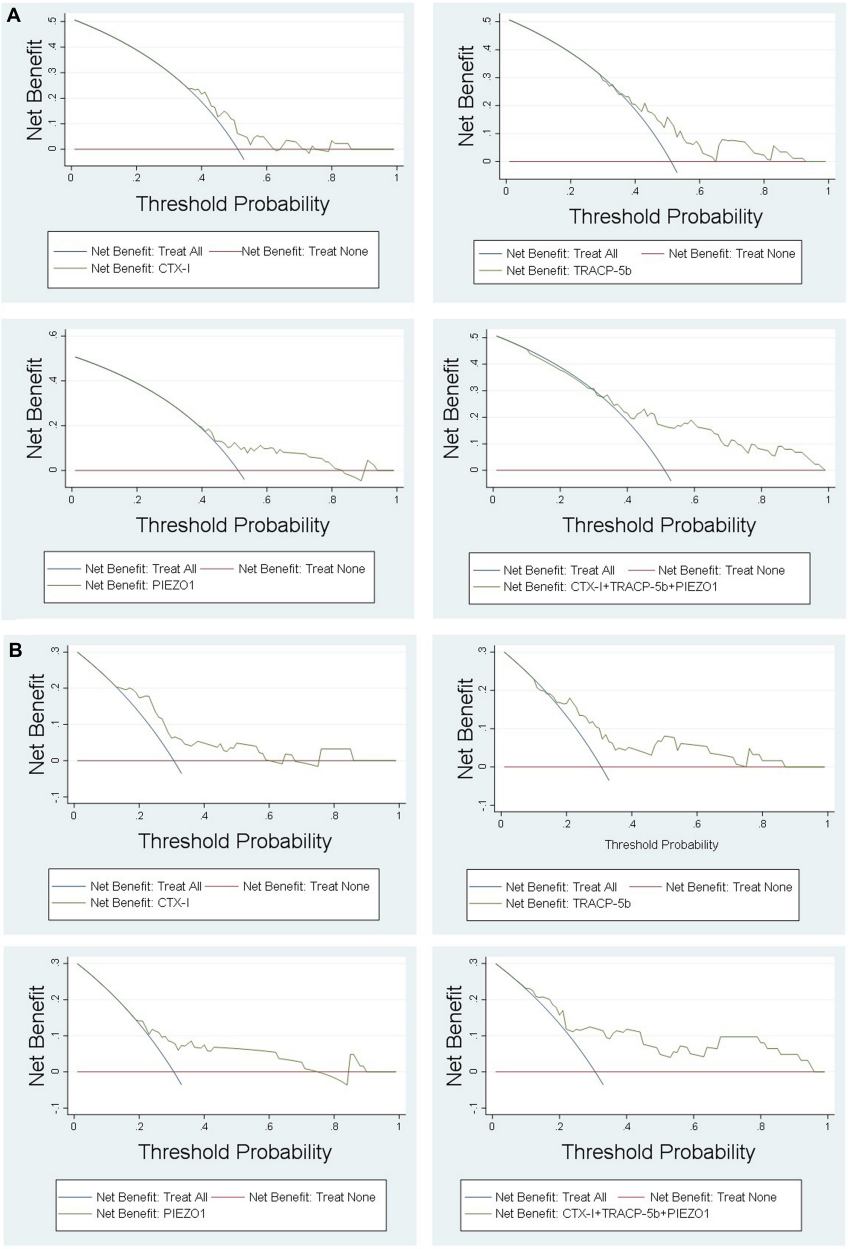

FIGURE 4
DCA results. **(A)** Sepsis vs. non-sepsis; **(B)** sepsis shock vs. non-sepsis.

between bone homeostasis and sepsis, sepsis shock. Moreover, whether bone homeostasis disorders aggravated sepsis severity had not been discussed.

In addition to the strong correlation between bone homeostasis and sepsis, we also used bone homeostasis indicators (CTX-I, TRACP-5b, and PIEZO1) to predict sepsis and sepsis shock. The ROC and PRC results showed that bone homeostasis indicators could predict the occurrence of sepsis and sepsis shock, which is a surprising result. Besides, the prediction efficiency was above 0.5. At present, there was no reports about the prediction of bone homeostasis on sepsis. DCA and IAC results confirmed the ROC results, which showed a high net benefit provided by bone homeostasis indicators on sepsis or sepsis shock.

Since bone homeostasis could predict sepsis and sepsis shock, we further evaluated the relationship between bone homeostasis and mortality of sepsis or sepsis shock. The Youden index was used to calculate the optimal cutoff value and the cutoff value was used to divided group. We found that patients with bone homeostasis indicators exceeded the cutoff value had

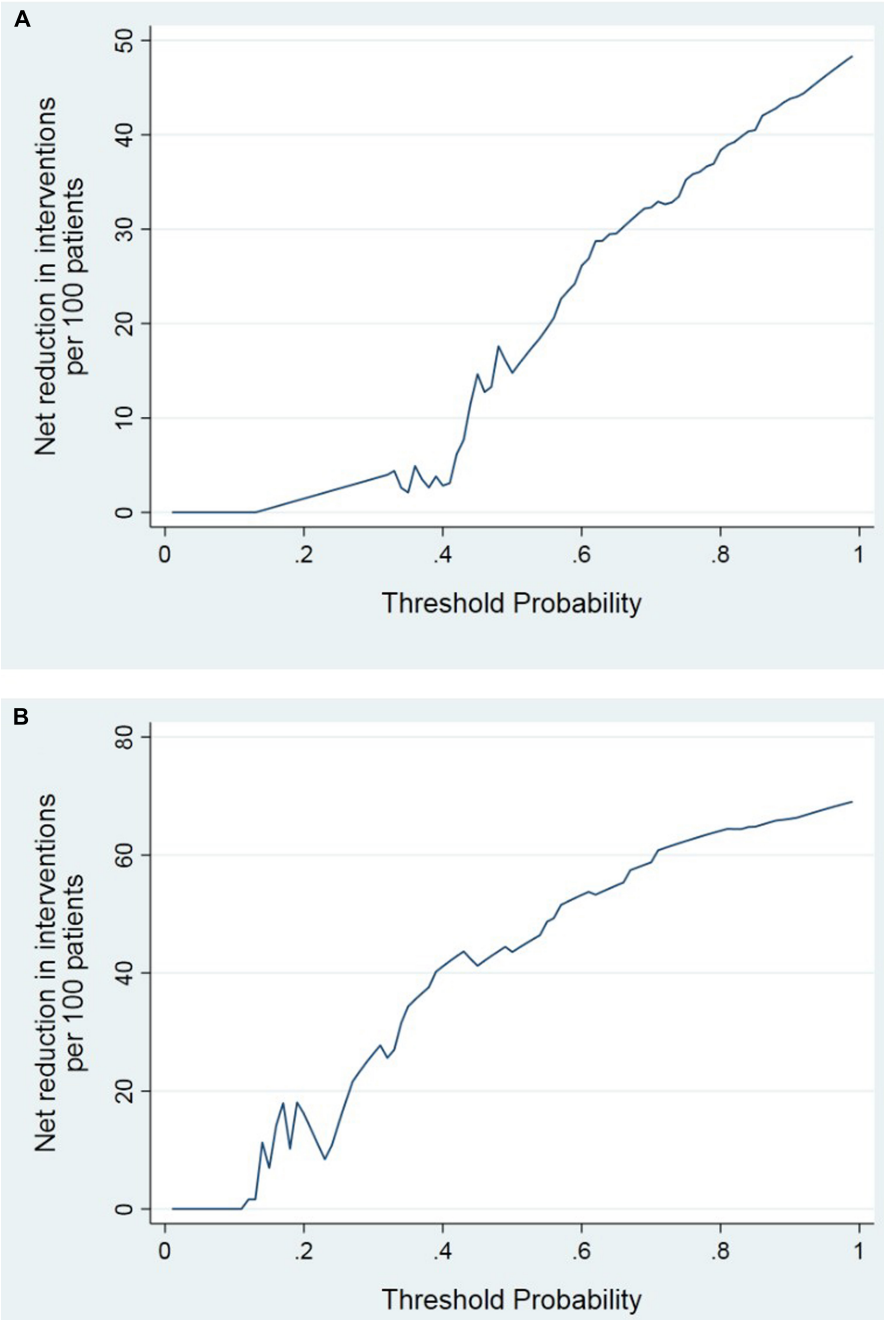

FIGURE 5
IAC analysis results. **(A)** Sepsis vs. non-sepsis; **(B)** sepsis shock vs. non-sepsis.

higher mortality, both in sepsis and sepsis shock patients. This indicated that bone homeostasis disorders could aggravate the illness degree of sepsis patients.

The innovation of this study was obvious. First of all, we found that sepsis and sepsis shock were related to bone homeostasis disorders through clinical observation. Secondly, we used a variety of statistical methods, including DCA, IAC, PRC, and found that bone homeostasis indicators could predict the occurrence of sepsis and sepsis shock. Finally, we found that bone homeostasis disorders could increase the mortality of sepsis and sepsis shock patients through Kaplan–Meier curve.

The limitations of this study were also obvious. Firstly, although we calculated the sample size, the sample size of this study was still small. Secondly, this study was a retrospective study, not a prospective cohort study. Besides, this study included a single center and we planned to

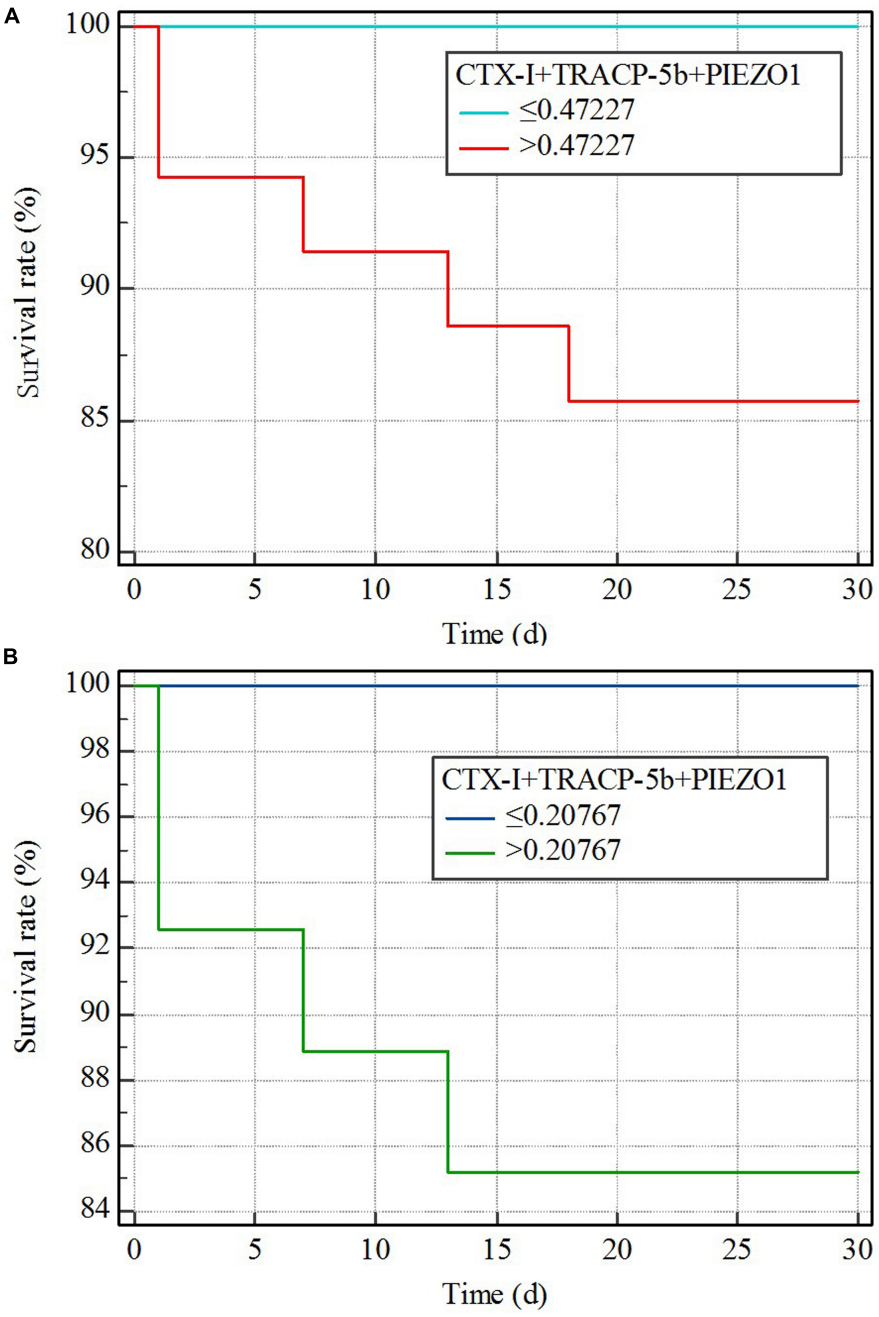

FIGURE 6
Kaplan–Meier curves. **(A)** Sepsis vs. non-sepsis; **(B)** sepsis shock vs. non-sepsis.

carry out a multicenter prospective cohort study in future. Thirdly, because this study did not use external data including public databases to validate, the results have limitations. Besides, there was no significant difference in age, gender, BMI, combination diseases between sepsis and non-sepsis group, which might be the result of limited sample size. Fourthly, the bone homeostasis biomarkers could be time-varying in a longitudinal dataset, which was not currently captured (32). In addition, the causal relationship between these bone homeostasis biomarkers and sepsis outcomes was not clear. This was required animal experiments or cell experiments to explore the relationship. Finally, we intended to explore the regulatory mechanism between bone homeostasis and sepsis by animal or cell experiments, so as to provide a research basis for the treatment of sepsis.

Conclusion

Bone homeostasis was closely related to the occurrence of sepsis and sepsis shock. The indicators, such as CTX-I, TRACP-5b, and PIEZO1, could predict the occurrence of sepsis and sepsis shock. Using these indicators to predict sepsis could get good net benefit. In additions, bone homeostasis disorders could increase the mortality of sepsis and sepsis shock patients, which were a notable problem.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Beijing Chaoyang Hospital of Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Acknowledgments

We thank Beijing Chaoyang Hospital and Capital Medical University for providing the related software used in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1017411/full#supplementary-material

References

1. Kjørholt KE, Kristensen NR, Prieto-Alhambra D, Johnsen SP, Pedersen AB. Increased risk of mortality after postoperative infection in hip fracture patients. *Bone.* (2019) 127:563–70. doi: 10.1016/j.bone.2019.07.023

2. Hagel S, Bach F, Brenner T, Bracht H, Brinkmann A, Annecke T, et al. Effect of therapeutic drug monitoring-based dose optimization of piperacillin/tazobactam on sepsis-related organ dysfunction in patients with sepsis: a randomized controlled trial. *Intensive Care Med.* (2022) 48:311–21. doi: 10.1007/s00134-021-06609-6

3. Moskowitz A, Huang DT, Hou PC, Gong J, Doshi PB, Grossestreuer AV, et al. Effect of ascorbic acid, corticosteroids, and thiamine on organ injury in septic shock: the acts randomized clinical trial. *JAMA.* (2020) 324:642–50. doi: 10.1001/jama.2020.11946

4. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA.* (2016) 315:801–10. doi: 10.1001/jama.2016.0287

5. Scheer CS, Kuhn SO, Fuchs C, Vollmer M, Modler A, Brunkhorst F, et al. Do sepsis-3 criteria facilitate earlier recognition of sepsis and septic shock? a retrospective cohort study. *Shock.* (2019) 51:306–11. doi: 10.1097/SHK.0000000000001177

6. Zhu Y, Zhang R, Ye X, Liu H, Wei J. SAPS III is superior to SOFA for predicting 28-day mortality in sepsis patients based on sepsis 3.0 criteria. *Int J Infect Dis.* (2022) 114:135–41. doi: 10.1016/j.ijid.2021.11.015

7. Garbero E, Livigni S, Ferrari F, Finazzi S, Langer M, Malacarne P, et al. High dose coupled plasma filtration and adsorption in septic shock patients. Results of the COMPACT-2: a multicentre, adaptive, randomised clinical trial. *Intensive Care Med.* (2021) 47:1303–11. doi: 10.1007/s00134-021-06501-3

8. Sevransky JE, Rothman RE, Hager DN, Bernard GR, Brown SM, Buchman TG, et al. Effect of vitamin C, thiamine, and hydrocortisone on ventilator- and vasopressor-free days in patients with sepsis: the VICTAS randomized clinical trial. *JAMA.* (2021) 325:742–50. doi: 10.1001/jama.2020.24505

9. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol.* (2018) 14:121–37. doi: 10.1038/nrneph.2017.165

10. Zhao Y, Feng X, Li B, Sha J, Wang C, Yang T, et al. Dexmedetomidine protects against lipopolysaccharide-induced acute kidney injury by enhancing autophagy through inhibition of the PI3K/AKT/mTOR pathway. *Front Pharmacol.* (2020) 11:128. doi: 10.3389/fphar.2020.00128

11. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature.* (2014) 507:323–8. doi: 10.1038/nature13145

12. Senda M, Hamano T, Fujii N, Ito T, Sakaguchi Y, Matsui I, et al. Exercise-induced hypercalcemia and vasopressin-mediated bone resorption. *Osteoporos Int.* (2021) 32:2533–41. doi: 10.1007/s00198-021-06030-1

13. Xu X, Zhang M, Fei Z, Sheng H, Qu S, Cui R. Calcification of lower extremity arteries is related to the presence of osteoporosis in postmenopausal women with type 2 diabetes mellitus: a cross-sectional observational study. *Osteoporos Int.* (2021) 32:1185–93. doi: 10.1007/s00198-020-05775-5

14. Guder C, Gravius S, Burger C, Wirtz DC, Schildberg FA. Osteoimmunology: a current update of the interplay between bone and the immune system. *Front Immunol.* (2020) 11:58. doi: 10.3389/fimmu.2020.00058

15. Kumar G, Roger PM. From crosstalk between immune and bone cells to bone erosion in infection. *Int J Mol Sci.* (2019) 20:5154. doi: 10.3390/ijms20205154

16. Yang N, Liu Y. The role of the immune microenvironment in bone regeneration. *Int J Med Sci.* (2021) 18:3697–707. doi: 10.7150/ijms.61080

17. Tsukasaki M, Takayanagi H. Osteoimmunology: evolving concepts in bone-immune interactions in health and disease. *Nat Rev Immunol.* (2019) 19:626–42. doi: 10.1038/s41577-019-0178-8

18. Han S, Kim NR, Kang JW, Eun JS, Kang YM. Radial BMD and serum CTX-I can predict the progression of carotid plaque in rheumatoid arthritis: a 3-year prospective cohort study. *Arthritis Res Ther.* (2021) 23:258. doi: 10.1186/s13075-021-02642-4

19. Eick GN, Devlin MJ, Cepon-Robins TJ, Kowal P, Sugiyama LS, Snodgrass JJ. A dried blood spot-based method to measure levels of tartrate-resistant acid phosphatase 5b (TRACP-5b), a marker of bone resorption. *Am J Hum Biol.* (2019) 31:e23240. doi: 10.1002/ajhb.23240

20. Wang L, You X, Lotinun S, Zhang L, Wu N, Zou W. Mechanical sensing protein PIEZO1 regulates bone homeostasis via osteoblast-osteoclast crosstalk. *Nat Commun.* (2020) 11:282. doi: 10.1038/s41467-019-14146-6

21. Otto E, Knapstein PR, Jahn D, Appelt J, Frosch KH, Tsitsilonis S, et al. Crosstalk of brain and bone-clinical observations and their molecular bases. *Int J Mol Sci.* (2020) 21:4946. doi: 10.3390/ijms21144946

22. Cannata-Andía JB, Martín-Carro B, Martín-Vírgala J, Rodríguez-Carrio J, Bande-Fernández JJ, Alonso-Montes C, et al. Chronic kidney disease-mineral and bone disorders: pathogenesis and management. *Calcif Tissue Int.* (2021) 108:410–22. doi: 10.1007/s00223-020-00777-1

23. Jeong HM, Kim DJ. Bone diseases in patients with chronic liver disease. *Int J Mol Sci.* (2019) 20:4270. doi: 10.3390/ijms20174270

24. Gäddnäs F, Koskela M, Koivukangas V, Risteli J, Oikarinen A, Laurila J, et al. Markers of collagen synthesis and degradation are increased in serum in severe sepsis: a longitudinal study of 44 patients. *Crit Care.* (2009) 13:R53. doi: 10.1186/cc7780

25. Gäddnäs FP, Koskela M, Koivukangas V, Laurila J, Saarnio J, Risteli J, et al. Skin collagen synthesis is depressed in patients with severe sepsis. *Anesth Analg.* (2010) 111:156–63. doi: 10.1213/ANE.0b013e3181e1db48

26. Mukhopadhyay S, Thatoi PK, Pandey AD, Das BK, Ravindran B, Bhattacharjee S, et al. Transcriptomic meta-analysis reveals up-regulation of gene expression functional in osteoclast differentiation in human septic shock. *PLoS One.* (2017) 12:e0171689. doi: 10.1371/journal.pone.0171689

27. Liu L, Wang H, Zhang X, Chen R. Identification of potential biomarkers in neonatal sepsis by establishing a competitive endogenous RNA network. *Comb Chem High Throughput Screen.* (2020) 23:369–80. doi: 10.2174/1386207323666200401121204

28. Aykut B, Chen R, Kim JI, Wu D, Shadaloey SAA, Abengozar R, et al. Targeting Piezo1 unleashes innate immunity against cancer and infectious disease. *Sci Immunol.* (2020) 5:eabb5168. doi: 10.1126/sciimmunol.abb5168

29. Puthucheary ZA, Sun Y, Zeng K, Vu LH, Zhang ZW, Lim RZL, et al. Sepsis reduces bone strength before morphologic changes are identifiable. *Crit Care Med.* (2017) 45:e1254–61. doi: 10.1097/CCM.0000000000002732

30. Bayer J, Vaghela R, Drechsler S, Osuchowski MF, Erben RG, Andrukhova O. The bone is the major source of high circulating intact fibroblast growth factor-23 in acute murine polymicrobial sepsis induced by cecum ligation puncture. *PLoS One.* (2021) 16:e0251317. doi: 10.1371/journal.pone.0251317

31. Terashima A, Takayanagi H. The role of bone cells in immune regulation during the course of infection. *Semin Immunopathol.* (2019) 41:619–26. doi: 10.1007/s00281-019-00755-2

32. Zhang, Z, Jin P, Feng M, Yang J, Huang J, Chen L, et al. Causal inference with marginal structural modeling for longitudinal data in laparoscopic surgery: a technical note. *Laparosc Endosc Robot Surg.* (2022). doi: 10.1016/j.lers.2022.10.002

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 11 January 2023
DOI 10.3389/fendo.2022.1021083

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

RECEIVED 06 September 2022
ACCEPTED 16 December 2022
PUBLISHED 11 January 2023

CITATION
Jiang L, Jiang Y, Wang A, Wu C and Shen Y (2023) The causal association between bone mineral density and risk of osteoarthritis: A Mendelian randomization study.
Front. Endocrinol. 13:1021083.
doi: 10.3389/fendo.2022.1021083

The causal association between bone mineral density and risk of osteoarthritis: A Mendelian randomization study

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Objectives: The causal direction and magnitude of the association between total body bone mineral density (TB-BMD) and osteoarthritis (OA) risk is uncertain owing to the susceptibility of observational studies to confounding and reverse causation. The study aimed to explore the relationships between TB-BMD concentration and OA using Mendelian randomization (MR).

Methods: In this study, we used two-sample MR to obtain unconfounded estimates of the effect of TB-BMD on hip and knee OA. Single nucleotide polymorphisms (SNPs) strongly associated with TB-BMD in a large genome-wide association study (GWAS) were identified and selected as instrumental variables (IVs). In addition to the main analysis using inverse-variance weighted (IVW) method, we applied 2 additional methods to control for pleiotropy(MR-Egger regression, weighted median estimator) and compared the respective MR estimates.

Results: MR analyses suggested that genetically predicted higher TB-BMD is associated with risks of hip OA (For IVW: OR=1.199, 95%CI: 1.02-1.42, *P*=0.032; for WM: OR=1.257, 95%CI: 1.09-1.45, *P*=0.002). There was no evidence that the observed causal effect between TB-BMD and the risk of hip OA was affected by genetic pleiotropy(*P*=0.618). Additionally, our study didn't support causal effects of a genetically increased TB-BMD risk on knee OA risk(OR=1.121, 95%CI: 0.99-1.28, *P*=0.084 using IVW; OR=1.132, 95%CI: 0.99-1.29, *P*=0.068 using WM; OR=1.274, 95%CI: 0.88-1.85, *P*=0.217 using MR-Egger).

Conclusions: Our findings support a causal effect that a genetic predisposition to systematically higher TB-BMD was associated with the risk of OA. And, TB-BMD likely exerts an effect on the risk of hip OA not knee OA.

KEYWORDS

osteoarthritis, bone mineral density, single nucleotide polymorphisms, Mendelian randomization, genome-wide association studies

Introduction

Osteoarthritis (OA) is the most prevalent musculoskeletal disease and the most common form of arthritis (1, 2). The hallmarks of OA are the degeneration of articular cartilage, remodeling of the underlying bone and synovial (3). As the leading cause of disability worldwide, it affects 40% of individuals over 70 years old and carries a substantial public health and health economic burden (4, 5). The health economic burden of OA is continually rising, commensurate with longevity and obesity rates, and current disease-modifying treatment is minimally effective (6). As a highly heterogeneous disease, the pathological features of OA include pain, inflammation, cartilage degradation and bone spurs. The heritability of the disease is over 50%, and previous genetic studies have identified 21 loci in total across hip, knee, and hand sites with limited overlap (5).

Bone mineral density (BMD) is considered to be one of the integral influences in the pathogenesis of OA. The association between BMD and OA was first reported by Foss in 1972 when they observed that higher BMD in femoral heads resected during OA-related hip replacement surgery (7). A cross-sectional study suggested that higher BMD was associated with an increased risk of incident OA defined by osteophyte or Kellgren-Lawrence(KL) grade, suggesting that increased BMD is a risk factor for OA development (8). Women with radiographic hip OA had a 9%-10% higher BMD of the femoral neck compared to those without OA (9). Previous studies indicated that male hand OA patients have reduced radial trabecular density and thickness in the distal radius (10).

Genome-wide association studies (GWAS) have made an important contribution to the identification of genetic variants associated with numerous potential risk factors for health-related outcomes. The GWAS results have facilitated the use of Mendelian randomization(MR) to evaluate causal relationships making use of summary-level data from GWAS between modifiable exposures and outcomes (11). MR uses genetic variants as instrumental variables(IVs) to detect whether the exposure phenotype has a causal effect on the outcome phenotype (12). This approach can overcome common pitfalls of traditional research, such as confounding factors and reverse causality (13). MR method has been applied to estimate the causal associations between parathyroid Hormone (14), insulin-like growth factor-1(IGF-1) (15), and smoking behavior (16), and OA.

Compelling evidence has suggested that a causal effect of high femoral neck BMD on the risk of knee OA and hip OA is predominantly reflecting cortical bone mass (17). However, these studies are susceptible to confounding and reverse causation, and thus it remains unclear whether these associations are accurate. Inferring causality from such studies is challenging because it is difficult to exclude reverse causality, confounding, or measurement error. The relationship between BMD and OA has always been a controversial issue, especially for different sites. The identification of risk factors for primary prevention is therefore of major interest as a method to reduce the incidence and consequences of the disease. Understanding the role of BMD in the pathogenesis of OA may have important therapeutic significance, and this would be of clinical relevance for OA prevention in high-risk individuals since lower total body BMD(TB-BMD) is common and safely correctable in elderly adults.

To the best of our knowledge, the role of TB-BMD in OA risk has not been well established through observational studies. Herein, we used genetic variations strongly associated with TB-BMD traits as unconfounded instruments to investigate the potential causal effect of TB-BMD on the risk of integrating knee OA and hip OA in individuals of similar European origin. In our study, we applied two-sample MR, an approach that combines summary statistics on the genetic variant to exposure and genetic variant to outcome associations from different samples, to provide estimates on the strength of the association between exposure and outcome.

Materials and methods

Study overview

This study determined whether TB-BMD was causally related to OA using MR analysis. First, the genetic variants utilized as instrumental variables (IVs) should not be associated with confounders. Second, genetic variants should not be associated with confounders. Third, the genetic variants should affect the risk of the outcome through the risk factor, not *via*

other pathways. The MR analysis followed strictly the STROBE-MR Statement (18).

As this study is based on existing publications and public databases, no additional ethical approval or consent to participate is required. Further details on the design strategy are shown in Figure 1.

Selection of genetic variants

Publicly accessible data for genetic variants associated with TB-BMD were retrieved obtained from the GEnetic Factors for OSteoporosis(GEFOS) consortium (http://www.gefos.org/). Summary data from a previous meta-analysis of 30 GWASs of TB-BMD including 66,628 individuals (56,284 individuals of European ancestry) was performed to investigate genetic determinants of TB-BMD variation (19). The SNPs of TB-BMD were already corrected covariates (age, weight, height and so on) *via* linear regression models (19).

Screening criteria for TB-BMD-associated SNPs: (i)SNPs have significance in genome-wide studies($P<5\times10^{-8}$). (ii)All IVs were independent with linkage disequilibrium(LD) ($r^2<0.001$), which validated the independence of selected genetic variants (20).

OA GWAS summary statistics

We searched the NHGRI-EBI GWAS catalog (www.ebi.ac.uk/gwas), which is a manually curated collection of complete GWAS summary datasets. Summary statistics for the effect of TB-BMD-associated SNPs on OA were derived from a genome-wide meta-analysis that included up to 455,221 European individuals (77,052 cases and 378,169 controls), complying with genotype data across 17.5 million variants from the UK Biobank and Arthritis Research UK OA Genetics(arcOGEN) resources (21).

Statistical analysis

The MR approach was used to identify the potential causal link of TB-BMD with knee OA and hip OA. All statistical data analyses were carried out using the package "TwoSampleMR" of the R program (version 4.1.0). The two-sample MR method was estimated for the genetically causal associations were obtained by applying the inverse-variance weighted (IVW) analysis, weighted median(WM) estimator, and MR-Egger regression (22–25). The IVW method examines the causal link by performing a meta-analysis of each Wald ratio for the included SNPs (23). Significantly, the IVW analysis is predicated on the assumption that all of the contained SNPs are genuine variables (23). Unlike the IVW method, the MR-Egger regression can work even if all of the SNPs are invalid. However, MR-Egger may be inaccurate, especially when the correlation coefficient between SNPs and the exposure is similar or the number of genetic instruments is small (22). The WM estimator produced the median when the effect estimations of each single SNP were sorted in order of weight values (24). The estimation of the causal relationship between TB-BMD and OA was expressed as odds ratio(OR) and 95% confidence interval(CI). In addition, $P<0.05$ was the threshold for a significant difference.

MR analysis assumes that the selected IVs should act on the outcome only through the exposure variable. To eliminate the impact of known confounding factors on causality estimates,

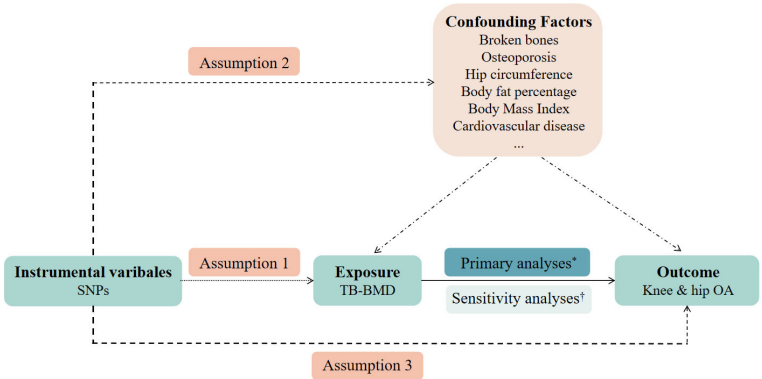

FIGURE 1
An overview of the study design. SNP, single-nucleotide polymorphism; TB-BMD, total body bone mineral density; OA, osteoarthritis. Assumption 1: the instrumental variables (IVs) must be robustly associated with the exposure. Assumption 2: the IVs must not be associated with any confounding factors of the exposure-outcome relationship. Assumption 3: the IVs must only affect the outcome through the exposure. *Primary analyses: inverse-variance weighted (IVW) method, weighted median estimator, and MR-Egger regression. †Sensitivity analyses: cochran's Q test and leave-one-out analyses.

potential pleiotropic effects of selected SNPs on BMD were verified *via* the Ensembl project (http://www.ensembl.org) and the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk). We systematically checked each SNP on the both websites and comprehensively aggregated the results. Finally, we excluded one SNP(rs2043230) at-a-time and performed analysis on the remaining SNPs to identify outlying IVs.

Heterogeneity and sensitivity test

In analyses of two-sample MR, pleiotropy represents those genetic variants or SNPs with multiple effects. That is, pleiotropic genetic variants or SNPs may have an effect on the outcome, not the exposure, which could cause a bias in the MR estimate and potential confounding effects; thus, investigating pleiotropy is essential. In the study, we removed SNPs one by one using the "leave-one-out" analysis and calculated the combined effect of the remaining SNPs separately to determine the magnitude of the effect of each SNP on the results. If the results of the "leave-one-out" analysis are inconsistent with the results of the causal effects analysis, it indicates that there may be effect on the estimated causal effects (22). Also, heterogeneity test is essential. We used Cochran's Q statistics to explore heterogeneity among these SNPs (26). In cases where Cochran's Q test indicated there was pleiotropy, we adopted the results of a random effects model instead of a fixed effects IVW model. For the sensitivity test, we performed a subgroup analysis utilizing only IV SNPs with genome-wide significance($P<5\times10^{-8}$).

Results

The detail of studies and datasets was presented in Table 1. The participants were all of European(100%), overcoming the issue of ethnic differences.

Characteristics of instrumental variables

The original data of IVs can be obtained from the link (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5777980/). Genetic variants were screened according the criteria of screening($P<5\times10^{-8}$, $r^2<0.001$, kb=10000). Eventually, 29 SNPs were employed as IVs for TB-BMD in the present study (Table 2). The associations between IVs for knee OA and hip OA are summarized in Supplementary Tables 1, 2, including the chromosome location, genes, effect allele(EA), other allele and effect allele frequency(EAF). In addition, estimations of the association between each SNP with TB-BMD and OA, including beta value, standard error(SE) and *P* value, were also presented.

Causal association with knee OA and hip OA

One palindromic SNPs (rs2043230) with intermediate allele frequencies was removed. Thus, 28 independent SNPs associated with TB-BMD in European ancestry were chosen to perform the MR analysis for the causal link between TB-BMD and knee OA and hip OA. None of the individual 28 SNPs was associated with knee OA in the study(OR: 1.121, 95%CI: 0.99-1.28, *P*=0.084 using IVW; OR: 1.132, 95%CI: 0.99-1.29, *P*=0.068 using WM; OR: 1.274, 95%CI: 0.88-1.85, *P*=0.217 using MR-Egger)(shown in Table 3).

The association between genetically predicted TB-BMD and hip OA were detected using the IVW method (OR=1.199), although with a wider CI(95%CI: 1.02-1.42, *P*=0.032). The association between genetically predicted TB-BMD and hip OA was consistent with IVW method using WM method (OR=1.257, 95%CI=1.09-1.45, *P*=0.002) (Table 3; Figure 2). There was no causal association between TB-BMD and hip OA using MR-Egger analysis(OR: 1.347, 95% CI: 0.83-2.18, *P*=0.235), and this was inconsistent with WM and IVM methods.

Sensitivity analysis

According to Cochran's Q test, there was no evidence of heterogeneity between the MR estimates based on individual variants using IVW method (For knee OA, Q value=76.61, *P*=0.484; for hip OA, Q value=79.22, *P*=0.618) (Table 3). The "leave one out" analysis revealed that no single SNP play a decisive role in the causal inference (Figure 3).

TABLE 1 Details of Studies and Datasets Used in the Study.

Exposure/Outcome	Sample Size	Web Source	First Author	Consortium	Year	Population
TB-BMD	56,284	http://www.gefos.org/	Medina C	GEFOS	2018	European
Knee OA	403,124	https://gwas.mrcieu.ac.uk/	Tachmazidou I	–	2019	European
Hip OA	393,873	https://gwas.mrcieu.ac.uk/	Tachmazidou I	–	2019	European

TB-BMD, total body bone mineral density; OA, osteoarthritis; GEFOS, the GEnetic Factors for OSteoporosis.

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TABLE 2 Characteristics of SNPs for TB-BMD.

SNPs	Chr	Effect allele	Other allele	EAF	Beta	SE	*P*-value
rs10249736	7:120737177	A	G	0.45	-0.0370	0.0062	2.25E-09
rs10777212	12:90334829	T	G	0.35	0.0454	0.0066	5.00E-12
rs10838622	11:46856536	T	C	0.36	0.0491	0.0067	3.04E-13
rs117557198	12:49655948	A	G	0.93	-0.0887	0.0128	3.84E-12
rs11910328	21:40350744	A	G	0.84	-0.0489	0.0085	8.51E-09
rs12293302	11:15776444	A	T	0.03	0.1441	0.0209	5.05E-12
rs12612325	2:119632252	A	G	0.20	-0.0600	0.0086	2.95E-12
rs1286079	14:91445162	T	C	0.19	0.0552	0.0080	5.42E-12
rs1385162	11:15689391	A	G	0.21	0.0466	0.0077	1.24E-09
rs144279715	2:119548256	A	G	0.98	-0.2178	0.0309	1.89E-12
rs1452102	21:28773868	T	G	0.58	-0.0343	0.0062	3.29E-08
rs2043230	2:85483350	A	T	0.44	0.0339	0.0062	4.77E-08
rs2289410	2:42284110	A	T	0.87	0.0528	0.0096	4.05E-08
rs344024	3:156474152	A	G	0.77	0.0504	0.0072	3.11E-12
rs3757493	7:96656572	T	G	0.42	-0.0359	0.0063	1.28E-08
rs6716216	2:202803881	A	G	0.88	-0.0658	0.0095	4.71E-12
rs6965122	7:96133319	A	G	0.68	0.0766	0.0066	4.64E-31
rs71390846	16:86714715	C	G	0.19	-0.0498	0.0081	6.95E-10
rs73305797	7:30997087	A	T	0.75	0.0424	0.0072	3.71E-09
rs7364724	1:110480220	A	G	0.40	-0.0377	0.0063	1.84E-09
rs73719811	7:121200844	T	C	0.93	-0.0944	0.0130	3.42E-13
rs746627	17:63850776	T	C	0.32	0.0414	0.0069	1.56E-09
rs7586085	2:166577489	A	G	0.52	0.0511	0.0062	1.72E-16
rs7740042	6:151971720	A	T	0.20	-0.0552	0.0077	7.86E-13
rs7741085	6:44636919	T	C	0.59	0.0465	0.0063	1.19E-13
rs78667121	13:43200103	A	G	0.03	0.1557	0.0192	5.96E-16
rs8047501	16:392318	A	G	0.49	0.0559	0.0065	6.83E-18
rs884205	18:60054857	A	C	0.25	-0.0530	0.0073	3.96E-13
rs9976876	21:36970350	T	G	0.46	-0.0381	0.0063	1.35E-09

TB-BMD, total body bone mineral density; SNP, single nucleotide polymorphism; EAF, effect allele frequency; SE, standard error.

Discussion

The causal direction and magnitude of the association between TB-BMD and risk of OA is uncertain, and observational studies make up the majority of previous research. Observational studies are susceptible to demonstrate confounding, reverse causation bias, and measurement error, all of which limit their ability to provide causal estimates of the effect of exposures on outcomes, thereby reducing their ability to inform prevention and treatment strategies against the disease. Unlike observational studies, MR uses exceptional genetic variants that are assumed to satisfy the IVs hypothesis to investigate the question of causality in epidemiological studies, which minimizes the possibility of inherent bias (27). Compared with randomized controlled trials, MR analysis is cost-effective and feasible (28).

In this study, we explored the relationship between TB-BMD and OA risk *via* a two-sample MR analysis based on 28 SNPs

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TABLE 3 MR estimates of associations between TB-BMD with knee and hip OA using various analysis methods.

Exposure	MR method	Number of SNPs	OR	95% CI	Association *P*-value	Cochran's Q Statistic	Heterogeneity *P*-value
Knee OA							
TB-BMD	IVW	28	1.121	0.99-1.28	0.084	76.61	0.484
	WM	28	1.132	0.99-1.29	0.068		
	MR-Egger	28	1.274	0.88-1.85	0.217		
Hip OA							
TB-BMD	IVW	28	1.199	1.02-1.42	0.032*	79.22	0.618
	WM	28	1.257	1.09-1.45	0.002*		
	MR-Egger	28	1.347	0.83-2.18	0.235		

*Statistically significant P-value.
MR, Mendelian randomization; TB-BMD, total body bone mineral density; OA, osteoarthritis; SNP, single nucleotide polymorphism; IVW, inverse variance weighted; WM, weighted median.

significantly related to TB-BMD as genetic variants. Despite the fact that the MR estimates from IVW, MR-Egger, and WM analysis were inconsistent, the IVW and WM estimators support a causal inverse relationship between TB-BMD and hip OA. The MR analysis suggests that a causal relationship between TB-BMD and hip OA, as the weighted average retains greater precision in the estimates compared to the MR-Egger analysis. However, there is no evidence of a causal association between TB-BMD and knee OA based on these three analyses. MR provide insights into the nature of the genetic aetiology we observed. Large-scale whole genome sequencing studies of well phenotyped individuals across diverse populations will capture the full allele frequency and variation type spectrum, and prompt us to explore the causes of this debilitating disease. This MR approach offers an alternative analytical technique being able to reduce conventional patterns of confounding and reverse causation and re-estimate observations in a framework allowing causal inference. These findings present important novel evidence on the etiology of OA.

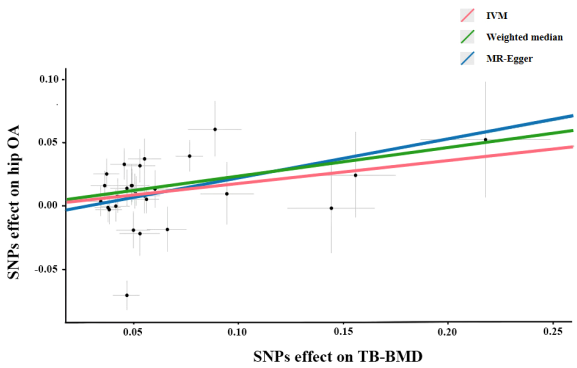

FIGURE 2
Scatter plots of the Mendelian randomization analyses for the association of TB-BMD with the risk of hip OA. The red line represents the inverse variance weighted estimate, the green line represents the Weighted median estimate, the blue line represents the MR-Egger estimate.

OA was previously thought to be a cartilage disease, whereas it is now considered as a disease of the entire joint with other articular components playing a key role in the pathogenesis of OA (29). The central feature of OA is joint cartilage degradation and loss, which is manifested radiographically as a narrowing of the joint space. Because of cartilage loss and metabolic disturbances, the biomechanics of the joint change during the process of OA development result in altered subchondral bone elasticity and stiffness (30). Possible mechanisms whereby changed BMD in the subchondral bone(the region of bone immediately beneath the cartilage that includes the cortical plate and the subchondral cancellous bone) and altered gene expression might be potential players in cartilage degeneration pathogenesis (31). The potential mechanisms underlying the association need to be more thoroughly evaluated.

The reliability of the findings from a MR study depends on 3 key assumptions, which could be violated by population stratification, canalization, pleiotropy and linkage disequilibrium. Since not all genetic variants used as proxies for the exposure of interest may fulfill the "no pleiotropy" assumption, several approaches were undertaken to assess and adjust for potential confounding or pleiotropic effects. Sensitivity analyses using Cochran's Q statistics and the "leave-one-out" method to explore

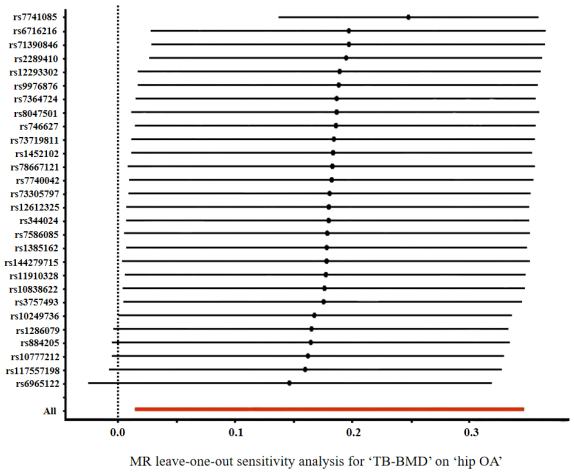

FIGURE 3
Forrest plot of the causal effects of TB-BMD-associated single nucleotide polymorphisms (SNPs) on hip OA.

and adjust for pleiotropy were also conducted. Pleiotropy is defined as the phenomenon in which a single locus affects two or more distinct phenotypic traits, resulting in compromises between trait adaptations because a mutation that benefits one trait may harm another (32, 33). The credibility of MR study results is likely to be affected by whether pleiotropy leads to bias. The results were consistent across these analyses, indicating that confounding is unlikely to explain the observed associations. Linkage disequilibrium with directly causal variants (violating the third assumption) was likely avoided owing to the use of multiple SNPs, most of which were positively associated with hip OA.

Recently, the temporal relationship between BMD and OA has been confirmed. In Johnston County, North Carolina (NC), higher BMD was associated with a lower risk of hip OA among adults aged 45 years old. Intermediate, but not high, BMD levels were linked to an increased risk of knee and hip OA (34). A case-control study provides strong evidence that high baseline femoral neck BMD is significantly related to the incidence of knee and hip OA, but not the incidence of hand OA. Whereas high baseline BMD is not associated with the progression of knee OA (35). Both men and women have presented lower fracture risk when their BMD is higher, particularly in the hip site (36). In middle-aged and older adults, lower fracture risk may be offset by an increased risk of incident knee OA (37). In postmenopausal women, there has been a similar link between higher BMD and incident radiographic hip OA (38). These studies are consistent with the results of the causal analysis.

The strengths of this MR analysis include the availability of larger sample size and the use of multiple uncorrelated SNPs associated with BMD based on published GWAS meta-analysis data, which increase the precision of the estimates. Moreover, a two-sample MR obtains IV-exposure and IV-outcome associations from two different sets of participants. Two-sample MR that uses the effect of IVs on the exposure and the outcome phenotype from two independent studies can greatly increase the power of detecting causality compared with a single sample study (39). Most importantly, population stratification arises when different subgroups of the population vary substantially. This bias can be mitigated by limiting the analysis to ethnically homogenous groups. The participants contributing to this study were composed of European descent populations, and the SNPs have been consistently associated with BMD in populations of different ancestries.

This study has several limitations that need to be considered. First, the overall estimate of the genetic association was based on a publicly available summary meta-analysis, and the study lacked complete information on sex and age. Therefore, subgroup analyses could not be conducted to explore the association separated by study-specific characteristics(e.g. age, gender), and a potential non-linear association between serum BMD levels and OA could not be further evaluated. A potential limitation is that datasets with a larger number of OA cases and non-cases were unavailable in the study. Genetic variants generally have small effects on the exposure and possibly explain only a small proportion of the variance, and large sample sizes are necessary to obtain statistically significant results. Other study limitations include an inability to exclude the existence of smaller associations and a lack of evidence from non-European populations.

In conclusion, our findings provide some support for a causal effect, whereby a systematically higher genetic susceptibility to TB-BMD is associated with an increased risk of OA. The use of multiple SNPs in MR analysis has also established that increased BMD is associated with the risk of hip OA, but not with an increased knee OA risk. Whether the risk of hip OA associated with lifelong genetic exposure to increased BMD can be translated into a risk associated with short-term to medium-term body load is unknown. Given that TB-BMD status is a modifiable trait, these results may have clinical and public health implications that need confirmation by further larger MR studies and clinical trials.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ Supplementary Material.

Author contributions

LJ drafted the protocol and wrote the final paper. YJ contributed to data analysis and interpretation of results. YS

made critical revisions. AW and CW participated in the data collection. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by teacher professional progress project in Shanghai, and Local High-level University (cultivation) project in Shanghai (E1-2602-21-201006-6), and supported by the Shanghai Municipal Health Commission (201840297), and supported by The Training Planned Fund of Academic Leaders from Shanghai Pudong New Area Health System (PWR 12018-09).

Acknowledgments

Summary-level data for genetic associations with OA was collected by Tachmazidou et al., and downloaded from GWAS Catalog website (https://www.ebi.ac.uk/gwas/). The authors thank all investigators for sharing these data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1021083/full#supplementary-material

References

1. Sun X, Zhen X, Hu X, Li Y, Gu S, Gu Y, et al. Osteoarthritis in the middle-aged and elderly in China: Prevalence and influencing factors. *Int J Environ Res Public Health* (2019) 16(23):4701. doi: 10.3390/ijerph16234701

2. Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, et al. 2019 American College of Rheumatology/Arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. *Arthritis Rheumatol* (2020) 72(2):220–33. doi: 10.1002/art.41142

3. Zhao L, Huang J, Fan Y, Li J, You T, He S, et al. Exploration of CRISPR/Cas9-based gene editing as therapy for osteoarthritis. *Ann Rheum Dis* (2019) 78(5):676–82. doi: 10.1136/annrheumdis-2018-214724

4. Cibrian Uhalte E, Wilkinson JM, Southam L, Zeggini E. Pathways to understanding the genomic aetiology of osteoarthritis. *Hum Mol Genet* (2017) 26 (R2):R193–201. doi: 10.1093/hmg/ddx302

5. Zengini E, Hatzikotoulas K, Tachmazidou I, Steinberg J, Hartwig FP, Southam L, et al. Genome-wide analyses using UK biobank data provide insights into the genetic architecture of osteoarthritis. *Nat Genet* (2018) 50(4):549–58. doi: 10.1038/s41588-018-0079-y

6. Usenbo A, Kramer V, Young T, Musekiwa A. Prevalence of arthritis in Africa: A systematic review and meta-analysis. *PloS One* (2015) 10(8):e0133858. doi: 10.1371/journal.pone.0133858

7. Foss MV, Byers PD. Bone density, osteoarthrosis of the hip, and fracture of the upper end of the femur. *Ann Rheum Dis* (1972) 31(4):259–64. doi: 10.1136/ard.31.4.259

8. Zhang Y, Hannan MT, Chaisson CE, McAlindon TE, Evans SR, Aliabadi P, et al. Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: the framingham study. *J Rheumatol* (2000) 27(4):1032–7.

9. Nevitt MC, Lane NE, Scott JC, Hochberg MC, Pressman AR, Genant HK, et al. Radiographic osteoarthritis of the hip and bone mineral density. the study of osteoporotic fractures research group. *Arthritis Rheumatol* (1995) 38(7):907–16. doi: 10.1002/art.1780380706

10. Simon D, Tascilar K, Unbehend S, Bayat S, Berlin A, Liphardt AM, et al. Bone mass, bone microstructure and biomechanics in patients with hand osteoarthritis. *J Bone Miner Res* (2020) 35(9):1695–702. doi: 10.1002/jbmr.4046

11. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* (2003) 32(1):1–22. doi: 10.1093/ije/dyg070

12. Greenland S. Randomization, statistics, and causal inference. *Epidemiology* (1990) 1(6):421–9. doi: 10.1097/00001648-199011000-00003

13. Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-egger method. *Eur J Epidemiol.* (2017) 32(5):377–89. doi: 10.1007/s10654-017-0255-x

14. Huang G, Zhong Y, Li W, Liao W, Wu P. Causal relationship between parathyroid hormone and the risk of osteoarthritis: A mendelian randomization study. *Front Genet* (2021) 12:686939. doi: 10.3389/fgene.2021.686939

15. Hartley A, Sanderson E, Paternoster L, Teumer A, Kaplan RC, Tobias JH, et al. Mendelian randomization provides evidence for a causal effect of higher serum IGF-1 concentration on risk of hip and knee osteoarthritis. *Rheumatol (Oxford)* (2021) 60(4):1676–86. doi: 10.1093/rheumatology/keaa597

16. Lee YH. Causal association between smoking behavior and the decreased risk of osteoarthritis: a mendelian randomization. *Z Rheumatol* (2019) 78(5):461–6. doi: 10.1007/s00393-018-0505-7

17. Funck-Brentano T, Nethander M, Moverare-Skrtic S, Richette P, Ohlsson C. Causal factors for knee, hip, and hand osteoarthritis: A mendelian randomization study in the UK biobank. *Arthritis Rheumatol* (2019) 71(10):1634–41. doi: 10.1002/art.40928

18. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomization: The STROBE-MR statement. *JAMA* (2021) 326(16):1614–21. doi: 10.1001/jama.2021.18236

19. Medina-Gomez C, Kemp JP, Trajanoska K, Luan J, Chesi A, Ahluwalia TS, et al. Life-course genome-wide association study meta-analysis of total body BMD and assessment of age-specific effects. *Am J Hum Genet* (2018) 102(1):88–102. doi: 10.1016/j.ajhg.2017.12.005

20. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* (2008) 27(8):1133–63. doi: 10.1002/sim.3034

21. Tachmazidou I, Hatzikotoulas K, Southam L, Esparza-Gordillo J, Haberland V, Zheng J, et al. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK biobank data. *Nat Genet* (2019) 51(2):230–6. doi: 10.1038/s41588-018-0327-1

22. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. *Elife* (2018) 7:e34408. doi: 10.7554/eLife.34408

23. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data mendelian randomization. *Stat Med* (2017) 36(11):1783–802. doi: 10.1002/sim.7221

24. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40(4):304–14. doi: 10.1002/gepi.21965

25. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data mendelian randomization *via* the zero modal pleiotropy assumption. *Int J Epidemiol.* (2017) 46(6):1985–98. doi: 10.1093/ije/dyx102

26. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. *BMJ* (1997) 315(7121):1533–7. doi: 10.1136/bmj.315.7121.1533

27. Bowden J, Holmes MV. Meta-analysis and mendelian randomization: A review. *Res Synth Methods* (2019) 10(4):486–96. doi: 10.1002/jrsm.1346

28. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA* (2017) 318(19):1925–6. doi: 10.1001/jama.2017.17219

29. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheumatol* (2012) 64(6):1697–707. doi: 10.1002/art.34453

30. Amini M, Nazemi SM, Lanovaz JL, Kontulainen S, Masri BA, Wilson DR, et al. Individual and combined effects of OA-related subchondral bone alterations on proximal tibial surface stiffness: a parametric finite element modeling study. *Med Eng Phys* (2015) 37(8):783–91. doi: 10.1016/j.medengphy.2015.05.011

31. Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. *Clin Orthop Relat Res* (1986) 213):34–40. doi: 10.1097/00003086-198612000-00005

32. Stearns FW. One hundred years of pleiotropy: a retrospective. *Genetics* (2010) 186(3):767–73. doi: 10.1534/genetics.110.122549

33. Wagner GP, Zhang J. The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. *Nat Rev Genet* (2011) 12(3):204–13. doi: 10.1038/nrg2949

34. Barbour KE, Murphy LB, Helmick CG, Hootman JM, Renner JB, Jordan JM. Bone mineral density and the risk of hip and knee osteoarthritis: The Johnston county osteoarthritis project. *Arthritis Care Res (Hoboken)* (2017) 69(12):1863–70. doi: 10.1002/acr.23211

35. Bergink AP, Rivadeneira F, Bierma-Zeinstra SM, Zillikens MC, Ikram MA, Uitterlinden AG, et al. Are bone mineral density and fractures related to the incidence and progression of radiographic osteoarthritis of the knee, hip, and hand in elderly men and women? the Rotterdam study. *Arthritis Rheumatol* (2019) 71 (3):361–9. doi: 10.1002/art.40735

36. Stone KL, Seeley DG, Lui LY, Cauley JA, Ensrud K, Browner WS, et al. BMD at multiple sites and risk of fracture of multiple types: long-term results from the study of osteoporotic fractures. *J Bone Miner Res* (2003) 18(11):1947–54. doi: 10.1359/jbmr.2003.18.11.1947

37. Nevitt MC, Zhang Y, Javaid MK, Neogi T, Curtis JR, Niu J, et al. High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study. *Ann Rheum Dis* (2010) 69(1):163–8. doi: 10.1136/ard.2008.099531

38. Hochberg MC. Do risk factors for incident hip osteoarthritis (OA) differ from those for progression of hip OA? *J Rheumatol Suppl* (2004) 70:6–9.

39. Pierce BL, Burgess S. Efficient design for mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol.* (2013) 178(7):1177–84. doi: 10.1093/aje/kwt084

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 02 February 2023
DOI 10.3389/fendo.2023.1098841

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

RECEIVED 15 November 2022
ACCEPTED 04 January 2023
PUBLISHED 02 February 2023

CITATION
Rajput S, Dutta A, Rajender S, Mithal A and Chattopadhyay N (2023) Efficacy of antiresorptive agents bisphosphonates and denosumab in mitigating hypercalcemia and bone loss in primary hyperparathyroidism: A systematic review and meta-analysis.
Front. Endocrinol. 14:1098841.
doi: 10.3389/fendo.2023.1098841

Efficacy of antiresorptive agents bisphosphonates and denosumab in mitigating hypercalcemia and bone loss in primary hyperparathyroidism: A systematic review and meta-analysis

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Purpose: Primary hyperparathyroidism (PHPT) is characterized by increased bone remodeling and hypercalcemia. Parathyroidectomy (PTX), the current standard of care, is recommended in all symptomatic and some groups of asymptomatic patients. Anti-resorptive therapies (bisphosphonates and denosumab) have been used in patients where PTX is refused or contraindicated. In this meta-analysis, we investigated the effectiveness of anti-resorptives in preventing/treating PHPT-induced bone loss and mitigating hypercalcemia.

Method: PubMed, Scopus, and Cochrane Library databases were searched for articles with keywords containing PHPT, bisphosphonates, and denosumab in various combinations. We extracted and tabulated areal BMD (aBMD), serum mineral, and bone turnover parameters from the qualified studies and used comprehensive meta-analysis software for analysis.

Results: Of the 1,914 articles screened, 13 were eligible for meta-analysis. In the pooled analysis, 12 months of anti-resoptives (bisphosphonates and denosumab) therapy significantly increased aBMD at the lumbar spine (Standard difference in means (SDM)=0.447, 95% CI=0.230 to 0.664, p=0.0001), femoral neck (SDM=0.270, 95% CI=0.049 to 0.491, p=0.017) and increased serum PTH (SDM=0.489, 95% CI=0.139 to 0.839, p=0.006), and decreased serum calcium (SDM=-0.545, 95% CI=-0.937 to -0.154, p=0.006) compared with baseline. 12 months of bisphosphonate use significantly increased aBMD only at the lumbar spine (SDM=0.330, 95% CI=0.088 to 0.571, p=0.007) with a significant increased in serum PTH levels (SDM=0.546, 95% CI= 0.162 to 0.930, p=0.005), and a decreased in serum calcium (SDM=-0.608, 95% CI=-1.048 to -0.169, p=0.007) and bone-turnover markers (BTMs) compared with baseline. Denosumab use for 12 months significantly increased aBMD at both the lumbar spine (SDM=0.828, 95% CI=0.378 to 1.278, p=0.0001) and femur neck (SDM=0.575, 95% CI=0.135 to 1.015, p=0.010) compared with baseline. Mean lumbar spine aBMD

(SDM=0.350, 95% CI=0.041 to 0.659, p=0.027) and serum PTH (SDM=0.602, 95% CI= 0.145 to 1.059, p=0.010) were significantly increased after 12 months of alendronate use compared with placebo. When compared with baseline, alendronate significantly decreased BTMs after 12 months and increased aBMD without altering the PTH and calcium levels after 24 months.

Conclusion: Anti-resorptives are effective in mitigating bone loss and hypercalcemia in PHPT while maintaining or increasing aBMD. PTX reversed all changes in PHPT and normalized PTH levels.

KEYWORDS

primary hyperparathyroidism, bisphosphonates, denosumab, bone mineral density, bone turnover markers, anti-resorptives

1 Introduction

Primary hyperparathyroidism (PHPT) is a disorder of mineral metabolism that is commonly observed in women of age 50 to 60 years (1–3). It is characterized by autonomous parathyroid hormone (PTH) secretion resulting in myriad systemic manifestations such as bone mineral loss, osteoporosis, fractures, lytic lesions, renal stones, and hypercalcemia (3). Low bone mineral density (BMD), osteopenia, and osteoporosis are frequently observed in women with PHPT (4). PHPT is characterized by an increase in the activation frequency of bone multicellular units (BMUs), resulting in an enlarged bone remodeling space. Specifically, cortical bone porosity and endocortical bone resorption are increased, leading to cortical bone loss with relative preservation of trabecular bones (5). These skeletal events account for increased calcium and bone turnover markers (BTM) in both serum and urine (6). PHPT increases fracture risk; thus, treatment strategies aiming to ameliorate hypercalcemia and improving BMD are likely to be clinically relevant.

Parathyroidectomy (PTX) is the standard of care for treating symptomatic PHPT and, in some cases of asymptomatic PHPT (3). According to the guidelines issued by the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism, PTX has been recommended for those with osteoporosis (T-score ≤ −2.5 at the hip, spine, or one-third distal radial site), hypercalcemia (serum calcium > 0.25 mmol/L above normal), creatinine clearance below 60 mL/min, or age < 50 years (7). Besides restoring normocalcemia, PTX increases BMD and decreases fracture risk in patients with osteoporosis and osteopenia (8). However, up to 75-80% of PHPT patients are asymptomatic at the time of presentation (9), and not everyone fits the aforementioned criteria for surgery. Therefore, specific pharmacotherapy targeting hypercalcemia and/or low BMD may be beneficial if the patient does not meet surgical requirements or presents with some medical contraindication/is unwilling for surgery (7).

Current pharmacotherapy for PHPT consists of calcimimetics (cinacalcet) to suppress PTH secretion (10) and anti-resorptive drugs, including bisphosphonates (BPs) and denosumab (RANKL neutralizing antibody) (11). Anti-resorptives are attractive because they increase BMD and reduce fracture risk in postmenopausal and senile osteoporosis patients. Because the characteristics of bone loss in PHPT differ from those seen in postmenopausal osteoporosis, it is essential to establish the usefulness of these therapies in increasing bone mass in PHPT. A systematic review observed that BPs improved BMD in PHPT patients but lowered serum calcium transiently (12).

This meta-analysis was undertaken to determine the effect of anti-resorptives (BPs, and/or denosumab) on areal BMD (aBMD), bone turnover markers (BTMs), calcium and phosphate levels in patients with PHPT (asymptomatic or surgery contraindicated) compared with placebo or baseline.

2 Method

2.1 Search strategy

The electronic databases PubMed (1976 to May 2022), Scopus (1998 to May 2022), and Cochrane Library (until May 2022) were searched to identify the studies that assessed the effect of BPs, denosumab, and BPs or denosumab compared with PTX in PHPT patients. The search strategy included various combinations of keywords and Boolean operators. The search terms included “PHPT, bone, BPs”, “PHPT, bone, Denosumab”, “PHPT, bone, alendronate”, “PHPT, bone, zoledronate”, “PHPT, bone, risedronate”, “PHPT, bone, etidronate”, “PHPT, bone, ibandronate”, “PHPT, bone, clodronate”, “PHPT, bone, neridronate”. The PRISMA flow diagram shows the findings of literature search and screening of the studies (Figure 1).

2.2 Inclusion and exclusion criteria

Inclusion criteria were as follows (1): original research and full-text articles published in the English language (2), studies where the PHPT is confirmed by hypercalcemia and elevated PTH levels (3), studies where the bone parameters such as aBMD at any site such as the lumbar spine, femoral neck, total hip or distal radius and BTMs were measured (4), retrospective studies, prospective studies and randomized controlled trials, and (5) sufficient quantitative data (mean± SD/SEM) is presented. The exclusion criteria included (1)

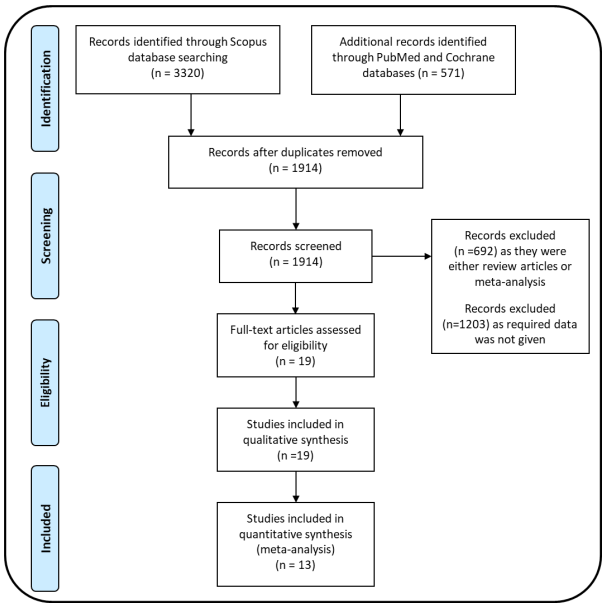

FIGURE 1
PRISMA flow diagram.

studies where the PTX and drug treatment were given simultaneously (2), data represented in median±interquartile range (3), aBMD represented in terms of t-score, z-score because reference value is not given, which can be used to convert t-score to g/cm^2 (4), reviews, case reports, book chapters, and letters to the editor, and (5) articles published in languages other than English. There were no limitations with regard to age and gender.

2.3 Data extraction

Two reviewers (SR and NC) independently assessed the studies for their eligibility. Disagreements with the eligibility of studies were resolved by discussion with all authors. Data were extracted from each article in the numeric form from tables and bar/XY plots using the WebPlotDigitilizer program (13). Data were tabulated from each eligible study for the following parameters: author name, year of publication, number of patients, aBMD at different sites such as the lumbar spine, femoral neck, total hip, and distal radius, serum PTH levels, serum calcium, serum phosphate, serum osteocalcin (OCN), serum bone alkaline phosphatase (BALP), serum CTX-I, age, and gender. Extracted data were transformed from % change in mean to absolute change in mean after treatment using (after treatment – before treatment)/before treatment * 100 = % change. Data extracted from all articles included in the meta-analysis showed in Table 1.

2.4 Outcome assessment

Based on the type of drugs used, studies were categorized into the following groups: BP, denosumab, and PTX. In the present study, bone loss was assessed by aBMD and BTMs (OCN, BALP, and CTX-I). The effect of various drugs in PHPT patients were assessed on serum PTH, calcium, and phosphate levels.

2.5 Quantitative data analysis

Cochrane's Q test determined the degree of heterogeneity among the studies and heterogeneity index (I^2), considering p value < 0.05 as statistically significant. The I^2 value lies between 0 and 100%; I^2> 75% indicates high heterogeneity, I^2> 50% indicates moderate heterogeneity, and I^2< 25% suggests low heterogeneity. Significant heterogeneity favors the use of the random effects model, while low heterogeneity favors the use of the fixed effect model. The comprehensive meta-analysis software (CMA) was used to perform the pooled data analysis.

2.6 Sensitivity analysis

The CMA software was used to determine the degree of sensitivity among these studies. The pooled effect size was determined using single-study exclusion statistics to identify the sensitive studies, the exclusion of which would bring drastic changes to the inference.

2.7 Publication bias analysis

Publication bias was assessed qualitatively and quantitatively using funnel plot and Egger's regression intercept test and Begg and Mazumdar rank correlation test, respectively. Publication bias was adjusted by calculating unbiased estimates using Duval and Tweedie's trim and fill method.

3 Results

3.1 Study characteristics and quality

A literature search for the effect of BPs and denosumab on PHPT-induced bone loss identified 3,891 articles: Scopus (3,320), PubMed (559), and Cochrane library (12). After the removal of duplicates, 1914 articles were screened. Out of these, 1,895 studies were excluded based on the set inclusion/exclusion criteria, and 19 were selected for qualitative analysis. Of the selected studies, 14 studies reported the use of BPs in PHPT patients. Of these, 8 studies were done with alendronate (ALN) (16–20, 23, 24, 27), and one each with etidronate (15), neridronate (14), and risedronate (28). Four studies (27–30) were excluded from this meta-analysis because of the following reasons; the types of BP were not mentioned, data were not presented in the required format, and the treatment duration was five years. Taken together, for ALN trials, 7 studies used only ALN, and one used a combination of BP and PTX for PHPT patients (22).

There were five studies in which denosumab was given to PHPT patients, of which two were excluded because data were not presented in the required format (31, 32). The remaining three studies were included in the meta-analysis (21, 25, 26). There were two studies in which the effect of BPs was compared with PTX (15, 20) and one study in which combination of BP and PTX was compared with PTX (22) in PHPT patients. These three studies were included in determining the effect of PTX on BMD and serum PTH in PHPT patients. In total, 13 studies were finally included for quantitative meta-analysis (Figure 1). A summary of all the results for various parameters is shown in Table 2.

10.3389/fendo.2023.1098841

TABLE 1 Data extracted from all articles included in the meta-analysis.

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
PTH (pMol/L)	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo=15.50 (2.80) ALN=14.40 (5.20)	Placebo=13.49 (3.58) ALN=16.56 (6.24)	Rossini et al. (14)
		EHDP= 9 PTX= 13	EHDP=76.3±5.2/F PTX=76.8±10.1/F	EHDP=8.42 (0.97) PTX=10.15 (1.11)	EHDP=11.88 (1.11) PTX=1.82 (1.32)	Horiuchi et al. (15)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo=10.74 (2.10) ALN= 10.37 (1.84)	Placebo=11.84 (1.52) ALN= 12.199 (2.01)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo=24.20 (15.10) ALN=19.90 (12.40)	Placebo=24.29 (2.86) ALN= 24.28 (2.87)	Chow et al. (17)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo=15.43 (1.41) ALN=17.31 (4.20)	Placebo=15.51 (2.57) ALN=21.12 (6.16)	Khan et al. (18)
		Neridronate IV= 54	Neridronate IV=64±8/F	Neridronate IV=16.60 (11)	Neridronate IV=18.04 (11.69)	Rossini et al. (14)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	Placebo=17.1 (6.50) ALN= 11.8 (3.35)	Placebo=18.1 (5.75) ALN=14 (4.20)	Akbaba et al. (19)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN=14.98 (6.24) PTX=23.89 (21.66)	ALN=16.36 (6.84) PTX=4.65 (2.40)	Szymczak et al. (20)
		Placebo= 15 ALN= 15	Placebo=57±5/F ALN=59±5/F	Placebo=10.60 (1.30) ALN=11.20 (2.10)	Placebo=11 (1.40) ALN=11 (1.30)	Cesareo et al. (19)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Placebo=11.16 (6.66) Denosumab=12.95 (9.92)	Placebo=10.97 (9.33) Denosumab=13.51 (8.00)	Leere et al. (21)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 24.37 (20.39)	PTX= 4.37 (3.34)	Choe et al. (22)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo=15.50 (2.80) ALN= 14.40 (5.20)	Placebo=16.34 (3.28) ALN= 16.27 (6.71)	Rossini et al. (23)
		Placebo= 18 ALN= 14 After 24 months Placebo= 13 ALN= 10	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo=10.74 (2.10) ALN=10.37 (1.84)	Placebo=12.45 (1.94) ALN=11.93 (1.69)	Parker et al. (16)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo=15.43 (1.41) ALN=17.31 (4.20)	ALN=15.67 (3.12)	Khan et al. (18)
		Neridronate IV= 54	Neridronate IV=64±8/F	Neridronate IV=16.60 (11)	Neridronate IV=26.30 (11.98)	Rossini et al. (14)
Calcium (mMol/L)	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 2.73 (0.08) ALN= 2.75 (0.10)	Placebo= 2.70 (0.08) ALN= 2.70 (0.10)	Rossini et al. (23)
		EHDP= 9 PTX= 13	EHDP=76.3±5.2/F PTX=76.8±10.1/F	EHDP= 2.71 (0.13) PTX= 2.76 (0.18)	EHDP= 2.58 (0.13) PTX= 2.39 (0.13)	Horiuchi et al. (15)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 2.82 (0.08) ALN= 2.84 (0.07)	Placebo= 2.78 (0.08) ALN=2.87 (0.07)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo= 2.81 (0.16) ALN= 2.82 (0.18)	Placebo= 2.83 (0.04) ALN=2.75 (0.05)	Chow et al. (17)
		Placebo= 19 ALN= 18				Khan et al. (18)

(Continued)

TABLE 1 Continued

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
			Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 2.64 (0.03) ALN= 2.68 (0.03)	Placebo= 2.67 (0.05) ALN= 2.64 (0.02)	
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 2.68 (0.15)	Neridronate IV = 2.64 (0.15)	Rossini et al. (14)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	Placebo= 2.78 (0.13) ALN= 2.80 (0.14)	Placebo= 2.75 (0.16) ALN= 2.68 (0.11)	Akbaba et al. (19)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Placebo= 2.71 (0.15) Denosumab= 2.72 (0.36)	Placebo= 2.72 (0.12) Denosumab= 2.68 (0.24)	Leere et al. (21)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 2.80 (0.60)	PTX=2.25 (0.15)	Choe et al. (22)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 2.73 (0.08) ALN= 2.75 (0.10)	Placebo= 2.73 (0.08) ALN= 2.77 (0.10)	Rossini et al. (23)
		Placebo= 18 ALN= 14 After 24 months Placebo= 13 ALN= 10	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 2.82 (0.08) ALN= 2.84 (0.07)	Placebo= 2.69 (0.07) ALN=2.89 (0.03)	Parker et al. (16)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 2.64 (0.03) ALN= 2.68 (0.03)	ALN= 2.62 (0.04)	Khan et al. (18)
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 2.68 (0.15)	Neridronate IV = 2.68 (0.15)	Rossini et al. (14)
Phosphate (mg/dL)	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 2.60 (0.50) ALN= 2.90 (0.60)	Placebo= 2.69 (0.62) ALN= 2.91 (0.66)	Rossini et al. (23)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 2.35 (0.13) ALN= 2.26 (0.23)	Placebo= 2.23 (0.13) ALN= 1.92 (0.23)	Parker et al. (16)
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 2.6 (0.4)	Neridronate IV = 2.48 (0.40)	Rossini et al. (14)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	Placebo= 2.70 (0.20) ALN=2.80 (0.45)	Placebo= 2.60 (0.35) ALN= 2.50 (0.18)	Akbaba et al. (19)
		Placebo= 15 ALN= 15	Placebo=57±5/F ALN=59±5/F	Placebo= 3.90 (0.30) ALN= 3.80 (0.20)	Placebo= 3.70 (0.40) ALN= 3.80 (0.30)	Cesareo et al. (24)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Placebo= 2.45 (0.09) Denosumab= 2.38 (0.74)	Placebo= 2.57 (0.15) Denosumab= 2.29 (0.87)	Leere et al. (21)
		Denosumab= 19	Denosumab=71.8 ± 7.1/ M=2, F=17	Denosumab= 3.20 (0.50)	Denosumab= 3 (0.60)	Miyaoka et al. (25)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 2.5 (0.7)	PTX= 3.2 (0.5)	Choe et al. (22)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 2.60 (0.50) ALN= 2.90 (0.60)	Placebo= 2.61 (0.62) ALN= 2.94 (0.66)	Rossini et al. (23)
		Placebo= 18 ALN= 14				Parker et al. (16)

(Continued)

10.3389/fendo.2023.1098841

TABLE 1 Continued

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
		After 24 months Placebo= 13 ALN= 10	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 2.35 (0.13) ALN= 2.26 (0.23)	Placebo= 2.20 (0.11) ALN= 2.35 (0.20)	
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 2.6 (0.4)	Neridronate IV = 2.57 (0.40)	Rossini et al. (14)
Osteocalcin (ng/mL)	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 3.60 (1.60) ALN= 4.10 (1.30)	Placebo= 3.67 (1.67) ALN= 2.81 (1.35)	Rossini et al. (23)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 9.52 (1.40) ALN= 6.98 (1.40)	Placebo= 7.38 (1.53) ALN= 4.06 (1.44)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo= 43.60 (28.5) ALN= 53 (28.90)	Placebo= 46.59 (7.86) ALN= 26.96 (4.32)	Chow et al. (17)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 31 (13.90) PTX= 61.90 (75.70)	ALN= 26.05 (11.40) PTX= 16.50 (5.80)	Szymczak et al. (20)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 3.60 (1.60) ALN= 4.10 (1.30)	Placebo= 3.45 (1.68) ALN= 2.65 (1.36)	Rossini et al. (23)
		Placebo= 18 ALN= 14 After 24 months Placebo= 13 ALN= 10	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 9.52 (1.40) ALN= 6.98 (1.40)	Placebo= 8.01 (1.04) ALN= 6.44 (1.26)	Parker et al. (16)
BALP(U/L)	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 43 (9) ALN= 42 (12)	Placebo= 45 (9.45) ALN= 26.57 (12.5)	Rossini et al. (14)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 136.53 (22.32) ALN= 147.59 (40.97)	Placebo= 113.38 (22.27) ALN= 58.08 (39.18)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo= 21.80 (15.9) ALN= 21.10 (12.8)	Placebo= 23.38 (3.23) ALN= 7.26 (0.96)	Chow et al. (17)
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 35 (14)	Neridronate IV = 25.86 (14.67)	Rossini et al. (14)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 43 (9) ALN= 42 (12)	Placebo= 43.69 (9.43) ALN= 25.42 (12.5)	Rossini et al. (23)
		Placebo= 18 ALN= 14 After 24 months Placebo= 13 ALN= 10	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 136.53 (22.32) ALN= 147.59 (40.97)	Placebo= 123.17 (11.57) ALN= 80.95 (30)	Parker et al. (16)
		Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 35 (14)	Neridronate IV = 29.18 (14.74)	Rossini et al. (14)
CTX-I (ng/mL)	6 months	Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 0.74 (0.39)	Neridronate IV = 0.47 (0.42)	Rossini et al. (14)
		Placebo= 15 ALN= 15	Placebo=57±5/F ALN=59±5/F			Cesareo et al. (24)

(Continued)

TABLE 1 Continued

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
				Placebo= 0.70 (0.10) ALN= 0.60 (0.10)	Placebo= 0.69 (0.10) ALN= 0.29 (0.05)	
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 4.90 (2.03) PTX= 5.77 (4.5)	ALN= 4.55 (1.74) PTX= 3.58 (1.19)	Szymczak et al. (20)
	12 months	Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 0.74 (0.39)	Neridronate IV = 0.45 (0.42)	Rossini et al. (14)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 4.90 (2.03) PTX= 5.77 (4.5)	ALN= 4.21 (1.58) PTX= 3.70 (1.68)	Szymczak et al. (20)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 1.08 (0.91)	PTX= 0.20 (0.14)	Choe et al. (22)
	24 months	Neridronate IV = 54	Neridronate IV=64±8/F	Neridronate IV = 0.74 (0.39)	Neridronate IV = 0.38 (0.41)	Rossini et al. (14)
BMD (g/cm^2) Whole body	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.82 (0.10) ALN= 0.82 (0.06)	Placebo= 0.81 (0.10) ALN= 0.83 (0.06)	Rossini et al. (23)
		EHDP= 9 PTX= 13	EHDP=76.3±5.2/F PTX=76.8±10.1/F	EHDP= 1.41 (0.23) PTX= 1.52 (0.26)	EHDP= 1.53 (0.20) PTX= 1.57 (0.32)	Horiuchi et al. (15)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 1.00 (0.10) PTX= 0.92 (0.10)	ALN= 1.02 (0.12) PTX= 0.962 (0.1)	Szymczak et al. (20)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.82 (0.10) ALN= 0.82 (0.06)	Placebo= 0.80 (0.09) ALN= 0.83 (0.06)	Rossini et al. (23)
Lumbar spine	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.73 (0.07) ALN= 0.70 (0.09)	Placebo= 0.74 (0.07) ALN= 0.75 (0.09)	Rossini et al. (23)
		EHDP= 9 PTX= 13	EHDP=76.3±5.2/F PTX=76.8±10.1/F	EHDP= 0.62 (0.11) PTX= 0.69 (0.21)	EHDP= 0.68 (0.15) PTX= 0.83 (0.23)	Horiuchi et al. (15)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 0.92 (0.08) ALN= 0.76 (0.07)	Placebo= 0.95 (0.04) ALN= 0.81 (0.11)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo= 0.71 (0.15) ALN= 0.71 (0.12)	Placebo= 0.71 (0.15) ALN= 0.74 (0.12)	Chow et al. (17)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.83 (0.13) ALN= 0.76 (0.11)	Placebo= 0.83 (0.14) ALN= 0.80 (0.12)	Khan et al. (18)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	ALN= 0.57 (0.13)	ALN= 0.61 (0.12)	Akbaba et al. (19)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 1.02 (0.17) PTX= 0.93 (0.20)	ALN= 1.04 (0.22) PTX= 0.99 (0.15)	Szymczak et al. (20)
		Denosumab= 7	Denosumab= 69.8 (range 62 – 81)/ Gender not given	Denosumab= 0.79 (0.11) (6 months)	Denosumab= 0.82 (0.13)	Grigorie et al. (26)

(Continued)

TABLE 1 Continued

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
		Placebo= 15 ALN= 15	Placebo=57±5/F ALN=59±5/F	Placebo= 0.77 (0.07) ALN= 0.78 (0.07)	Placebo= 0.76 (0.07) ALN= 0.81 (0.07)	Cesareo et al. (24)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Denosumab= 0.83 (0.02)	Denosumab= 0.88 (0.12)	Leere et al. (21)
		Denosumab= 19	Denosumab=71.8 ± 7.1/ M=2, F=17	Denosumab= 0.73 (0.03)	Denosumab= 0.77 (0.03)	Miyaoka et al. (25)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 0.76 (0.08)	PTX= 0.89 (0.12)	Choe et al. (22)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.73 (0.07) ALN= 0.70 (0.09)	Placebo= 0.73 (0.07) ALN= 0.76 (0.09)	Rossini et al. (23)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 0.92 (0.08) ALN= 0.76 (0.07)	Placebo= 0.96 (0.08) ALN= 0.85 (0.07)	Parker et al. (16)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.83 (0.13) ALN= 0.76 (0.11)	ALN= 0.81 (0.12)	Khan et al. (18)
Femoral neck	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.55 (0.04) ALN= 0.58 (0.06)	Placebo= 0.55 (0.04) ALN= 0.59 (0.06)	Rossini et al. (23)
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 0.70 (0.04) ALN= 0.52 (0.07)	Placebo= 0.69 (0.08) ALN= 0.54 (0.04)	Parker et al. (16)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo= 0.54 (0.12) ALN= 0.54 (0.11)	Placebo= 0.54 (0.12) ALN= 0.54 (0.11)	Chow et al. (17)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.62 (0.12) ALN= 0.59 (0.10)	Placebo= 0.61 (0.12) ALN= 0.61 (0.10)	Khan et al. (18)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	ALN= 0.74 (0.1)	ALN= 0.77 (0.08)	Akbaba et al. (19)
		ALN= 33 PTX= 33	F=60, M=3 54 post-menopausal, 6 pre-menopausal	ALN= 0.81 (0.12) PTX= 0.75 (0.10)	ALN= 0.83 (0.13) PTX= 0.79 (0.11)	Szymczak et al. (20)
		Denosumab= 7	Denosumab= 69.8 (range 62 – 81)/ Gender not given	Denosumab= 0.68 (0.07)	Denosumab= 0.69 (0.06)	Grigorie et al. (26)
		Placebo= 15 ALN= 15	Placebo=57±5/F ALN=59±5/F	Placebo= 0.64 (0.08) ALN= 0.62 (0.10)	Placebo= 0.63 (0.08) ALN= 0.64 (0.09)	Cesareo et al. (24)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Denosumab= 0.64 (0.08)	Denosumab= 0.67 (0.11)	Leere et al. (21)
		Denosumab= 19	Denosumab=71.8 ± 7.1/ M=2, F=17	Denosumab= 0.51 (0.02)	Denosumab= 0.53 (0.02)	Miyaoka et al. (25)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 0.68 (0.10)	PTX= 0.77 (0.12)	Choe et al. (22)
	24 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.55 (0.04) ALN= 0.58 (0.06)	Placebo= 0.55 (0.04) ALN= 0.59 (0.06)	Rossini et al. (23)

(Continued)

TABLE 1 Continued

Parameters	Duration of drug administration	Number of patients	Age (Mean±SD) years/ Gender	Baseline Mean (SD)	After drug administration Mean (SD)	Reference
		Placebo= 18 ALN= 14	Placebo= 63.4± 2.02/ ALN= 69.6± 2.91/ F=27 & M=5	Placebo= 0.70 (0.04) ALN= 0.52 (0.07)	Placebo= 0.69 (0.04) ALN= 0.55 (0.07)	Parker et al. (16)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.62 (0.12) ALN= 0.59 (0.10)	ALN= 0.62 (0.10)	Khan et al. (18)
Total hip	12 months	Placebo= 13 ALN= 13	Placebo=74±4/ F ALN=72±5/F	Placebo= 0.57 (0.08) ALN= 0.61 (0.07)	Placebo= 0.56 (0.08) ALN= 0.63 (0.07)	Rossini et al. (23)
		Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.72 (0.14) ALN= 0.67 (0.14)	Placebo= 0.72 (0.14) ALN= 0.70 (0.14)	Khan et al. (18)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Denosumab= 0.78 (0.08)	Denosumab= 0.82 (0.11)	Leere et al. (21)
		Denosumab= 19	Denosumab=71.8 ± 7.1/ M=2, F=17	Denosumab= 0.63 (0.02)	Denosumab= 0.64 (0.03)	Miyaoka et al. (25)
		PTX= 24	PTX= 61.4±9.8/ M=7, F=17	PTX= 0.71 (0.12)	PTX= 0.80 (0.13)	Choe et al. (22)
		Placebo= 20 ALN= 20	Placebo= 71.8± 8.8/F ALN= 68.2± 9.7/F	Placebo=0.49 (0.09) ALN=0.47 (0.08)	Placebo= 0.49 (0.09) ALN= 0.47 (0.08)	Chow et al. (17)
Distal radius (1/3)	12 months	Placebo= 19 ALN= 18	Placebo=70.09±10.36/M=6, F=13 ALN=63.73±9.36/ M=3, F=15	Placebo= 0.55 (0.12) ALN= 0.52 (0.11)	Placebo= 0.55 (0.12) ALN= 0.52 (0.12)	Khan et al. (18)
		Placebo= 10 ALN= 12	Placebo=63.2±8.3/F ALN=69.4±6.3/F	ALN= 0.72 (0.18)	ALN= 0.69 (0.61)	Akbaba et al. (19)
		Placebo= 15 Denosumab= 16	Placebo=68·0 ±1·8/ M=3, F=12 Denosumab= 65·4±2·2/ M=3, F=13	Denosumab= 0.57 (0.08)	Denosumab= 0.58 (0.10)	Leere et al. (21)

F, female; M, male; ALN, alendronate; EHDP, etidronate; PTX, parathyroidectomy.

The majority of the studies used baseline control, although some of the ALN studies used placebo control. Therefore, baseline and placebo control were used separately in this meta-analysis.

3.2 Effects of anti-resorptive drugs on aBMD and biochemical parameters compared with baseline

We pooled data from the studies that used BPs or denosumab to determine the overall effect of anti-resorptive therapies on aBMD and serum parameters. Ten datasets from as many studies were available for lumbar spine aBMD before and 12 months after BP or denosumab use. The fixed effect model was used for drawing inference because there was no significant heterogeneity between the studies (I^2=0.000, Q=8.880, p=0.448). Pooled analysis showed a significant increase in the mean lumbar spine aBMD after drug administration compared with baseline (SDM=0.447, 95% CI=0.230 to 0.664, p=0.0001) (Figure 2A).

Nine datasets from as many studies were available for femur neck aBMD before and 12 months after drug administration. No significant heterogeneity was observed among these studies (I^2=0.000, Q=5.748, p=0.675), suggesting the use of fixed effect model. Pooled analysis showed that the mean femoral neck aBMD was significantly increased after 12 months of drug administration compared with baseline (SDM=0.270, 95% CI=0.049 to 0.491, p=0.017) (Figure 2B).

Four datasets from as many studies were available for total hip aBMD before and 12 months after BP or denosumab use. No significant heterogeneity was found among these studies (I^2=0.000, Q=0.261, p=0.967), suggesting the use of fixed effect model for drawing inference. Pooled analysis showed no significant change in mean total hip aBMD after drug administration compared with baseline (SDM=0.330, 95% CI=-0.014 to 0.673, p=0.060) (Supplementary Figure 1A).

Four datasets from as many studies were available for aBMD of distal radius before and after 12 months of BP or denosumab use. No significant heterogeneity was observed among these studies (I^2=0.000, Q=0.097, p=0.992), suggesting the use of fixed effect model for drawing a conclusion. There was no change in the mean distal radius aBMD after 12 months of drug use compared with baseline (SDM=0.042, 95% CI=-0.300 to 0.383, p=0.810) (Supplementary Figure 1B).

Ten datasets from as many studies were available for serum PTH levels before and 12 months after drug administration, including BP or denosumab. Significant heterogeneity was observed among these studies (I^2=63.413, Q=24.599, p=0.003), which suggested using the

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TABLE 2 The summary of pooled analysis for various parameters.

Groups	Parameters	Heterogeneity analysis			Test model	Effect size			p-value	Conclusion
						SDM	95% CI			
		Q	p-value	I^2			Lower limit	Upper limit		
12 months ALN use compared with Placebo	Lumbar spine BMD	1.892	0.756	0.000	**Fixed**	**0.350**	**0.041**	**0.659**	**0.027**	Significant
					Random	0.350	0.041	0.659	0.027	
	Femur neck BMD	2.538	0.638	0.000	**Fixed**	**0.250**	**-0.058**	**0.558**	**0.111**	Non-significant
					Random	0.250	-0.058	0.558	0.111	
	PTH	11.574	0.041	56.799	Fixed	0.612	0.313	0.911	0.0001	Significant
					Random	**0.602**	**0.145**	**1.059**	**0.010**	
	Calcium (Ca)	32.902	0.000	87.843	Fixed	-0.414	-0.749	-0.079	0.015	Non-significant
					Random	**-0.381**	**-1.345**	**0.583**	**0.439**	
	Phosphate	12.232	0.007	75.474	Fixed	-0.338	-0.727	0.050	0.088	Non-significant
					Random	**-0.369**	**-1.156**	**0.418**	**0.358**	
	OCN	35.212	0.000	94.320	Fixed	-1.346	-1.831	-0.860	0.0001	Non-significant
					Random	**-1.947**	**-4.064**	**0.170**	**0.072**	
	BALP	31.008	0.000	93.550	Fixed	-2.552	-3.134	-1.970	0.0001	Significant
					Random	**-3.422**	**-5.844**	**-1.000**	**0.006**	
12 months anti-resorptives use compared with baseline	Lumbar spine BMD	8.880	0.448	0.000	**Fixed**	**0.447**	**0.230**	**0.664**	**0.0001**	Significant
					Random	0.447	0.230	0.664	0.0001	
	Femur neck BMD	5.748	0.675	0.000	**Fixed**	**0.270**	**0.049**	**0.491**	**0.017**	Significant
					Random	0.270	0.049	0.491	0.017	
	Total hip BMD	0.261	0.967	0.000	**Fixed**	**0.330**	**-0.014**	**0.673**	**0.060**	Non-Significant
					Random	0.330	-0.014	0.673	0.060	
	Distal radius BMD	0.097	0.992	0.000	**Fixed**	**0.042**	**-0.300**	**0.383**	**0.810**	Non-significant
					Random	0.042	-0.300	0.383	0.810	
	PTH	24.599	0.003	63.413	Fixed	0.363	0.165	0.562	0.0001	Significant
					Random	**0.489**	**0.139**	**0.839**	**0.006**	
	Calcium	18.785	0.009	62.736	Fixed	-0.471	-0.696	-0.245	0.0001	Significant
					Random	**-0.545**	**-0.937**	**-0.154**	**0.006**	
	Phosphate	10.834	0.094	44.617	**Fixed**	**-0.357**	**-0.594**	**-0.120**	**0.003**	Significant
					Random	-0.393	-0.733	-0.054	0.023	
12 months BP use compared with baseline	Total BMD	0.506	0.776	0.000	**Fixed**	**0.235**	**-0.141**	**0.610**	**0.221**	Non-significant
					Random	0.235	-0.141	0.610	0.221	
	Lumbar spine BMD	1.774	0.971	0.000	**Fixed**	**0.330**	**0.088**	**0.571**	**0.007**	Significant
					Random	0.330	0.088	0.571	0.007	
	Femur neck	0.622	0.996	0.000	**Fixed**	**0.170**	**-0.079**	**0.418**	**0.181**	Non-significant
					Random	0.170	-0.079	0.418	0.181	

(Continued)

TABLE 2 Continued

Groups	Parameters	Heterogeneity analysis			Test model	Effect size			p-value	Conclusion
						SDM	95% CI			
		Q	p-value	I^2			Lower limit	Upper limit		
	PTH	23.808	0.002	66.398	Fixed	0.390	0.183	0.598	0.0001	Significant
					Random	**0.546**	**0.162**	**0.930**	**0.005**	
	Calcium	17.753	0.007	66.203	Fixed	-0.511	-0.749	-0.273	0.0001	Significant
					Random	**-0.608**	**-1.048**	**-0.169**	**0.007**	
	Phosphate	10.296	0.036	61.148	Fixed	-0.394	-0.668	-0.120	0.005	Non-significant
					Random	**-0.478**	**-0.969**	**0.012**	**0.056**	
	Osteocalcin	10.967	0.012	72.645	Fixed	-0.901	-1.234	-0.568	0.0001	Significant
					Random	**-1.097**	**-1.774**	**-0.420**	**0.001**	
	BALP	12.372	0.006	75.751	Fixed	-1.035	-1.333	-0.736	0.0001	Significant
					Random	**-1.339**	**-2.035**	**-0.643**	**0.0001**	
	CTX-I (6months)	30.992	0.000	93.547	Fixed	-0.677	-0.971	-0.383	0.0001	Significant
					Random	**-1.417**	**-2.741**	**-0.092**	**0.036**	
Denosumab use compared with baseline	Lumbar spine BMD	3.526	0.172	43.283	**Fixed**	**0.828**	**0.378**	**1.278**	**0.0001**	Significant
					Random	0.793	0.179	1.407	0.011	
	Femur neck BMD	2.664	0.264	24.937	**Fixed**	**0.575**	**0.135**	**1.015**	**0.010**	Significant
					Random	0.559	0.042	1.077	0.034	
Parathyroidectomy compared with baseline	Lumbar spine BMD	4.550	0.103	56.045	**Fixed**	**0.662**	**0.319**	**1.005**	**0.0001**	Significant
					Random	0.700	0.162	1.238	0.011	
	PTH	28.090	0.000	92.880	Fixed	-1.513	-1.909	-1.117	0.0001	Significant
					Random	**-2.723**	**-4.466**	**-0.980**	**0.002**	
12 months ALN use compared with baseline	Lumbar spine BMD	1.701	0.945	0.000	**Fixed**	**0.321**	**0.071**	**0.571**	**0.012**	Significant
					Random	0.321	0.071	0.571	0.012	
	Femur neck BMD	0.622	0.996	0.000	**Fixed**	**0.170**	**-0.079**	**0.418**	**0.181**	Non-significant
					Random	0.170	-0.079	0.418	0.181	
	Distal radius	0.048	0.976	0.000	**Fixed**	**0.020**	**-0.372**	**0.412**	**0.921**	Non-significant
					Random	0.020	-0.372	0.412	0.921	
	PTH	5.620	0.467	0.000	**Fixed**	**0.416**	**0.164**	**0.668**	**0.001**	Significant
					Random	0.416	0.164	0.668	0.001	
	Calcium	14.201	0.007	71.833	Fixed	-0.618	-0.949	-0.287	0.0001	Significant
					Random	**-0.632**	**-1.261**	**-0.004**	**0.049**	
	Phosphate	9.732	0.021	69.175	Fixed	-0.504	-0.899	-0.108	0.013	Non-significant
					Random	**-0.567**	**-1.283**	**0.150**	**0.121**	
	Osteocalcin	10.967	0.012	72.645	Fixed	-0.901	-1.234	-0.568	0.0001	Significant

(Continued)

TABLE 2 Continued

Groups	Parameters	Heterogeneity analysis			Test model	Effect size			p-value	Conclusion
						SDM	95% CI			
		Q	p-value	I^2			Lower limit	Upper limit		
					Random	**-1.097**	**-1.774**	**-0.420**	**0.001**	
	BALP	2.394	0.302	16.475	**Fixed**	**-1.617**	**-2.086**	**-1.149**	**0.0001**	Significant
					Random	-1.625	-2.142	-1.109	0.0001	
24 months ALN use compared with baseline	Lumbar spine BMD	2.057	0.358	2.766	**Fixed**	**0.724**	**0.295**	**1.153**	**0.001**	Significant
					Random	0.726	0.290	1.161	0.001	
	Femur neck BMD	0.164	0.922	0.000	**Fixed**	**0.274**	**-0.141**	**0.690**	**0.195**	Non-significant
					Random	0.274	-0.141	0.690	0.195	
	PTH	6.091	0.048	67.163	Fixed	0.136	-0.296	0.569	0.537	Non-Significant
					Random	**0.213**	**-0.551**	**0.977**	**0.585**	
	Calcium	21.064	0.000	90.505	Fixed	-0.311	-0.767	0.145	0.181	Non-significant
					Random	**-0.245**	**-1.728**	**1.237**	**0.746**	

Bold values were used for drawing conclusion.

random effects model to draw a conclusion. Pooled analysis showed a significant increase in mean PTH levels after drug administration compared with baseline (SDM=0.489, 95% CI=0.139 to 0.839, p=0.006) (Figure 2C). The funnel plot showed an asymmetric distribution of studies, suggesting the presence of publication bias (Egger's regression intercept=3.618; p=0.018). So, we used trim and fill analysis to compute unbiased estimates and adjusted the values (SDM= 0.228, 95% CI=-0.172 to 0.628).

Eight datasets from as many studies were available for serum calcium levels before and 12 months after drug administration. The random effects model was used in the pooled analysis to draw a conclusion because significant heterogeneity was found in these studies (I^2=62.736, Q=18.785, p=0.009). Pooled analysis showed a significant decrease in mean serum calcium levels after drug administration compared with baseline (SDM=-0.545, 95% CI=-0.937 to -0.154, p=0.006) (Figure 2D).

Seven datasets from as many studies were available for serum phosphate levels before and 12 months after drug use. No significant heterogeneity was observed among these studies (I^2=44.617, Q=10.834, p=0.094), suggesting the use of fixed effect model for drawing inference. The pooled analysis showed that mean serum phosphate levels were significantly decreased after drug administration (SDM=-0.357, 95% CI=-0.594 to -0.120, p=0.003) (Supplementary Figure 1C).

3.3 Effects of BPs on aBMD and biochemical parameters compared with baseline

In the previous section, we found that anti-resorptive therapies improved aBMD at many sites while decreasing serum calcium level in PHPT patients. Here, we analyzed the effect of only BPs on aBMD and biochemical parameters. Three datasets from as many studies were available for total aBMD before and 12 months after BP use. Heterogeneity was not significant between these studies (I^2=0.000, Q=0.506, p=0.776), suggesting the use of fixed effect model for drawing inference. There was no significant change in the mean total aBMD after BP administration compared with baseline (SDM=0.235, 95% CI=-0.141 to 0.610, p=0.221) (Supplementary Figure 2A).

Eight datasets from as many studies were available for the lumbar spine aBMD before and 12 months after BP use. No significant heterogeneity was observed between these studies (I^2=0.000, Q=1.774, p=0.971), suggesting the use of fixed effect model for drawing a conclusion. The mean lumbar spine aBMD was significantly increased after BP administration compared with baseline (SDM=0.330, 95% CI=0.088 to 0.571, p=0.007) (Figure 3A). Studies were distributed asymmetrically in the funnel plot (Egger's regression intercept= 2.018, p= 0.009), suggesting the presence of publication bias. So, we used the adjusted values according to the trim and fill method for unbiased estimates (SDM=0.240, 95% CI=0.029 to 0.451).

Seven datasets from as many studies were available for femur neck aBMD before and 12 months after BP use. No significant heterogeneity was observed between these studies (I^2=0.000, Q=0.622, p=0.996), suggesting the use of fixed effect model for data analysis. Pooled analysis indicated no significant change in mean femur neck aBMD after BP use (SDM=0.170, 95% CI=-0.079 to 0.418, p=0.181) (Supplementary Figure 2B).

Nine datasets from as many studies were available for serum PTH levels before and 12-months after BP use. Significant heterogeneity was found between these studies (I^2=66.398, Q=23.808, p=0.002), suggesting that the random effects model should be used for drawing inference. Mean serum PTH levels were significantly increased after BP therapy in PHPT patients (SDM=0.546, 95% CI=0.162 to 0.930,

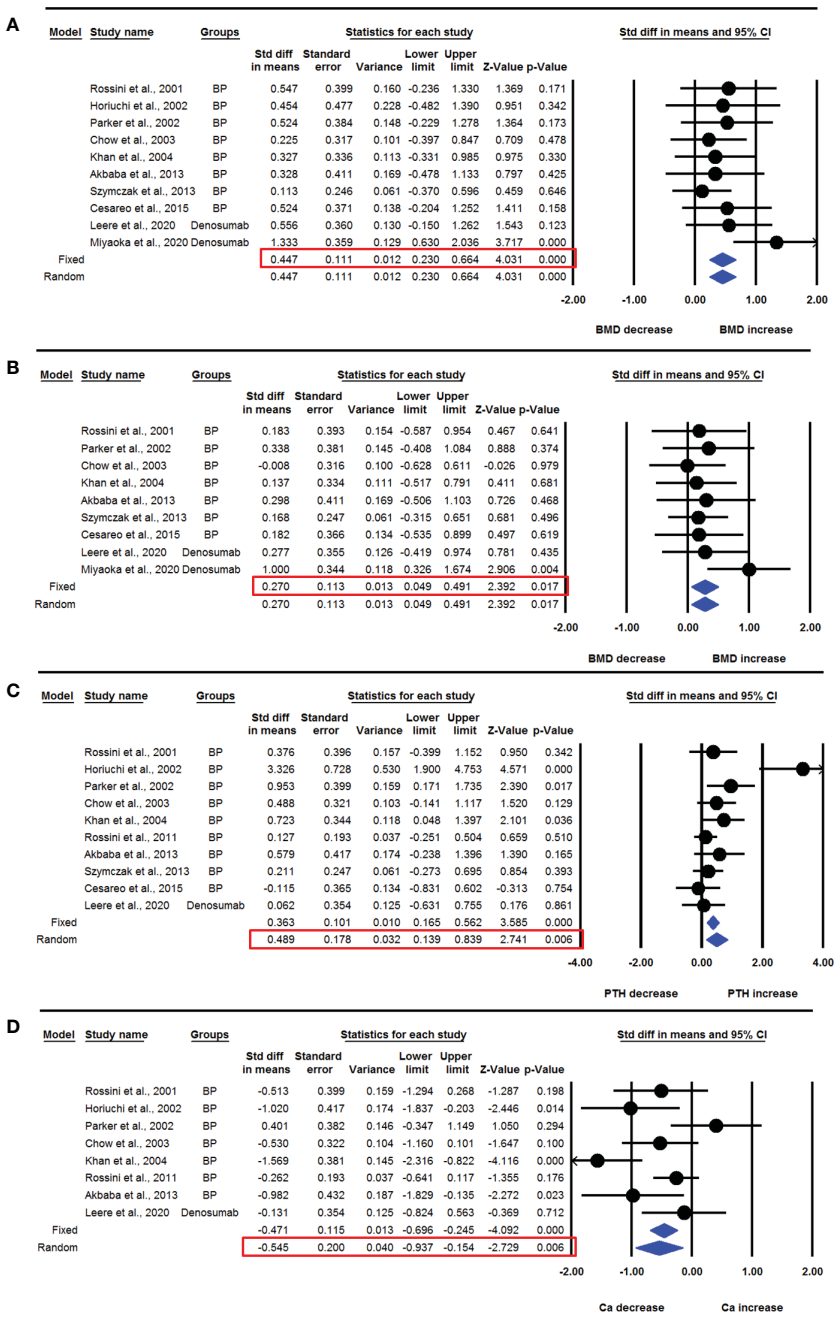

FIGURE 2
The effect of anti-resorptive drug administration on various parameters in PHPT patients compared with baseline; **(A)** lumbar spine aBMD, **(B)** femoral neck aBMD, **(C)** serum PTH, and **(D)** serum calcium (Ca).

p=0.005) (Figure 3B). The funnel plot showed an asymmetric distribution of studies (Egger's regression intercept=3.832, p=0.016), suggesting the presence of publication bias. We used trim and fill analysis to compute unbiased estimates and adjusted the values (SDM= 0.250, 95% CI= -0.181 to 0.681).

Seven datasets from as many studies were available for serum calcium levels before and 12-months after BP use. The random effects model was used for drawing conclusion because significant heterogeneity was observed in these studies (I^2=66.203, Q=17.753, p=0.007). Pooled analysis showed that the mean serum calcium level was significantly decreased after BP administration compared with baseline (SDM=-0.608, 95% CI=-1.048 to -0.169, p=0.007) (Figure 3C).

Five datasets from as many studies were available for serum phosphate levels before and 12-months after BP use. Significant heterogeneity was found in these studies (I^2=61.148, Q=10.296, p=0.036), resulting in the use of the random effects model for data analysis. The pooled analysis showed no significant change in serum phosphate levels after BP administration (SDM=-0.478, 95% CI=-0.969 to -0.012, p=0.056) (Supplementary Figure 2C).

Four datasets from as many studies were available for serum OCN levels before and 12 months after BP use. I^2 values showed that heterogeneity was significant in these studies (I^2=72.645, Q=10.967, p=0.012), which suggested that the random effects model should be used for drawing a conclusion. Pooled analysis showed that the mean

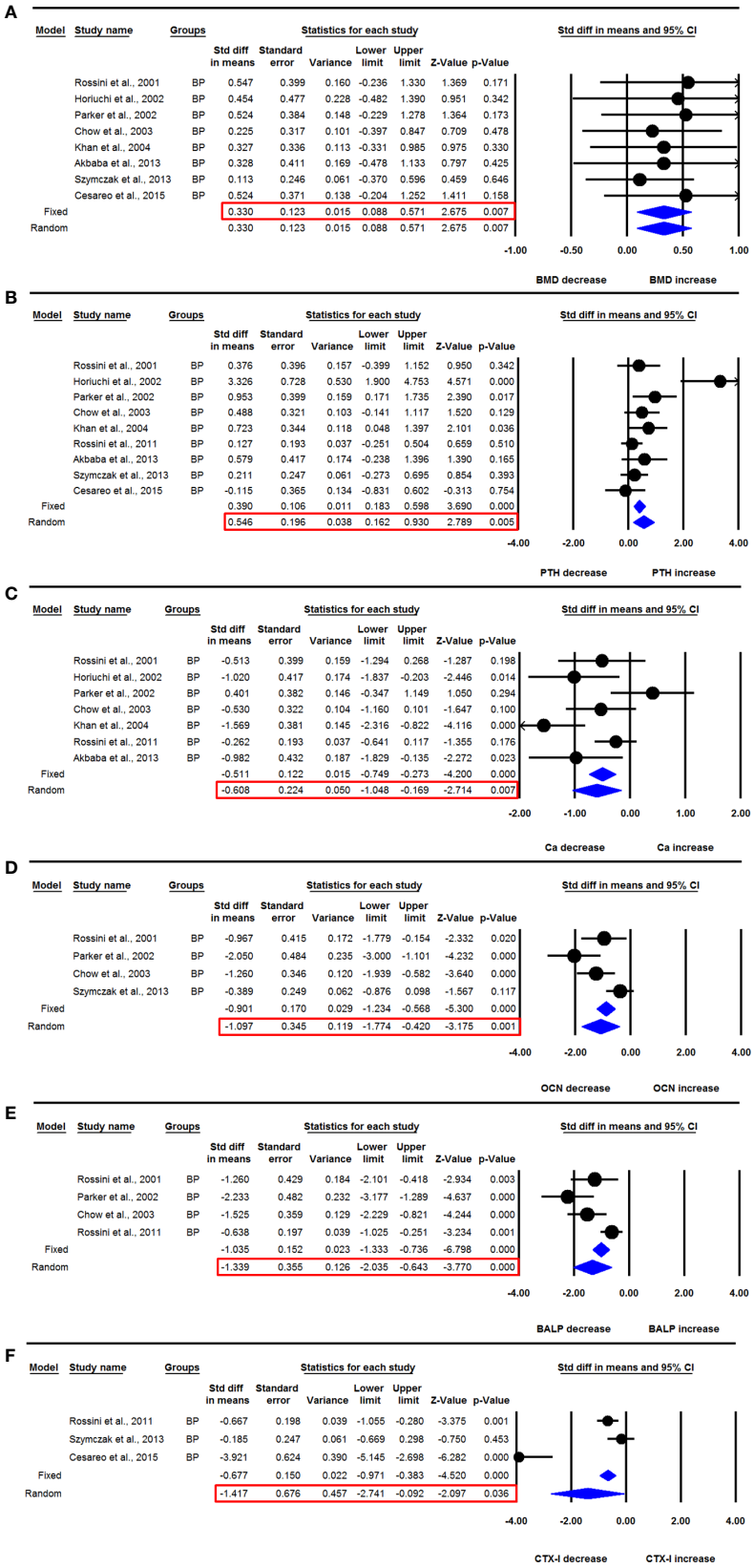

FIGURE 3
The effect of BP administration on various parameters in PHPT patients compared with baseline; **(A)** lumbar spine aBMD, **(B)** serum PTH, **(C)** serum calcium (Ca), **(D)** serum OCN, **(E)** serum BALP, and **(F)** serum CTX-I.

serum OCN levels were significantly decreased after BP use (SDM= -1.097, 95% CI=-1.774 to -0.420, p=0.001) (Figure 3D).

Four datasets from as many studies were available for serum BALP levels before and 12 months after BP use. There was significant heterogeneity between the studies (I^2=75.751, Q=12.372, p=0.006), suggesting the use of random effects model for drawing inference. The mean serum BALP level was significantly reduced after BP administration compared with baseline (SDM=-1.339, 95% CI= -2.035 to -0.643, p=0.0001) (Figure 3E).

Three datasets from as many studies were available for serum CTX-1 before and 6 months after BP use. Significant heterogeneity was observed in these studies (I^2=93.547, Q=30.992, p=0.000), suggesting the use of random effects model for data analysis. The mean serum CTX-1 level was significantly decreased after BP administration compared with baseline (SDM=-1.417, 95% CI=-2.741 to -0.092, p=0.036) (Figure 3F).

3.4 Effects of ALN on aBMD and biochemical parameters

3.4.1 Comparison with the baseline values

In the previous section, we found that BP administration significantly improved aBMD, and decreased serum calcium as well as BTMs. Here, we analyzed the effect of ALN on PHPT patients because a sufficient number of studies was available to perform a meta-analysis.

Seven datasets from as many studies were available for lumbar spine aBMD before and 12 months after ALN use. The heterogeneity between these studies was not significant (I^2=0.000, Q=1.701, p=0.945), suggesting the use of the fixed effect model for drawing inferences. Pooled analysis showed a significant increase in the mean lumbar spine aBMD after ALN administration compared with baseline (SDM=0.321, 95% CI=0.071 to 0.571, p=0.012) (Figure 4A). The funnel plot showed an asymmetric distribution of studies (Egger's regression intercept=2.455, p=0.009), suggesting the presence of publication bias. Unbiased estimates were used from the trim and fill method, and the values were adjusted (SDM= 0.199, 95% CI=-0.011 to 0.408).

Seven datasets from as many studies were available for femoral neck aBMD before and 12 months after ALN use. I^2 value showed no significant heterogeneity between these studies (I^2=0.000, Q=0.622, p=0.996), suggesting the use of the fixed effect model for drawing conclusions. Pooled analysis showed that there was no significant change in the mean femoral neck aBMD after ALN administration (SDM=0.170, 95% CI=-0.079 to 0.418, p=0.181) (Supplementary Figure 3A).

Three datasets from as many studies were available for the distal radius aBMD before and 12 months after ALN administration. There was no significant heterogeneity between the studies (I^2=0.000, Q=0.048, p=0.976), suggesting the use of the fixed effect model for data analysis. Pooled analysis showed no significant change in distal radius aBMD after ALN use in PHPT patients (SDM=0.020, 95% CI=-0.372 to 0.412, p=0.921) (Supplementary Figure 3B).

Seven datasets from as many studies were available for serum PTH before and 12 months after ALN administration. Since the heterogeneity between these studies was not significant (I^2=0.000, Q=5.620, p=0.467), we used the fixed effect model for drawing inference. Pooled analysis showed a significant increase in serum PTH after ALN use (SDM=0.416, 95% CI=0.164 to 0.668, p=0.001) (Figure 4B).

Five datasets from as many studies were available for serum calcium before and 12 months after ALN use. There was significant heterogeneity between these studies (I^2=71.833, Q=14.201, p=0.007), suggesting the use of the random effects model for drawing a conclusion. Pooled analysis showed a significant decrease in serum calcium after ALN use (SDM=-0.632, 95% CI=-1.261 to -0.004, p=0.049) (Figure 4C).

Four datasets from as many studies were available for serum phosphate before and 12 months after ALN use. Significant heterogeneity was found between these studies (I^2=69.175, Q= 9.732, p=0.021), which suggested the use of the random effects model. Pooled analysis showed no significant change in serum phosphate after ALN use (SDM=-0.567, 95% CI=-1.283 to 0.150, p=0.121) (Supplementary Figure 3C).

Four datasets from as many studies were available for serum OCN before and 12 months after ALN use. Significant heterogeneity was found between these studies (I^2=72.645, Q=10.967, p=0.012), suggesting the use of the random effects model for drawing a conclusion. Pooled analysis showed a significant decrease in serum OCN after ALN use (SDM=-1.097, 95% CI=-1.774 to -0.420, p=0.001) (Figure 4D).

Three datasets from as many studies were available for serum BALP before and 12 months after ALN use. The heterogeneity between these studies was not significant (I^2=16.475, Q=2.394, p=0.302), suggesting the use of the fixed effect model for drawing inference. Pooled analysis showed a significant decrease in serum BALP after ALN use (SDM=-1.617, 95% CI=-2.086 to -1.149, p= 0.0001) (Figure 4E).

3.4.2 Comparison with the placebo control

Five datasets from as many studies were available for lumbar spine aBMD in PHPT patients treated with ALN for 12 months and compared with placebo control. No significant heterogeneity was found among these studies (I^2=0.000, Q=1.892, p=0.756), suggesting that the fixed effect model should be used for data analysis. The pooled analysis showed that the mean lumbar spine aBMD in the ALN group was significantly increased compared with the placebo group (SDM=0.350, 95% CI=0.041 to 0.659, p=0.027) (Figure 5A).

Five datasets from as many studies were available for femoral neck aBMD in the ALN and placebo groups. No significant heterogeneity was observed among these studies (I^2=0.000, Q=2.538, p=0.638), suggesting the use of the fixed effect model for drawing inference. The mean femoral neck aBMD was not significantly different in the ALN and placebo group (SDM=0.250, 95% CI=-0.058 to 0.558, p=0.111) (Supplementary Figure 4A).

Six datasets from as many studies were analyzed for serum PTH in the ALN and placebo groups. Significant heterogeneity was found in these studies (I^2=56.799, Q=11.574, p=0.041), which suggested the use of the random effects model for drawing conclusions. The pooled analysis shows that PTH was significantly higher in the ALN group compared with placebo (SDM=0.602, 95% CI=0.145 to 1.059, p=0.010) (Figure 5B).

Five datasets from as many studies were available for serum calcium in the ALN and placebo group. I^2 values showed significant heterogeneity (I^2=87.843, Q=32.902, p=0.000), suggesting the use of

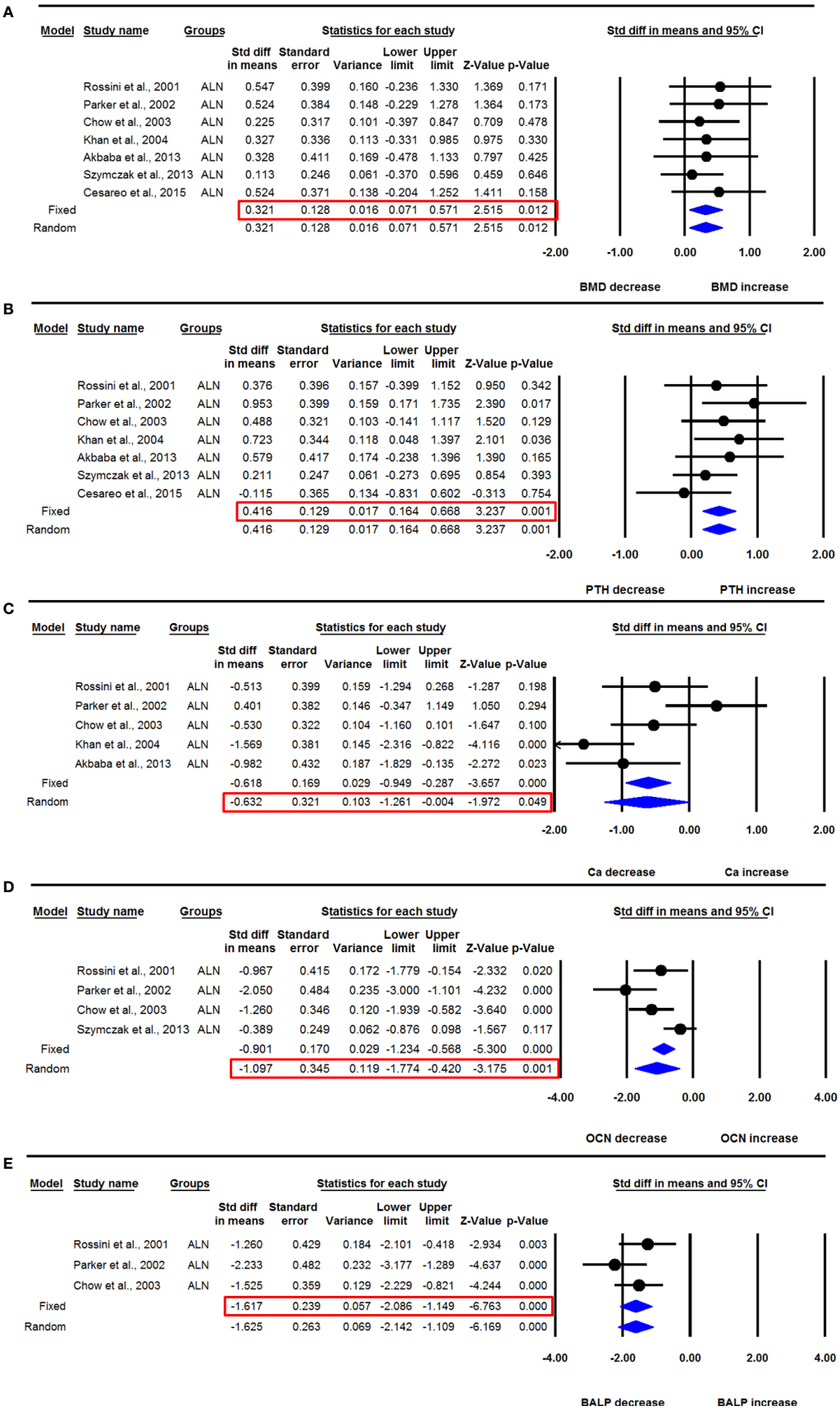

FIGURE 4
The effect of ALN administration in PHPT patients compared with baseline; **(A)** lumbar spine aBMD, **(B)** serum PTH, **(C)** serum calcium (Ca), **(D)** serum OCN, and **(E)** serum BALP.

the random effects model. The mean serum calcium was not different in the ALN and placebo groups (SDM=-0.381, 95% CI= -1.345 to 0.583, p=0.439) (Figure 5C).

Four datasets from as many studies were available for serum phosphate in both groups. Since no significant heterogeneity was found among these studies (I^2=75.474, Q=12.232, p=0.007), we applied the random effects model to draw the inference. No significant change was found between the ALN and placebo groups (SDM=-0.369, 95% CI=-1.156 to 0.418, p=0.358) (Supplementary Figure 4B).

Three datasets from as many studies were available for serum BALP in the ALN and placebo groups. Significant heterogeneity was observed among these studies (I^2=93.550, Q=31.008, p=0.000), so the random effects model was used for drawing conclusions. The mean BALP was significantly decreased in the ALN group compared with the placebo (SDM=-3.422, 95% CI=-5.844 to -1.000, p=0.006) (Figure 5D).

Three datasets from as many studies were available for serum OCN. Significant heterogeneity was found in these studies (I^2=94.320, Q=35.212, p=0.000), suggesting the use of the random effects model for inference. The mean serum OCN was not significantly different in the ALN and placebo groups (SDM=-1.947, 95% CI=-4.064 to 0.170, p=0.072) (Supplementary Figure 4C).

3.5 Effect of denosumab on aBMD compared with baseline

Three datasets from as many studies were available for lumbar spine aBMD before and 6- or 12 months after denosumab use. The fixed effect model was used for drawing inference because no significant heterogeneity was observed between these studies (I^2=43.283, Q=3.526, p=0.172). Pooled analysis showed that the mean lumbar spine aBMD significantly increased after denosumab administration compared with the baseline (SDM=0.828, 95% CI=0.378 to 1.278, p=0.0001) (Figure 6A).

Three datasets from as many studies were available for femur neck aBMD before and 6- or 12 months after denosumab use. No significant heterogeneity was observed between these studies (I^2=24.937, Q=2.664, p=0.264), suggesting the use of the fixed effect model for drawing a conclusion. Pooled analysis showed that the mean femoral neck aBMD significantly increased after denosumab administration compared with the baseline (SDM=0.575, 95% CI=0.135 to 1.015, p=0.010) (Figure 6B).

Only one study (31) had denosumab data for 24 months, and it was not in the required format, thus resulting in its exclusion. We could not analyze the biochemical parameters due to the limitation in data availability.

3.6 Effect of PTX on aBMD and serum PTH levels compared with baseline

In this meta-analysis, we focused on the effect of BPs and denosumab but not PTX in PHPT patients. We included only those studies where the data associated with PTX was provided as additional information for the selected studies.

Three datasets from as many studies were available for lumbar spine aBMD before and 12 months after PTX. There was no significant heterogeneity between these studies (I^2=56.045, Q=4.550, p=0.103), suggesting the use of the fixed effect model for data analysis. In a pooled analysis, the mean lumbar spine aBMD significantly increased after PTX compared with baseline (SDM=0.662, 95% CI=0.319 to1.005, p=0.0001) (Figure 6C).

Three datasets from as many studies were available for serum PTH after 12 months of PTX. Significant heterogeneity was observed between these studies (I^2=92.880, Q=28.090, p=0.0001), suggesting the use of the random effects model for drawing inference. In the pooled analysis, the mean serum PTH significantly decreased after PTX compared with baseline (SDM=-2.723, 95% CI=-4.466 to -0.980, p=0.002) (Figure 6D).

3.7 Effect of 24 months of ALN use on aBMD, PTH, and calcium compared with baseline

The majority of the data was available for 12 months, and 24 months of ALN administration from 3 studies. Three datasets from as many studies were available for lumbar aBMD before and 24 months after ALN use. The heterogeneity was not significant between these studies (I^2=2.766, Q=2.057, p=0.358), suggesting the use of the fixed effect model for drawing a conclusion. Pooled analysis showed a significant increase in mean lumbar spine aBMD after ALN use (SDM=0.724, 95% CI=0.295 to 1.153, p=0.001) (Figure 7A).

Three datasets from as many studies were available for femoral neck aBMD before and 24 months after ALN use. The heterogeneity in these studies was not significant (I^2=0.000, Q=0.164, p=0.922), suggesting the use of the fixed effect model for data analysis. Pooled analysis showed no significant change in the mean femoral neck aBMD after ALN administration (SDM=0.274, 95% CI=-0.141 to 0.690, p=0.195) (Figure 7B).

Three datasets from as many studies were available for serum PTH before and 24 months after ALN use. The heterogeneity among these studies was significant (I^2=67.163, Q=6.091, p=0.048), suggesting the use of the random effects model for drawing inference. Pooled analysis showed that the mean PTH was not significantly changed after ALN administration (SDM=0.213, 95% CI=-0.551 to 0.977, p=0.585) (Figure 7C). Of the three studies, one (18) was sensitive; however, meta-analysis excluding this could not be performed due to the paucity of the number of studies.

Three datasets from as many studies were available for serum calcium before and 24-months after ALN use. The heterogeneity was significant between these studies (I^2=90.505, Q=21.064, p=0.0001), suggesting the use of the random effects model for drawing conclusion. Pooled analysis showed a significant decrease in serum calcium after ALN administration (SDM=-0.245, 95% CI=-1.728 to 1.237, p=0.746) (Figure 7D).

3.8 Publication bias

The majority of the parameters were unaffected by publication bias. The unbiased estimates based on the trim and fill procedure have been mentioned where they have been observed.

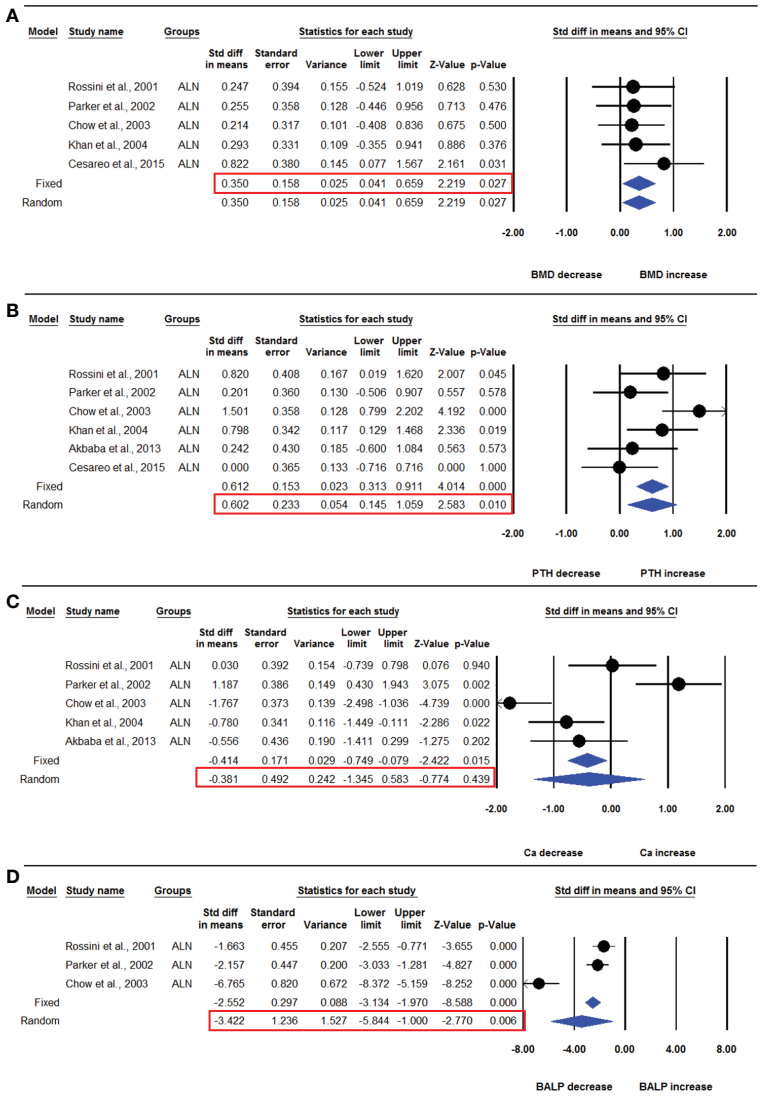

FIGURE 5
The effect of ALN administration on different parameters in PHPT patients compared with the placebo group; **(A)** lumbar spine aBMD, **(B)** serum PTH, **(C)** serum calcium (Ca), and **(D)** serum BALP.

3.9 Sensitivity analysis

The sensitivity analysis was performed with the exclusion of one study at a time. No study was found to be sensitive.

4 Discussion

A recent paper performed meta-analyses of all available medical and surgical modalities for PHPT in comparison to placebo control and excluded non-RCT studies where endpoint effects were compared with baseline (33). The parameters included in the meta-analysis were BMD, PTH, and calcium, although for BMD, only a single dataset was used for performing the meta-analysis. Our study examined whether anti-resorptive medicines may improve BMD in PHPT patients, and we focused on BPs and denosumab because there were sufficient studies on these medications to do meta-analysis. The parameters included in our study were BMD, calcium, phosphate, PTH, and BTMs. We included studies having placebo control as well as comparison between endpoints and baseline, i.e., both RCT and non-RCT. This way, we could most comprehensively capture the effects of anti-resorptives on bone and mineral homeostasis in order to determine their efficacy in protecting those PHPT patients from osteoporotic fractures who are ineligible for surgery.

We found aBMD gains in nine and three studies with BPs (7 ALN, 1 etidronate, 1 neridronate) and denosumab for 12 months, respectively; three with ALN and one with denosumab for 24 months, respectively. In the pooled analysis, BPs and denosumab use in PHPT patients for 12 months increased aBMD in the lumbar spine and femoral neck while decreasing serum calcium and phosphate. When the effects of only BPs were considered at this treatment duration, significant increases in lumbar spine aBMD, decreases in BTMs (OCN, BALP, and CTX-I), and decreases in serum calcium were observed. Of the BPs, sufficient studies were available only with ALN to conduct a meta-analysis, which revealed that ALN increased aBMD at the lumbar spine at 12- and 24 months

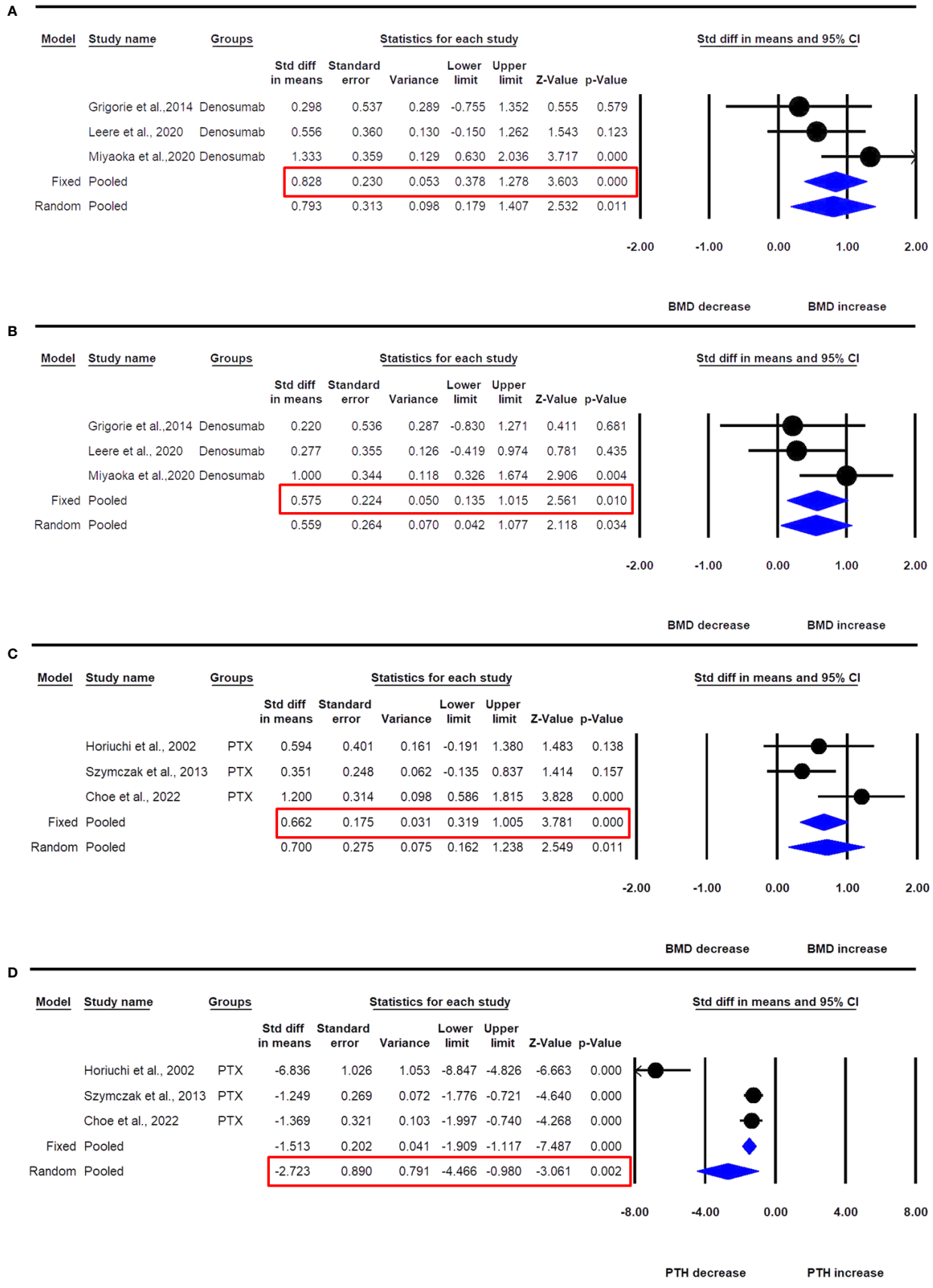

FIGURE 6
The effect of denosumab use in PHPT patients compared with baseline; **(A)** lumbar spine aBMD, **(B)** femur neck aBMD; and PTX on **(C)** lumbar spine aBMD, and **(D)** PTH in PHPT patients.

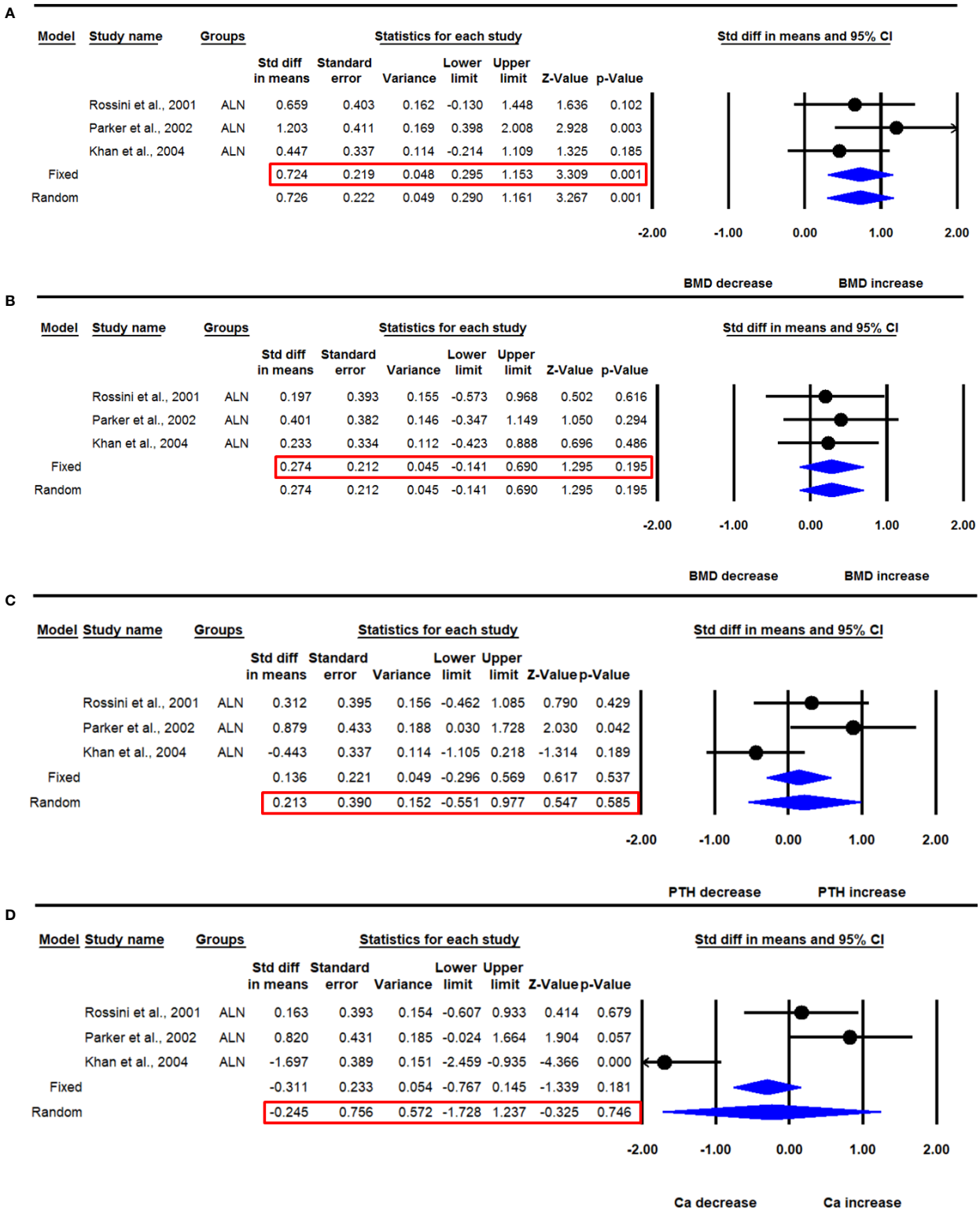

FIGURE 7
The effect of 24 months ALN administration on various parameters in PHPT patients compared with baseline; **(A)** lumbar spine aBMD, **(B)** femur neck aBMD, **(C)** serum PTH, and **(D)** serum calcium (Ca).

but it did not affect aBMD at the femoral neck or the distal radius. ALN also lowered serum calcium while increasing serum PTH, but PTX normalized the hormone after 12 months. Serum PTH increased significantly after 12 months but returned to baseline 24 months after ALN use. Serum calcium levels dropped significantly after 12 months and returned to baseline after 24 months of ALN therapy. Regarding the skeletal effect, denosumab was superior to ALN and PTX as it increased aBMD at the lumbar spine as well as at the femoral neck.

PTX is the standard of care for symptomatic PHPT as well as in selected patients of asymptomatic PHPT. However, PTX can result in

uncontrolled bone mineralization and hypocalcemia, a condition known as "hungry bone syndrome (HBS)" (34). A significant number of PHPT patients are unable to undergo PTX (asymptomatic, personal wish, severe comorbidities, and advanced age) (35). Mitigating hypercalcemia and its consequences, including improving BMD and reducing fracture risk, are priorities in managing such cases. Medical management for PHPT is not new. Anti-resorptives have been used to prevent bone loss, reduce the risk of fracture, and correct hypercalcemia although often temporarily. Cinacalcet has been used to decrease PTH secretion and hypercalcemia. Mithramycin has been historically used to normalize calcium (36). The main purpose of anti-resorptive therapy is to provide functional remission, i.e., normalizing calcium and preventing BMD loss.

Our meta-analysis findings support the long-term use of ALN and denosumab in providing skeletal protection in PHPT patients who are ineligible for surgery or in cases of surgery delay. ALN raised aBMD at the lumbar spine as compared to placebo (Figure 5A), while denosumab increased both aBMD at the lumbar spine and femoral neck when compared with baseline (Figures 6A, B). The effect on aBMD was accompanied by a reduction in BTM parameters, including serum calcium, OCN, BALP, and CTX-I. ALN had similar effects on BMD improvement and BTM reduction in men and women with PHPT (27). ALN at 12 months decreased serum calcium; however, the effect disappeared at 24 months. The effect of ALN in lowering serum calcium is inconsistent due to study heterogeneity and insufficient number of studies that spanned for 24 months. There was no effect on serum phosphate with ALN use. Only two denosumab studies measured serum calcium; in one, a decline in serum calcium was observed during the first month, after which it returned to baseline levels and continued until 50 weeks (21); and in the second, a decrease in serum calcium was observed in the first two weeks but then returned to the baseline levels and continued throughout the study (6 months) (26). PHPT is characterized by cortical bone loss with relative preservation of trabecular bone. In our meta-analysis, we observed that the increase in aBMD was greater at the trabecular site of the lumbar spine than the cortical site of the femur neck with both ALN and denosumab. From these results, it appears that the use of anti-resorptives in PHPT may have an impact similar to postmenopausal osteoporosis in terms of slowing bone remodeling but may fall short of significant reductions in the modeling (at the cortical bone) and continuous BMU activation at the endosteal surface as a consequence of increased PTH levels.

BMD is an important predictor of fracture risk, but fracture data is essential to determine the treatment efficacy in osteoporosis. We found that only two studies addressed fractures, and both observed no significant effect of BPs on the rate of fragility fracture (30) and fracture risk (29) in PHPT patients. Given insufficient data, we could not perform a meta-analysis of the effect of BPs in modifying the risk of fracture and hence propose future studies to acquire fracture data.

Strong suppression of bone resorption by anti-resorptive therapy in PHPT could lead to the exacerbation of hyperparathyroidism. By a pooled analysis, we observed there was an increase in serum PTH following 12 months of ALN use. In the case of denosumab, one study reported a moderate yet significant rise in PTH after 12 months (25); while in another study conducted for 50 weeks, a rapid increase in PTH level over the baseline was quickly followed by its return to the baseline level till the end of the study (21). Unlike ALN, pooled analysis of the effect of denosumab on PTH levels after 12 months could not be done due to the paucity of data. Only one study examined the short-term effect of denosumab (3 and 6 months) on PTH levels in PHPT patients and found that the drug had no effect (26). Future studies are required to assess the effect of denosumab on PTH levels in PHPT patients. These studies are essential for assessing the safety of long term denosumab in PHPT as elevation of PTH has been reported to be associated with adverse effects, including hypertension, left ventricular hypertrophy, heart failure, and renal insufficiency (37).

Furthermore, the production of fibroblast growth factor 23 (FGF23) is increased by PTH, and the former is an independent marker for left ventricular function (38). Thus, further elevation of serum PTH using BPs in PHPT could heighten cardiovascular risk. Future studies should measure FGF23 levels and monitor for any cardiovascular event in PHPT patients treated with anti-resorptive drugs.

Theoretically, a calcimimetic drug that inhibits both PTH and FGF23 can be combined with anti-resorptives for greater efficacy and preventing cardiovascular morbidity. A clinical trial has considered this combination and found that cinacalcet improved the biochemical abnormalities and alendronate increased BMD at 24-months follow-up (35). However, more such studies are required to determine the efficacy and safety of these combinations through a meta-analysis.

The strengths of this meta-analysis are that a comparison of drug effects has been made with both placebo and baseline, and drug effects at the site-specific BMD and BTMs have been compared with PTX. The limitations include the inclusion of both RCT and non-RCT studies and the lack of fracture data due to insufficient data availability. Head-to-head comparison between anti-resorptive and PTX therapy is not possible because of the paucity of the studies.

5 Conclusion

Anti-resorptive therapies, including ALN and denosumab, increase aBMD, decrease serum calcium, and inhibit BTMs in PHPT patients. Alendronate significantly increases PTH levels in PHPT patients compared with both baseline and placebo without affecting normal mineral levels. Future studies should measure FGF23 and monitor cardiovascular events in PHPT patients receiving anti-resorptive drugs. A combination of calcimimetic and anti-resorptive drugs could provide an improved clinical profile over monotherapy to treat aberrant bone and mineral homeostasis in PHPT patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

SwR conducted literature screening, statistical analyses of the extracted data and wrote the manuscript; AD performed statistical analyses of the extracted data and wrote the manuscript; SiR, AM, and NC conceived the idea, conducted literature screening, and finalized

the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

This study was supported by a grant from the Council of Scientifc and Industrial Research, Government of India, (MLP2035). Swati Rajput acknowledges the Department of Biotechnology, Govt. of India, for graduate fellowship (Ref No. DBT/2018/CDRI/1047). The CDRI communication number for this paper is 10529.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1098841/full#supplementary-material

References

1. Silvano A, Claudio M, Gatti D. Epidemiology of primary hyperparathyroidism in Europe. *J Bone Min Res* (2002) 17:N18–23.

2. Melton LJ. Epidemiology of primary hyperparathyroidism. *J Bone Min Res* (1991) 6: S25–30. doi: 10.1002/jbmr.5650061409

3. Bilezikian J. Primary hyperparathyroidism. In: Favus MJ, editor. Primary hyperparathyroidism. In: Favus MJ, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. *Wiley-Blackwell* (1999). 1:187–92.

4. Bergström I, Landgren BM, Freyschuss B. Primary hyperparathyroidism is common in postmenopausal women with forearm fracture and low bone mineral density. *Acta Obs Gynecol Scand* (2007) 86:61–4. doi: 10.1080/00016340601033287

5. Marcocci C, Cianferotti L, Cetani F. Bone disease in primary hyperparathyrodism. *Ther Adv Musculoskelet Dis* (2012) 4:357–68. doi: 10.1177/1759720X12441869

6. Costa AG, Bilezikian JP. Bone turnover markers in primary hyperparathyroidism. *J Clin Densitom* (2013) 16:22–7. doi: 10.1016/J.JOCD.2012.11.004

7. Bilezikian JP, Khan AA, Potts JT. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. *J Clin Endocrinol Metab* (2009) 94:335–9. doi: 10.1210/JC.2008-1763

8. Silverberg SJ, Lewiecki EM, Mosekilde L, Peacock M, Rubin MR. Presentation of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metab* (2009) 94:351–65. doi: 10.1210/JC.2008-1760

9. Aslam M, Talukdar R, Jagtap N, Rao GV, Pradeep R, Rao U. Clinical profile and outcome of parathyroid adenoma – associated pancreatitis. *Saudi J Med Med Sci* (2018) 6:95–9. doi: 10.4103/sjmms.sjmms_80_17

10. Shoback DM, Bilezikian JP, Turner SA, Cary LCMC, Guo MD, Peacock M, et al. The calcimimetic cinacalcet normalizes serum calcium in subjects with primary hyperparathyroidism. *J Clin Endocrinol Metab* (2003) 88:5644–9. doi: 10.1210/jc.2002-021597

11. Dandurand K, Ali DS, Khan AA. Primary hyperparathyroidism: A narrative review of diagnosis and medical management. *J Clin Med* (2021) 10:1604. doi: 10.3390/jcm10081604

12. Leere JS, Karmisholt J, Robaczyk M, Vestergaard P. Contemporary medical management of primary hyperparathyroidism: A systematic review. *Front Endocrinol (Lausanne)* (2017) 8:79. doi: 10.3389/fendo.2017.00079

13. Rajput S, Mehta P, Mittal M, Rajender S, Chattopadhyay N. Human relevance of preclinical studies on the skeletal impact of inflammatory bowel Disease : A systematic review and meta – analysis. *Calcif Tissue Int* (2021) 108:708–24. doi: 10.1007/s00223-021-00808-5

14. Rossini M, Viapiana O, Kalpakcioglu B, Dhangana R, Gatti D, Braga V, et al. Long-term effects of neridronate and its discontinuation in patients with primary hyperparathyroidism. *Calcif Tissue Int* (2011) 89:21–8. doi: 10.1007/s00223-011-9489-x

15. Horiuchi T, Onouchi T, Inoue J, Shionoiri A, Hosoi T, Orimo H. A strategy for the management of elderly women with primary hyperparathyroidism: A comparison of etidronate therapy with parathyroidectomy. *Clin Sect Gerontol* (2002) 48:103–8. doi: 10.1159/000048935

16. Parker CR, Blackwell PJ, Fairbairn KJ, Hosking DJ. Alendronate in the treatment of primary hyperparathyroid-related osteoporosis: A 2-year study. *J Clin Endocrinol Metab* (2002) 87:4482–9. doi: 10.1210/jc.2001-010385

17. Chow CC, Chan WB, Li JKY, Chan NN, Chan MHM, Ko GTC, et al. Oral alendronate increases bone mineral density in postmenopausal women with primary hyperparathyroidism. *J Clin Endocrinol Metab* (2003) 88:581–7. doi: 10.1210/jc.2002-020890

18. Khan AA, Bilezikian JP, Kung AWC, Ahmed MM, Dubois SJ, Ho AYY, et al. Alendronate in primary hyperparathyroidism: A double-blind, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* (2004) 89:3319–25. doi: 10.1210/jc.2003-030908

19. Akbaba G, Isik S, Ates Tutuncu Y, Ozuguz U, Berker D, Guler S. Comparison of alendronate and raloxifene for the management of primary hyperparathyroidism. *J Endocrinol Invest* (2013) 36:1076–82. doi: 10.3275/9095

20. Szymczak J, Bohdanowicz-Pawlak A. Osteoprotegerin, RANKL, and bone turnover in primary hyperparathyroidism: The effect of parathyroidectomy and treatment with alendronate. . *Horm Metab Res* (2013) 45:759–64. doi: 10.1055/s-0033-1349842

21. Leere JS, Karmisholt J, Robaczyk M, Lykkeboe S, Handberg A, Steinkohl E, et al. Denosumab and cinacalcet for primary hyperparathyroidism (DENOCINA): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol* (2020) 8:407–24. doi: 10.1016/S2213-8587(20)30063-2

22. Choe HJ, Koo BK, Yi KH, Kong SH, Kim JH, Shin CS, et al. Skeletal effects of combined bisphosphonates treatment and parathyroidectomy in osteoporotic patients with primary hyperparathyroidism. *J Bone Miner Metab* (2022) 40:292–300. doi: 10.1007/s00774-021-01279-2

23. Rossini M, Gatti D, Isaia G, Sartori L, Braga V, Adami S. Effects of oral alendronate in elderly patients with osteoporosis and mild primary hyperparathyroidism. *J Bone Min Res* (2001) 16:113–9. doi: 10.1359/jbmr.2001.16.1.113

24. Cesareo R, Di Stasio E, Vescini F, Campagna G, Cianni R, Pasqualini V, et al. Effects of alendronate and vitamin d in patients with normocalcemic primary hyperparathyroidism. *Osteoporos Int* (2015) 26:1295–302. doi: 10.1007/s00198-014-3000-2

25. Miyaoka D, Imanishi Y, Kato E, Toi N, Nagata Y, Kurajoh M, et al. Effects of denosumab as compared with parathyroidectomy regarding calcium, renal, and bone involvement in osteoporotic patients with primary hyperparathyroidism. *Endocrine* (2020) 69:642–9. doi: 10.1007/s12020-020-02401-6

26. Grigorie D, Sucaliuc A. A single-dose, open-label, prospective clinical study of denosumab in patients with primary hyperparathyroidism. *Acta Endocrinol (Copenh)* (2014) 10:396–403. doi: 10.4183/aeb.2014.396

27. Khan AA, Bilezikian JP, Kung A, Dubois SJ, Standish TI, Syed ZA. Alendronate therapy in men with primary hyperparathyroidism. *Endocr Pract* (2009) 15:705–13. doi: 10.4158/EP08178.ORR

28. Tournis S, Fakidari E, Dontas I, Liakou C, Antoniou J, Galanos A, et al. Effect of parathyroidectomy versus risedronate on volumetric bone mineral density and bone geometry at the tibia in postmenopausal women with primary hyperparathyroidism. *J Bone Miner Metab* (2014) 32:151–8. doi: 10.1007/s00774-013-0473-6

29. Orr LE, Zhou H, Zhu CY, Haigh PI, Adams AL, Yeh MW. Skeletal effects of combined medical and surgical management of primary hyperparathyroidism. *Surgery* (2020) 167:144–8. doi: 10.1016/j.surg.2019.04.059

30. Segula D, Nikolova T, Marks E, Ranganath L, Mishra V. Long term outcome of bisphosphonate therapy in patients with primary hyperparathyroidism. *Int J Clin Med* (2014) 05:829–35. doi: 10.4236/ijcm.2014.514111

31. Eller-Vainicher C, Palmieri S, Cairoli E, Goggi G, Scillitani A, Arosio M, et al. Protective effect of denosumab on bone in older women with primary hyperparathyroidism. *J Am Geriatr Soc* (2018) 66:518–24. doi: 10.1111/jgs.15250

32. Mamedova E, Kolodkina A, Vasilyev EV, Petrov V, Belaya Z, Tiulpakov A. Successful use of denosumab for life-threatening hypercalcemia in a pediatric patient with primary hyperparathyroidism. *Horm Res Paediatr* (2020) 93:272–8. doi: 10.1159/000510625

33. Ye Z, Silverberg SJ, Sreekanta A, Tong K, Wang Y, Chang Y, et al. The efficacy and safety of medical and surgical therapy in patients with primary Hyperparathyroidism : A systematic review and meta-analysis of randomized controlled trials. *J Bone Miner Res* (2022) 37:2351–72. doi: 10.1002/jbmr.4685

34. Wayne IL, Tu HSS, Pei SKD. Bisphosphonate pretreatment attenuates hungry bone syndrome postoperatively in subjects with primary hyperparathyroidism. *J Bone Min Metab* (2006) 24:255–8. doi: 10.1007/s00774-005-0680-x

35. Faggiano A, Di SC, Ramundo V, Severino R, Vuolo L, Coppola A, et al. Cinacalcet hydrochloride in combination with alendronate normalizes hypercalcemia and improves bone mineral density in patients with primary hyperparathyroidism. *Endocr* (2011) 39:283–7. doi: 10.1007/s12020-011-9459-0

36. Perlia CP, Gubisch NJ, Wolter J, Edelberg D, Dederick MM, Taylor SG. Mithramycin treatment of hypercalcemia. *Cancer* (1970) 25:389–94. doi: 10.1002/1097-0142(197002)25:2<389::aid-cncr2820250217>3.0.co;2-x

37. Brown SJ, Ruppe MD, Tabatabai LS. The parathyroid gland and heart disease. *Methodist Debakey Cardiovasc J* (2017) 13:49–54. doi: 10.14797/mdcj-13-2-49

38. Faul C, Amaral AP, Oskouei B, Hu M, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest* (2011) 121:4393–408. doi: 10.1172/JCI46122

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TYPE Original Research
PUBLISHED 03 February 2023
DOI 10.3389/fendo.2023.1074574

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

RECEIVED 19 October 2022
ACCEPTED 24 January 2023
PUBLISHED 03 February 2023

CITATION
Hou W, Chen S, Zhu C, Gu Y, Zhu L and Zhou Z (2023) Associations between smoke exposure and osteoporosis or osteopenia in a US NHANES population of elderly individuals. *Front. Endocrinol.* 14:1074574. doi: 10.3389/fendo.2023.1074574

Associations between smoke exposure and osteoporosis or osteopenia in a US NHANES population of elderly individuals

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Background: Tobacco exposure is considered to be a risk factor for reduced bone mineral density (BMD), which may result in osteopenia. Cotinine, a metabolite of nicotine, is commonly utilized as a marker of tobacco exposure. Nevertheless, there are limited clinical data on the associations between osteoporosis (OP) or osteopenia and smoking status or serum cotinine level.

Methods: We thoroughly examined the NHANES cross-sectional data from 2005 to 2010, 2013 to 2014, and 2017 to 2018. Multivariate logistic regression models were applied to assess the associations among smoking status and serum cotinine levels as well as OP and osteopenia. The relationships between serum cotinine level and OP and osteopenia were also assessed using the restricted cubic spline (RCS) method.

Results: A total of 10,564 participants were included in this cross-sectional study. The mean age of the study population was 64.85 ± 9.54 years, and the patients were predominantly male (51.9%). We found that the relationships between higher serum cotinine levels (≥3 ng/ml) and the prevalence of osteoporosis (Model 1: OR=2.27 [1.91-2.69]; Model 2: OR=2.03 [1.70-2.43]; Model 3: OR=2.04 [1.70-2.45]; all *p* for trend <0.001) remained significant after adjustment for covariates by applying the lowest serum cotinine levels (<0.05 ng/ml) as the reference. Similar results were observed for current smokers, who were more likely to develop OP compared with nonsmokers (Model 1: OR=2.30 [1.90-2.79]; Model 2: OR=2.16 [1.77-2.64]; Model 3: OR=2.16 [1.77-2.65]). Moreover, higher serum cotinine levels were found to be strongly and positively correlated with the prevalence of osteopenia (OR=1.60 [1.42-1.80]). A similar relationship was observed between current smokers and the prevalence of osteopenia compared with nonsmokers (OR=1.70 [1.49-1.94]). RCS regression also showed that serum cotinine levels were nonlinearly and positively correlated with OP and osteopenia, with inflection points of 5.82 ng/ml and 3.26 ng/ml, respectively.

Conclusion: This study showed that being a smoker was associated with the prevalence of OP or osteopenia compared with being a nonsmoker and that there was a strong nonlinear positive dose–response relationship between serum cotinine levels and OP and osteopenia.

KEYWORDS

nonlinear associations, osteoporosis, osteopenia, serum cotinine, smoking, NHANES

Introduction

Osteoporosis (OP) is a systemic bone disorder characterized by low bone mineral density (BMD) and skeletal fragility, which increases the risk of fracture (1–3). It is the most widespread metabolic bone disease worldwide and a crucial source of morbidity and mortality (4, 5).

Studies have shown that subjects who are actively and passively exposed to tobacco are at an elevated risk for multiple health conditions, including osteopenia and an increased risk of osteoporotic fractures (6–8). Cigarette smoke contains a multitude of toxic and harmful substances, such as nicotine, heavy metals (arsenic, cadmium and lead), and tar, which may alter the skeletal system and reduce bone density (9–12). Cotinine, a significant proximal metabolite of nicotine, is regarded as a trustworthy and sensitive indicator of tobacco smoke exposure within the past 72-hours. As a result, cotinine is currently recognized as a distinctive chemical reflecting an individual's degree of tobacco smoke exposure (13–15). According to a new study, exposure to cigarette smoke induces oxidative stress by increasing superoxide radicals and decreasing intracellular glutathione in MSCs, which adversely affects osteogenic differentiation (16). Cessation of smoking led to increases in serum levels of osteocalcin and uncarboxylated osteocalcin and BMD in humans (17).

However, clinical data on the associations between tobacco smoke exposure and the prevalence of OP or osteopenia are scarce. The goal of this study was to demonstrate an association between serum cotinine levels and self-reported smoking status with the prevalence of OP and osteopenia in a large national sample using NHANES data from 2005 to 2010, 2013 to 2014, and 2017 to 2018. Our findings provide epidemiological evidence to further investigate the associations of tobacco smoke exposure with OP and osteopenia.

Methods

Study population and design

The NHANES is a study project that aims to assess the health and nutritional status of American adults and children (18). There are demographic, socioeconomic, nutritional, and health-related questions in the NHANES interviews. The National Center for Health Statistics (NCHS) Research Ethics Review Board approved the NHANES research protocols, and all participants provided written informed consent.

We looked at NHANES descriptive data from 2005–2010, 2013–2014, and 2017–2018. Participants aged ≥50 years were enrolled, and those with missing serum cotinine and bone mineral density (BMD) data were excluded.

Assessment of tobacco exposure

During the household interview, adults aged 20 and up self-reported their smoking status. Participants who claimed to have smoked fewer than 100 cigarettes in their lives were labeled 'never smokers'. Former smokers were individuals who had smoked more than 100 cigarettes in their lives but had quit, while current smokers were those who were currently smoking.

Cotinine is a primary nicotine metabolite used as a marker of active smoking and as an indicator of exposure to secondhand smoke (19). Serum cotinine was determined by isotope dilution-high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (20). As in previous investigations (21), those below the lower detection limit were considered unexposed. We generated cotinine categories representing smoking exposure and utilized the newly recommended cut-off point of 3 ng/ml by Benowitz et al. (22) to separate smokers from nonsmokers. Cotinine levels were ranked as follows: cotinine <0.05 ng/ml, cotinine 0.05–2.99 ng/ml, and cotinine ≥3 ng/ml.

BMD measurements and definition of osteopenia and osteoporosis

BMD (measured in grams/cm^2) was evaluated using a dual X-ray absorptiometry technique (QDR 4500A fan-beam densitometers [Hologic Inc]) while the participants visited mobile examination centers. The left hip (or right hip, in case of left hip replacement or metal object injection) was routinely scanned to report total BMD of the femur, femoral neck, and trochanter. The exclusion criteria for assessing participants' dual X-ray absorptiometry followed those of the NHANES recommendations.

The WHO criteria for osteopenia and osteoporosis (23) identify low bone mineral density for male and female individuals aged 50 years and older. This method uses the BMD data from young male and female individuals as threshold values. Male and female individuals aged between 20 and 29 years were selected as the reference group in the current study since prospective data demonstrated femur bone loss in female participants in their thirties (24). Osteopenia was defined as a BMD value that was between 1 and 2.5 standard deviations (SDs) below the mean of male and female participants aged between 20 and 29 years; osteoporosis was defined as a BMD value of more than 2.5 SD below the young reference mean. Both of these conditions were considered to have low bone density. This criteria has been applied to each region of interest.

Covariates

In the NHANES, data were collected using a standard participant questionnaire administered throughout a household interview, along with a medical assessment for each participant. The covariates considered in this study included age, sex, race, education level, poverty, drinking status, physical activity, energy intake level, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), serum calcium, history of prednisone or cortisone, self-reported diabetes, self-reported hypertension, self-reported cardiovascular disease (CVD), and self-reported cancer. Poverty was assessed using the poverty income ratio (PIR) and was defined as a PIR of 1 for a particular family. For drinking status, participants were categorized as

being nondrinkers, low-to-moderate drinkers (<2 drinks/day in men and <1 drink/day in women), or heavy drinkers (≥2 drinks/day in men and ≥1 drink/day in women). The intake of energy was calculated by averaging the two values for the two 24-hour recall interviews. In line with their physical activity levels, the participants were classified as active, insufficiently active, and inactive (25). Descriptions of each variable are presented in https://www.cdc.gov/Nchs/Nhanes/continuousnhanes/.

Statistical analysis

Continuous variables were reported as the means (standard deviations) or medians (interquartile ranges) and compared by adopting Student's t test (normal distribution) or the Mann–Whitney U test (nonnormal distribution). By adopting the chi-square test, categorical variables were represented as absolute values (percentages) and compared. As continuous variables, cotinine levels were log2-transformed to achieve a normal distribution. The "mice" package utilized the random forest algorithm for multiple interpolation of the missing data. All statistical analyses were conducted by utilizing R Statistical Software, version 4.2.0, and results with p values < 0.05 (two-sided) were considered statistically significant.

The connections between tobacco exposure and the prevalence of osteoporosis as well as osteopenia were investigated by adopting three consecutive multivariate logistic regression models. Model 1 was adjusted for age (continuous), sex (male or female), and race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, or other race). Model 2 was adjusted for Model 1 by adding education level (below high school, high school, or above high school), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), family income-poverty ratio (<1.0, or ≥1.0), physical activity (inactive, insufficiently active, active), and total energy intake (log2-transformed). Model 3 was based on Model 2 and included additional adjustments for TC (continuous), HDL-C (continuous), serum calcium (continuous), history of prednisone or cortisone (yes or no), self-reported hypertension (yes or no), self-reported diabetes (yes or no), self-reported cardiovascular disease (yes or no), and self-reported cancer (yes or no). Furthermore, restricted cubic spline (RCS) with three knots (10th, 50th, and 90th percentiles) was adopted to study dose–response associations. Nonlinearity was examined by analysis of variance (ANOVA). By adopting segmented regression, the threshold inflection of linearity was computed to fit the piecewise-linear relationship between tobacco exposure and the prevalence of osteoporosis and osteopenia.

Results

Characteristics of the study participants

A total of 50463 participants from NHANES 2005–2010, 2013–2014 and 2017–2018 were included. Of these, those aged < 50 (n=36297) and those with missing data on serum cotinine (n=1309) and BMD (n=2293) were excluded. In total, 10564 eligible participants were enrolled (Figure 1). Table S1 presents the baseline characteristics of participants according to NHANES 2005-2010, 2013-2014, and 2017-2018.

In this study, the thresholds for osteoporosis in men were 0.73 gm/cm^2 or less for the total femur, 0.61 gm/cm^2 or less for the femoral neck, 0.50 gm/cm^2 or less for the trochanter, and 0.86 gm/cm^2 or less for the intertrochanter; among female smokers, the thresholds were 0.65 gm/cm^2 or less for the total femur, 0.56 gm/cm^2 or less for the femoral neck, 0.44 gm/cm^2 or less for the trochanter, and 0.77 gm/cm^2 or less for the intertrochanter. The cutoff values for osteopenia in men were 0.73 to 0.94 gm/cm^2, 0.61 to 0.83 gm/cm^2, 0.50 to 0.69 gm/cm^2 and 0.86 to 1.11 gm/cm^2 for the total femur, femoral neck, femoral rotor and intertrochanter, respectively; for women, they were 0.65 to 0.84 gm/cm^2, 0.56 to 0.76 gm/cm^2, 0.44 to 0.61 gm/cm^2 and 0.77 to 0.99 gm/cm^2 for the total femur, femoral neck, femoral rotor and intertrochanter, respectively (Table 1). Moreover, the overall mean age of the reference group (men or women aged 20 to 29 years)

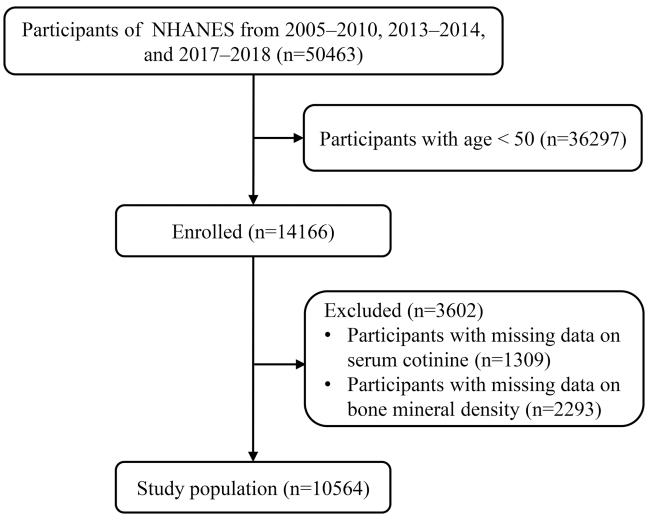

FIGURE 1
The flow chart of selection of included studies.

10.3389/fendo.2023.1074574

TABLE 1 Mean femoral bone mineral density (BMD) of 20–29-year-old men and women in NHANES 2005–2010, 2013–2014, and 2017–2018.

Region of interest	Mean (gm/cm^2)	SD (gm/cm^2)	BMD cutoff values for	
			Osteopenia	Osteoporosis
Men (n=1149)				
Total femur	1.09	0.15	0.73-0.94	<0.73
Femoral neck	0.97	0.14	0.61-0.83	<0.61
Trochanter	0.82	0.13	0.50-0.69	<0.50
Intertrochanter	1.28	0.17	0.86-1.11	<0.86
Women (n=1062)				
Total femur	0.97	0.13	0.65-0.84	<0.65
Femoral neck	0.89	0.13	0.56-0.76	<0.56
Trochanter	0.72	0.11	0.44-0.61	<0.44
Intertrochanter	1.14	0.15	0.77-0.99	<0.77

For each of the four regions of interest, low bone density was defined as: (1) osteopenia: a BMD value between 1 standard deviation (SD) and 2.5 SD below the mean of men or women age 20–29 years; and (2) osteoporosis: a BMD value >2.5 SD below the young reference mean.

was 24.45 ± 2.91 years (Table S2). A total of 797 (36.0%) participants had cotinine levels ≥3 ng/ml, and 663 (30.0%) participants in the reference group were current smokers. The proportion of cotinine levels was significantly higher in men than in women.

The characteristics of the enrolled individuals are presented in Table 2. The mean age of the study population was 64.85 ± 9.54 years, and the patients were predominantly male (51.9%). By applying cotinine levels, 2228 (21.1%) participants had cotinine levels ≥3 ng/ml, 2179 (20.6%) participants had cotinine levels between 0.05 and 2.99 ng/ml, and 6157 (58.3%) participants had cotinine levels <0.05 ng/ml. The self-reported smoking status revealed that there were 1747 (16.5%) current smokers, 3612 (34.2%) former smokers, and 5205 (49.3%) nonsmokers among the participants. Participants with cotinine level ≥3 ng/ml and self-reported smokers were more likely to be younger, male, and non-Hispanic Black, to have lower education, to have poverty, to be a drinker, to have a low level of physical activity, to have a history of prednisone or cortisone use, and to have more comorbidity than those with a cotinine level <0.05 ng/ml and those who were self-reported nonsmokers.

Associations between serum cotinine levels and smoking status with the prevalence of osteoporosis

Table 3 displays the associations between serum cotinine levels and the prevalence of osteoporosis in both continuous and categorical analyses. Regardless of adjustment for covariates, the continuous analysis revealed that log2-transformed cotinine levels showed a noticeable positive association with the prevalence of osteoporosis. The categorical analysis indicated that the association between higher serum cotinine levels (≥3 ng/ml) and the prevalence of osteoporosis (Model 1: OR=2.27 [1.91-2.69]; Model 2: OR=2.03 [1.70-2.43]; Model 3: OR=2.04 [1.70-2.45]; all p for trend <0.001) remained significant after adjustment for covariates using the lowest serum cotinine levels (<0.05 ng/ml) as a reference. Similar results were observed in that current smokers were highly associated with the prevalence of osteoporosis compared with nonsmokers (Model 1: OR=2.30 [1.90-2.79]; Model 2: OR=2.16 [1.77-2.64]; Model 3: OR=2.16 [1.77-2.65]; all p for trend <0.001).

Associations between serum cotinine levels and smoking status with the prevalence of osteopenia

Further examination of the associations between serum cotinine levels, smoking status, and the prevalence of osteopenia is presented in Table 4. As illustrated by the continuous analysis, after adjustment for covariates, there was a markedly positive relationship between log2-transformed serum cotinine levels and the prevalence of osteopenia. In Model 3, the categorical analysis revealed that the multivariate odds ratios (95% confidence intervals [CI]) for osteopenia increased monotonically to 0.95 (0.85-1.06) and 1.60 (1.42-1.80) (p for trend < 0.001) with higher serum cotinine levels (≥3 ng/ml). Similar relationships were observed between current smokers and the prevalence of osteopenia compared with nonsmokers (OR=1.70 [1.49-1.94]; p for trend < 0.001).

Nonlinear associations between serum cotinine levels and the prevalence of osteoporosis and osteopenia

RCS regression with multivariable-adjusted associations was adopted to demonstrate dose–response associations between log2-transformed serum cotinine levels and the prevalence of osteoporosis as well as osteopenia (Figure 2). Serum cotinine levels were nonlinearly and positively correlated with the prevalence of osteoporosis (p for nonlinearity = 0.001) and osteopenia (p for nonlinearity < 0.001), with inflection points of 5.82 ng/ml and 3.26 ng/ml, respectively.

10.3389/fendo.2023.1074574

TABLE 2 Baseline characteristics of participants in NHANES 2005–2010, 2013–2014, and 2017–2018.

Characteristics	Total	Cotinine category, %			*P* value	Self-reported smoking status, %			*P* value
		<0.05 ng/mL	0.05–2.99 ng/mL	≥3 ng/mL		Nonsmoker	Former smoker	Current smoker	
Participants, n	10564	6157	2179	2228		5205	3612	1747	
Age, years	64.85 (9.54)	66.00 (9.71)	64.95 (9.44)	61.59 (8.37)	<0.001	64.81 (9.74)	66.84 (9.31)	60.86 (8.05)	<0.001
Male, %	5478 (51.9)	2880 (46.8)	1190 (54.6)	1408 (63.2)	<0.001	2069 (39.8)	2363 (65.4)	1046 (59.9)	<0.001
Race/ethnicity, %					<0.001				<0.001
Mexican American	1473 (13.9)	1009 (16.4)	241 (11.1)	223 (10.0)		799 (15.4)	464 (12.8)	210 (12.0)	
Other Hispanic	913 (8.6)	625 (10.2)	160 (7.3)	128 (5.7)		521 (10.0)	278 (7.7)	114 (6.5)	
Non-Hispanic White	5262 (49.8)	3159 (51.3)	1017 (46.7)	1086 (48.7)		2386 (45.8)	2063 (57.1)	813 (46.5)	
Non-Hispanic Black	2033 (19.2)	803 (13.0)	574 (26.3)	656 (29.4)		932 (17.9)	594 (16.4)	507 (29.0)	
Other race	883 (8.4)	561 (9.1)	187 (8.6)	135 (6.1)		567 (10.9)	213 (5.9)	103 (5.9)	
Education level, %					<0.001				<0.001
Below high school	2959 (28.0)	1444 (23.5)	700 (32.1)	815 (36.6)		1323 (25.4)	979 (27.1)	657 (37.6)	
High school	2542 (24.1)	1334 (21.7)	586 (26.9)	622 (27.9)		1182 (22.7)	877 (24.3)	483 (27.6)	
Above high school	5063 (47.9)	3379 (54.9)	893 (41.0)	791 (35.5)		2700 (51.9)	1756 (48.6)	607 (34.7)	
Poverty, %	1624 (15.4)	627 (10.2)	398 (18.3)	599 (26.9)	<0.001	676 (13.0)	456 (12.6)	492 (28.2)	<0.001
Drinking status, %					<0.001				<0.001
Nondrinker	2501 (23.7)	1635 (26.6)	581 (26.7)	285 (12.8)		1826 (35.1)	466 (12.9)	209 (12.0)	
Low-to-moderate drinker	7397 (70.0)	4230 (68.7)	1484 (68.1)	1683 (75.5)		3196 (61.4)	2884 (79.8)	1317 (75.4)	
Heavy drinker	666 (6.3)	292 (4.7)	114 (5.2)	260 (11.7)		183 (3.5)	262 (7.3)	221 (12.7)	
Physical activity, %					<0.001				<0.001
Inactive	3276 (31.0)	1783 (29.0)	728 (33.4)	765 (34.3)		1597 (30.7)	1073 (29.7)	606 (34.7)	
Insufficiently active	3605 (34.1)	2185 (35.5)	733 (33.6)	687 (30.8)		1761 (33.8)	1304 (36.1)	540 (30.9)	
Active	3683 (34.9)	2189 (35.6)	718 (33.0)	776 (34.8)		1847 (35.5)	1235 (34.2)	601 (34.4)	
Energy intake, kcal/day	1776.75 [1371.00, 2285.00]	1757.50 [1371.00, 2226.00]	1759.50 [1344.50, 2290.50]	1852.75 [1404.00, 2437.62]	<0.001	1702.00 [1328.00, 2184.00]	1841.75 [1425.38, 2342.12]	1859.50 [1409.25, 2450.00]	<0.001
Total cholesterol, mg/dL	197.73 (42.93)	197.86 (42.20)	196.70 (43.49)	198.40 (44.34)	0.396	200.24 (42.05)	193.28 (43.49)	199.48 (43.64)	<0.001
HDL-C, mmol/L	1.40 (0.43)	1.42 (0.42)	1.38 (0.41)	1.36 (0.45)	<0.001	1.44 (0.42)	1.36 (0.41)	1.37 (0.46)	<0.001
Serum calcium, mg/dL	9.43 (0.38)	9.44 (0.38)	9.42 (0.37)	9.44 (0.39)	0.112	9.43 (0.38)	9.43 (0.38)	9.44 (0.39)	0.364
History of prednisone or cortisone, %	651 (6.2)	345 (5.6)	142 (6.5)	164 (7.4)	0.009	288 (5.5)	235 (6.5)	128 (7.3)	0.015
Hypertension, %	5551 (52.5)	3264 (53.0)	1199 (55.0)	1088 (48.8)	<0.001	2729 (52.4)	2002 (55.4)	820 (46.9)	<0.001
Diabetes, %	1990 (18.8)	1182 (19.2)	443 (20.3)	365 (16.4)	0.002	974 (18.7)	749 (20.7)	267 (15.3)	<0.001
CVD, %	1919 (18.2)	1016 (16.5)	446 (20.5)	457 (20.5)	<0.001	765 (14.7)	810 (22.4)	344 (19.7)	<0.001
Cancer, %	1696 (16.1)	1100 (17.9)	313 (14.4)	283 (12.7)	<0.001	787 (15.1)	701 (19.4)	208 (11.9)	<0.001
BMD, gm/cm^2									
Total femur	0.92 (0.16)	0.92 (0.16)	0.94 (0.17)	0.92 (0.17)	<0.001	0.91 (0.16)	0.94 (0.16)	0.91 (0.17)	<0.001

(Continued)

TABLE 2 Continued

Characteristics	Total	Cotinine category, %			*P* value	Self-reported smoking status, %			*P* value
		<0.05 ng/mL	0.05–2.99 ng/mL	≥3 ng/mL		Nonsmoker	Former smoker	Current smoker	
Femoral neck	0.77 (0.14)	0.76 (0.14)	0.78 (0.15)	0.77 (0.14)	<0.001	0.76 (0.15)	0.77 (0.14)	0.77 (0.14)	<0.001
Trochanter	0.70 (0.14)	0.70 (0.14)	0.71 (0.15)	0.69 (0.14)	<0.001	0.69 (0.14)	0.72 (0.14)	0.69 (0.14)	<0.001
Intertrochanter	1.10 (0.20)	1.09 (0.19)	1.12 (0.20)	1.10 (0.20)	<0.001	1.09 (0.20)	1.12 (0.19)	1.09 (0.20)	<0.001
Osteoporosis, %	1106 (10.5)	657 (10.7)	190 (8.7)	259 (11.6)	0.005	546 (10.5)	356 (9.9)	204 (11.7)	0.124
Osteopenia, %	6927 (65.6)	4105 (66.7)	1330 (61.0)	1492 (67.0)	<0.001	3373 (64.8)	2375 (65.8)	1179 (67.5)	0.119

Normally distributed continuous variables are described as means ± SEs, and continuous variables without a normal distribution are presented as medians [interquartile ranges]. Categorical variables are presented as numbers (percentages). HDL-C, high-density lipoprotein cholesterol; CVD, cardiovascular disease; BMD, bone mineral density.

Discussion

As shown by our findings, both serum cotinine levels and self-reported smoking status have an impact on OP and osteopenia. This relationship remained constant even after the addition of other factors (education level, drinking status, poverty status, physical activity status, total energy intake, total cholesterol level, high-density lipoprotein cholesterol level, serum calcium level, history of prednisone or cortisone use, and diagnoses of hypertension, diabetes, and cardiovascular disease, and cancer). Our dose–response analysis also showed nonlinear and positive associations between serum cotinine and the prevalence of OP as well as osteopenia.

A 2011-2018 NHANES cross-sectional study showed that elevated serum cotinine levels were associated with reduced lumbar BMD in 7905 participants aged 30 years and over, particularly in women (26). This study demonstrated that reducing cigarette exposure and maintaining serum cotinine at lower levels may be beneficial for bone health in adults. However, the study failed to calculate the specific breakpoints of the curve. Second, other confounding factors were not considered, so there is a possibility of bias. Finally, the sample size included was insufficient. Therefore, we further analyzed the NHANES cross-sectional study from 2005-2018, 10,564 participants aged ≥50 years, to assess not only the association between serum cotinine levels with OP and osteopenia, but also the association between smoking status with OP and osteopenia. In addition, we considered the effect of other confounders on the study results, such as (drinking status, poverty status, total energy intake, total cholesterol level, high-density lipoprotein cholesterol level, history of prednisone or cortisone use, and diagnoses of hypertension, diabetes, and cardiovascular disease, and cancer), reducing the possibility of biased results. In last, we analyzed the relationship between serum cotinine levels and OP and osteopenia, which were nonlinearly and positively correlated, with inflection points of 5.82 ng/ml and 3.26 ng/ml, respectively. This provides a preliminary basis for the study of OP or osteopenia and the mechanism of action of serum cotinine.

We found that associations between smoking and OP and osteopenia were consistent with other cross-sectional studies (27–29). For example, the results of a Swedish study investigating the association

TABLE 3 Association of smoking status, serum cotinine level and the prevalence of osteoporosis.

	OR (95% CI)		
	Model 1	Model 2	Model 3
Log2-transformed cotinine, ng/mL	1.07 (1.05-1.08)	1.06 (1.04-1.07)	1.06 (1.04-1.07)
Cotinine categories			
<0.05 ng/mL	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
0.05–2.99 ng/mL	0.99 (0.83-1.18)	0.91 (0.76-1.09)	0.91 (0.76-1.10)
≥3 ng/mL	2.27 (1.91-2.69)	2.03 (1.70-2.43)	2.04 (1.70-2.45)
P for trend	<0.001	<0.001	<0.001
Self-reported smoking status, %			
Nonsmokers	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Former smokers	0.96 (0.82-1.12)	1.01 (0.86-1.18)	1.02 (0.87-1.20)
Current smokers	2.30 (1.90-2.79)	2.16 (1.77-2.64)	2.16 (1.77-2.65)
P for trend	<0.001	<0.001	<0.001

Model 1 was adjusted as age, sex, and race;
Model 2 was adjusted as model 1 plus education level, drinking status, poverty, physical activity, and total energy intake.
Model 3 was adjusted as model 2 plus total cholesterol, high-density lipoprotein cholesterol, serum calcium, history of prednisone or cortisone, hypertension, diabetes, cardiovascular disease, and cancer.

10.3389/fendo.2023.1074574

TABLE 4 Association of smoking status, serum cotinine level and the prevalence of osteopenia.

	OR (95% CI)		
	Model 1	Model 2	Model 3
Log2-transformed cotinine, ng/mL	1.04 (1.03-1.05)	1.04 (1.03-1.05)	1.04 (1.03-1.05)
Cotinine categories			
<0.05 ng/mL	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
0.05–2.99 ng/mL	0.96 (0.86-1.07)	0.94 (0.84-1.04)	0.95 (0.85-1.06)
≥3 ng/mL	1.65 (1.47-1.85)	1.59 (1.41-1.79)	1.60 (1.42-1.80)
P for trend	<0.001	<0.001	<0.001
Self-reported smoking status, %			
Nonsmokers	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Former smokers	0.99 (0.90-1.09)	1.01 (0.92-1.12)	1.04 (0.94-1.15)
Current smokers	1.72 (1.52-1.95)	1.69 (1.49-1.93)	1.70 (1.49-1.94)
P for trend	<0.001	<0.001	<0.001

Model 1 was adjusted as age, sex, and race;
Model 2 was adjusted as model 1 plus education level, drinking status, poverty, physical activity, and total energy intake.
Model 3 was adjusted as model 2 plus total cholesterol, high-density lipoprotein cholesterol, serum calcium, history of prednisone or cortisone, hypertension, diabetes, cardiovascular disease, and cancer.

between smoking status and skeletal parameters (area BMD, volume BMD, etc.) in 1068 young (mean age 18.9 years) men showed that smokers had lower whole-body (-2.1%), lumbar spine (-4.3%), femoral neck (- 5.3%) and femoral rotor (-6.6%) BMD, but there was no difference in the distribution of volumetric BMD between smokers and nonsmokers (27). In addition, an Icelandic quantitative computed tomography scan of the hip including 2673 older adults (55.9% female) aged 66 to 92 years at baseline demonstrated that the volumetric BMD of cortical bone in the hip was lower in current smokers than in those who were never smokers and that the volumetric BMD of whole hip, volumetric BMD of trabecular bone, and volumetric BMD of cortical bone were lower in current smokers than in nonsmokers. The lower proportions of total volumetric BMD, trabecular bone volumetric BMD, and cortical bone volumetric BMD among current smokers suggest that smoking may accelerate aging-induced osteopenia (28). As reported by relevant studies, male smokers had lower trabecular bone mineral density (-26.6 to -30.3%), and lower trabecular bone scores (-13.5 to -15.3%) in the radius and tibia than current quitters or those who were never smokers. There were fewer trabeculae, thinner areas, and larger intertrabecular spaces, but there was no significant variability in cortical bone parameters. Among female smokers, radial cortical porosity was twice as large in smokers as in nonsmokers, and tibial cortical porosity was 50% larger in smokers than in nonsmokers, with no statistically significant differences in trabecular parameters between smokers and nonsmokers (29).

As revealed by our in-depth research on the nonlinear and positive correlation of serum cotinine levels with OP and osteopenia, the relationship between smoking and osteopenia is at least partially correlated with the effects of nicotine. Indeed, studies have demonstrated that nicotine not only has a direct toxic effect on osteoblasts but is also associated with an increase in osteoclasts (9, 30). In addition, nicotine inhibits aromatase activity and exerts antiestrogenic effects, and the decrease in gonadal hormone levels gives rise to a decrease in osteoblast activity and proliferation and an

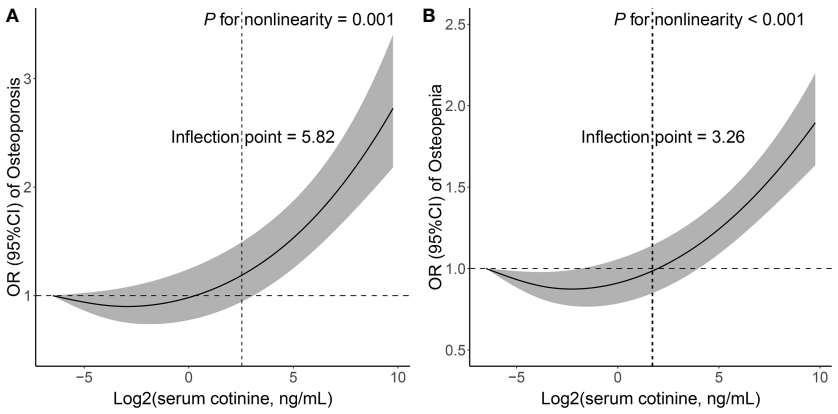

FIGURE 2
Associations between log2-transformed serum cotinine levels and the prevalence of osteoporosis **(A)** as well as osteopenia **(B)**.

increase in the resorptive activity of osteoclasts (31, 32). Finally, nicotine heightens inflammation, disrupts the body's oxidative-antioxidant balance, elevates malondialdehyde levels, and decreases superoxide dismutase and catalase levels, all of which may contribute to osteoporosis (16, 33).

NHANES data were collected and screened by adopting a standardized, uniform protocol to ensure the accuracy, consistency, and reliability of the study data and results. The large community sample ensured the reliability of the results. The inclusion of some important confounding factors in the regression analysis was more intuitive and comprehensive than the mechanism study. Nevertheless, the study still has some limitations. Above all, because this study was based on a cross-sectional survey of NHANES, causal relationships among dependent, independent, and covariate variables could not be inferred. Moreover, because the half-life of nicotine varies among and within individuals, a single indicator of cotinine may not necessarily reflect long-term nicotine exposure. Last but not least, NHANES data sources are measured or collected only once, which heightens the potential for data bias. Accordingly, the database may be replicated multiple times in subsequent studies.

In summary, our study demonstrated that tobacco smoke exposure was correlated with the prevalence of OP or osteopenia in a representative sample of the elderly population in the U.S. The dose-effect phenomenon exhibited a nonlinear and positive relationship between serum cotinine levels and the prevalence of OP or osteopenia. Consequently, the mechanism of OP or osteopenia and serum cotinine needs to be further explored in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The National Center for Health Statistics (NCHS) Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WH and SC designed the study, analyzed the data, and wrote the manuscript. CZ, LZ and YG analyzed the data. ZZ and LZ revised the manuscript. WH and SC have contributed equally to this work. All authors contributed to the article and approved the submitted version.

Funding

The work was funded by The Provincial Natural Science Foundation of Anhui (2008085MH281).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1074574/full#supplementary-material

References

1. Reid IR, Billington EO. Drug therapy for osteoporosis in older adults. *Lancet (Lond Engl)* (2022) 399(10329):1080–92. doi: 10.1016/S0140-6736(21)02646-5

2. Gregson CL, Armstrong DJ, Bowden J, Cooper C, Edwards J, Gittoes NJL, et al. UK Clinical guideline for the prevention and treatment of osteoporosis. *Arch Osteoporosis* (2022) 17(1):58. doi: 10.1007/s11657-022-01061-5

3. Anam AK, Insogna K. Update on osteoporosis screening and management. *Med Clinics North Am* (2021) 105(6):1117–34. doi: 10.1016/j.mcna.2021.05.016

4. Lorentzon M, Johansson H, Harvey NC, Liu E, Vandenput L, McCloskey EV, et al. Osteoporosis and fractures in women: the burden of disease. *Climacteric: J Int Menopause Society* (2022) 25(1):4–10. doi: 10.1080/13697137.2021.1951206

5. Ensrud KE, Crandall CJ. Osteoporosis. *Ann Internal Med* (2017) 167(3):Itc17–itc32. doi: 10.7326/AITC201708010

6. Yang CY, Cheng-Yen Lai J, Huang WL, Hsu CL, Chen SJ. Effects of sex, tobacco smoking, and alcohol consumption osteoporosis development: Evidence from Taiwan biobank participants. *Tobacco Induced Dis* (2021) 19:52. doi: 10.18332/tid/136419

7. Cusano NE. Skeletal effects of smoking. *Curr Osteoporosis Rep* (2015) 13(5):302–9. doi: 10.1007/s11914-015-0278-8

8. Li H, Wallin M, Barregard L, Sallsten G, Lundh T, Ohlsson C, et al. Smoking-induced risk of osteoporosis is partly mediated by cadmium from tobacco smoke: The MrOS Sweden study. *J Bone Mineral Res: Off J Am Soc Bone Mineral Res* (2020) 35 (8):1424–9. doi: 10.1002/jbmr.4014

9. Marinucci L, Balloni S, Fettucciari K, Bodo M, Talesa VN, Antognelli C. Nicotine induces apoptosis in human osteoblasts *via* a novel mechanism driven by H(2)O(2) and entailing glyoxalase 1-dependent MG-H1 accumulation leading to TG2-mediated NF-kB desensitization: Implication for smokers-related osteoporosis. *Free Radical Biol Med* (2018) 117:6–17. doi: 10.1016/j.freeradbiomed.2018.01.017

10. Chung SM. Long-term sex-specific effects of cadmium exposure on osteoporosis and bone density: A 10-year community-based cohort study. *J Clin Med* (2022) 11 (10):2899. doi: 10.3390/jcm11102899

11. Papa V, Bimonte VM, Wannenes F, D'Abusco AS, Fittipaldi S, Scandurra R, et al. The endocrine disruptor cadmium alters human osteoblast-like saos-2 cells homeostasis

in vitro by alteration of wnt/β-catenin pathway and activation of caspases. *J Endocrinological Invest* (2015) 38(12):1345–56. doi: 10.1007/s40618-015-0380-x

12. Banjabi AA, Kurunthachalam K, Kumosani TA, Abulnaja KO, Al-Malki AL, Moselhy SS. Serum heavy metals of passive smoker females and its correlation to bone biomarkers and risk of osteoporosis. *Environ Sci Pollut Res Int* (2022) 29(5):6943–8. doi: 10.1007/s11356-021-16186-2

13. Hukkanen J, Jacob P3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* (2005) 57(1):79–115. doi: 10.1124/pr.57.1.3

14. Benowitz NL, Bernert JT, Foulds J, Hecht SS, Jacob P, Jarvis MJ, et al. Biochemical verification of tobacco use and abstinence: 2019 update. *Nicotine tobacco research: Off J Soc Res Nicotine Tobacco* (2020) 22(7):1086–97. doi: 10.1093/ntr/ntz132

15. Kim S. Overview of cotinine cutoff values for smoking status classification. *Int J Environ Res Public Health* (2016) 13(12):1236. doi: 10.3390/ijerph13121236

16. Aspera-Werz RH, Ehnert S, Heid D, Zhu S, Chen T, Braun B, et al. Nicotine and cotinine inhibit catalase and glutathione reductase activity contributing to the impaired osteogenesis of SCP-1 cells exposed to cigarette smoke. *Oxid Med Cell Longevity* (2018) 2018:3172480. doi: 10.1155/2018/3172480

17. Kiyota Y, Muramatsu H, Sato Y, Kobayashi T, Miyamoto K, Iwamoto T, et al. Smoking cessation increases levels of osteocalcin and uncarboxylated osteocalcin in human sera. *Sci Rep* (2020) 10(1):16845. doi: 10.1038/s41598-020-73789-4

18. Huyett P, Siegel N, Bhattacharyya N. Prevalence of sleep disorders and association with mortality: Results from the NHANES 2009-2010. *Laryngoscope* (2021) 131(3):686–9. doi: 10.1002/lary.28900

19. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiologic Rev* (1996) 18(2):188–204. doi: 10.1093/oxfordjournals.epirev.a017925

20. Bernert JTJr., Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clin Chem* (1997) 43(12):2281–91. doi: 10.1093/clinchem/43.12.2281

21. Dove MS, Dockery DW, Connolly GN. Smoke-free air laws and secondhand smoke exposure among nonsmoking youth. *Pediatrics* (2010) 126(1):80–7. doi: 10.1542/peds.2009-3462

22. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the united states between 1999 and 2004. *Am J Epidemiol* (2009) 169(2):236–48. doi: 10.1093/aje/kwn301

23. Kanis JA, Melton LJ3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone mineral research: Off J Am Soc Bone Mineral Res* (1994) 9(8):1137–41. doi: 10.1002/jbmr.5650090802

24. Slemenda C, Longcope C, Peacock M, Hui S, Johnston CC. Sex steroids, bone mass, and bone loss. a prospective study of pre-, peri-, and postmenopausal women. *J Clin Invest* (1996) 97(1):14–21. doi: 10.1172/JCI118382

25. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, et al. Physical activity and public health. a recommendation from the centers for disease control and prevention and the American college of sports medicine. *Jama* (1995) 273(5):402–7. doi: 10.1001/jama.1995.03520290054029

26. Fang JG, Wang DJ, Yang HY, Zhang H, Tong JY, Lin ZJ. Association between serum cotinine levels and bone mineral density: An analysis of the national health and nutrition examination survey (NHANES). *Int J Endocrinol* (2022) 2022:6830705. doi: 10.1155/2022/6830705

27. Lorentzon M, Mellström D, Haug E, Ohlsson C. Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. *J Clin Endocrinol Metab* (2007) 92(2):497–503. doi: 10.1210/jc.2006-1294

28. Marques EA, Elbejjani M, Gudnason V, Sigurdsson G, Lang T, Sigurdsson S, et al. Cigarette smoking and hip volumetric bone mineral density and cortical volume loss in older adults: The AGES-Reykjavik study. *Bone* (2018) 108:186–92. doi: 10.1016/j.bone.2018.01.014

29. Agarwal S, Germosen C, Kil N, Bucovsky M, Colon I, Williams J, et al. Smoking is associated with sex-specific effects on bone microstructure in older men and women. *J Clin densitometry: Off J Int Soc Clin Densitometry* (2021) 24(3):341–50. doi: 10.1016/j.jocd.2020.07.002

30. Costa-Rodrigues J, Rocha I, Fernandes MH. Complex osteoclastogenic inductive effects of nicotine over hydroxyapatite. *J Cell Physiol* (2018) 233(2):1029–40. doi: 10.1002/jcp.25956

31. Ruan X, Mueck AO. Impact of smoking on estrogenic efficacy. *Climacteric: J Int Menopause Soc* (2015) 18(1):38–46. doi: 10.3109/13697137.2014.929106

32. Cassidenti DL, Pike MC, Vijod AG, Stanczyk FZ, Lobo RA. A reevaluation of estrogen status in postmenopausal women who smoke. *Am J Obstetrics Gynecol* (1992) 166(5):1444–8. doi: 10.1016/0002-9378(92)91617-J

33. Valavanidis A, Vlachogianni T, Fiotakis K. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int J Environ Res Public Health* (2009) 6(2):445–62. doi: 10.3390/ijerph6020445

frontiers | Frontiers in Physiology

TYPE Mini Review
PUBLISHED 05 June 2023
DOI 10.3389/fphys.2023.1177829

OPEN ACCESS

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RECEIVED 02 March 2023
ACCEPTED 23 May 2023
PUBLISHED 05 June 2023

CITATION
Aguilar A, Gifre L, Ureña-Torres P, Carrillo-López N, Rodriguez-García M, Massó E, da Silva I, López-Báez V, Sánchez-Bayá M, Prior-Español Á, Urrutia M, Paul J, Bustos MC, Vila A, Garnica-León I, Navarro-González JF, Mateo L and Bover J (2023), Pathophysiology of bone disease in chronic kidney disease: from basics to renal osteodystrophy and osteoporosis. *Front. Physiol.* 14:1177829. doi: 10.3389/fphys.2023.1177829

Pathophysiology of bone disease in chronic kidney disease: from basics to renal osteodystrophy and osteoporosis

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Chronic kidney disease (CKD) is a highly prevalent disease that has become a public health problem. Progression of CKD is associated with serious complications, including the *systemic* CKD-mineral and bone disorder (CKD-MBD). Laboratory, bone and vascular abnormalities define this condition, and all have been independently related to cardiovascular disease and high mortality rates. The "old" cross-talk between kidney and bone (classically known as "renal osteodystrophies") has been recently expanded to the cardiovascular system, emphasizing the importance of the bone component of CKD-MBD. Moreover, a recently recognized higher susceptibility of patients with CKD to falls and bone fractures led to important paradigm changes in the new CKD-MBD guidelines. Evaluation of bone mineral density and the diagnosis of "osteoporosis" emerges in nephrology as a new possibility "if results will impact clinical decisions". Obviously, it is still reasonable to perform a bone biopsy if knowledge of the type of renal osteodystrophy will be clinically useful (low *versus* high turnover-bone disease). However, it is now considered that the inability to perform a bone biopsy may not justify withholding antiresorptive therapies to patients with high risk of fracture. This view adds to the effects of parathyroid hormone in CKD patients and the classical treatment of secondary hyperparathyroidism. The availability of new antiosteoporotic treatments bring the opportunity to come back to the basics, and the knowledge of new pathophysiological pathways [OPG/RANKL (LGR4); Wnt-ß-catenin pathway], also affected in CKD, offers great opportunities to further unravel the complex physiopathology of CKD-MBD and to improve outcomes.

KEYWORDS

CKD-MBD, renal osteodystrophy, osteoporosis, adynamic bone disease, sclerostin, RANKL (receptor activator for nuclear factor k B ligand), parathyroid hormone, Wnt

Introduction

Chronic kidney disease (CKD) is a highly prevalent and progressive condition that affects more than 10% of the general population around the globe (Kalantar-Zadeh et al., 2021; Csaba, 2022). CKD has also emerged as one of the leading causes of morbidity and mortality and represents significant challenges for healthcare systems and societies worldwide (Elshahat et al., 2020; Kalantar-Zadeh et al., 2021; Alberto Ortiz, 2022; Csaba, 2022). Importantly, progression of CKD is associated with a number of serious complications, including mineral metabolism disorders and bone pathology, and these are independently associated with fractures, accelerated vascular calcification, cardiovascular disease, and dismal outcomes (Moe et al., 2006; Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Wang et al., 2018; Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009). Bone is no longer regarded simply as an organ that supports and protects internal organs and we even considered to be as a new endocrine organ at the heart of the CKD-mineral and bone disorders (CKD-MBD) (Vervloet et al., 2014). Bone is capable of secreting countless hormones and molecules essential for the normal physiology of many other body systems (Vervloet et al., 2014). Consequently, CKD-MBD is a currently accepted term which refers to a *systemic* disorder of mineral and bone metabolism due to CKD manifested by various laboratory, bone, and vascular abnormalities (Moe et al., 2006; Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009; Torregrosa et al., 2022).

Previously, the term *renal osteodystrophy* (ROD) had been coined in 1943 (Llach et al., 2000), 60 years after the identification of an association between bone disease and kidney failure (Lucas, 1883; Llach et al., 2000). ROD was a very broad term that classically included all the skeletal manifestations in patients suffering from CKD or end-stage kidney disease (CKD G5D) (Llach et al., 2000). In children, rickets and skeletal deformities were also included, while osteosclerosis and osteoporosis (OP) were globally considered less common (Llach et al., 2000). Nevertheless, ROD is nowadays considered to be only one component of the wider complex CKD-MBD after a histomorphometric analysis of a bone biopsy has been performed (Moe et al., 2006; Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017). Derangements induced by CKD are multiple, ranging from the classically described disturbances of vitamin D metabolism, calcium, and phosphate balance, through increased levels of parathyroid hormone (PTH) (secondary hyperparathyroidism) to the more recently recognized increases in fibroblast growth factor 23 (FGF23) and sclerostin, or decreased serum klotho levels, among others (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Llach et al., 2000; Hruska et al., 2017). Chronic metabolic acidosis, the use of drugs such as prednisone or calcineurin inhibitors (used to treat certain kidney diseases), diabetes mellitus, accelerated aging, female gender, and early menopause can additionally affect one or more bone properties. A detailed description of all the pathophysiological pathways leading to different forms of ROD is beyond the scope of this article, and we refer interested readers to excellent reviews elsewhere (Goltzman et al., 2018). Nevertheless, in this narrative review we will briefly address the relevant basics as well as evolving topics in bone pathophysiology of interest beyond nephrology. In fact, important paradigm changes from ROD to OP have occurred in recent CKD-MBD guidelines and these need to be more widely known.

Bone cells

The most important cells of bone tissue are osteoblasts (OBs), osteoclasts (OCs), osteocytes, and bone-lining cells.

a) OBs develop from pluripotential mesenchymal stem cells (MSCs). MSCs can differentiate into adipocytes, chondrocytes, myocytes, or OBs depending on the transcription factor acting on them. Bone morphogenic proteins (BMPs) and the Wnt signaling pathway are related to OB differentiation. The *canonical* Wnt signaling pathway induces transcription factors that favor OB differentiation, and the *non-canonical* Wnt pathway inhibits the differentiation of MSCs to other cell types, resulting overall in a positive balance towards OB formation. The main function of OBs is the *formation* of the bone matrix through the synthesis and secretion of type 1 collagen and other non-collagenous proteins which will later be mineralized. OBs also collaborate in this function by releasing phosphate contained in their vesicles and, together with the calcium and phosphate contained in the extracellular fluid, compose the main mineral of cortical bone (calcium hydroxyapatite crystals) (Day et al., 2005; Takada et al., 2007; Guo et al., 2010). The N-terminal propeptide of type I procollagen (P1NP) has been identified by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry (IFCC) to be one of the reference markers of bone turnover (formation) for fracture risk prediction and monitoring of OP treatment (Vasikaran et al., 2011). It is important to take into account the fact that only the measurement of *intact* P1NP is not affected by the decreased renal function in patients with CKD (Bover et al., 2021a; Tridimas et al., 2021). Alkaline phosphatase (AP, especially the bone isoform) can also be used to evaluate bone turnover in CKD (Bover et al., 2021a). Actually, AP can reflect not only OB activity in bone but also OB-like cell activity in the active process of *ossification* of vascular smooth muscle cells (Bover et al., 2018; Bover et al., 2021b).
b) OCs derive from precursor cells of the monocyte-macrophage lineage. OC differentiation and survival require the presence of molecules such as the macrophage colony-stimulating factor (M-CSF) and the important receptor activator of NF-κB

ligand (RANKL). OB-synthesized osteoprotegerin (OPG) acts as a high-affinity decoy receptor for RANKL, inhibiting RANKL action on the OC-RANK receptor (Wada et al., 2006). The ratio between RANKL and OPG determines the degree of osteoclastic differentiation (Gori et al., 2000), although blood measurements are not of clinical use. It has been recently described another RANKL receptor, the leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4), which competes with RANK to bind RANKL and suppresses canonical RANK signaling during OC differentiation (Luo et al., 2016). It also regulates OB differentiation *in vivo* and *in vitro* (Luo et al., 2009). LGR4 is also present in different tissues and consequently it has been linked with systemic roles from development to metabolic regulation (Filipowska et al., 2022).

The main function of the OC is bone *resorption*. OCs must be activated by binding to the bone matrix, polarizing and forming podosomes and different membrane domains (the sealing zone, the characteristic ruffled border, and the functional secretory domain). Each of these domains is extremely important for bone resorption, collagen degradation, and the return of calcium and phosphate to the bloodstream (Luxenburg et al., 2007). Lysosomal enzymes derived from OCs are responsible for breakdown of the collagenous bone matrix at specific sites (Bover et al., 2021a). Resultant products such as carboxy-terminal crosslinking telopeptide of type 1 collagen (CTX) are considered reference markers for bone resorption in the general population (Wheater et al., 2013). However, CTX is highly dependent on kidney function; therefore, the use of CTX cannot be recommended in patients with CKD (Bover et al., 2021a). For this reason, tartrate-resistant acid phosphatase 5b (TRAP5b) is gaining increasing importance, given that its concentration is not kidney dependent (Bover et al., 2021a).

c) Osteocytes represent 95% of all bone cells. These cells are mature OBs that occupy the lacunar space and are surrounded by the unmineralized osteoid matrix. After mineralization, these buried cells become osteocytes and acquire long dendritic-like processes, giving them a star-shaped appearance. Dendritic processes extend along the canaliculi in the bone matrix, interacting with other osteocytes or with OBs on the surface. Osteocytes have a position that allows detection of both mechanical and metabolic signals and act accordingly, directly activating OBs and indirectly OCs, thus initiating the classic remodeling cycle. Osteocytes influence OBs in two directions, either upregulating them through the production of messengers such as nitric oxide and prostaglandin E2 or downregulating them through the secretion of sclerostin (Rochefort et al., 2010). As we will discuss later, osteocytes and sclerostin have gained increased attention in bone pathophysiology, nephrology, and medicine in general since their discovery and the development of new treatments for bone diseases such as OP. Osteocytes are also the main source of FGF23, a pleiotropic hormone responsible of suppressing phosphate reabsorption and calcitriol synthesis in the kidney (Orlando, 2020). Although FGF23 monitoring is not yet included in the regular management of CKD-MBD, it is important to emphasize its role in the development of left ventricular hypertrophy (Richter and Faul, 2018), among other systemic effects (Vervloet, 2020), and its powerful inverse association with survival in CKD patients (Gutiérrez et al., 2008).

Normal bone anatomy and physiology

Two histologically different regions can be distinguished in bone: a) *cortical or compact bone*, which represents up to 80% of the skeleton, is composed mainly of calcium hydroxyapatite, and has the main function of providing mechanical support, and b) *trabecular or cancellous bone,* which is less abundant, is mainly composed of an organic matrix rich in type 1 collagen, has an important endocrine function, and contains the bone marrow.

Several biological processes occur in bone tissue during life. Bone undergoes *modelling and remodelling* in order to grow or change shape (Katsimbri, 2017). Bone *modelling* is a process by which bones change shape or size in response to physiological influences or mechanical forces that are encountered by the skeleton (Katsimbri, 2017). *Remodelling* is the process which allows bone to maintain its mineral homeostasis and strength (Katsimbri, 2017). Once bone growth stops when adult age is reached (after bone formation and shaping), the bone tissue requires dynamic remodelling to maintain adequate resistance and properties (Katsimbri, 2017). Old bone tissue is removed and replaced by new tissue through an organized process occurring within temporary anatomical structures described as basic multicellular units. Five stages are described in the remodelling process. They are widely known as activation, resorption, rest or reversal, bone formation, and termination; however, a detailed description of these mechanisms is beyond the scope of this review (Burger et al., 2003; Martin and Sims, 2005; Bonewald, 2007; Bonewald and Johnson, 2008; Katsimbri, 2017).

Metabolic bone biopsy

Bone dynamics can only be assessed by a *bone biopsy*, a classic procedure previously performed frequently by nephrologists in order to precisely distinguish among different forms of ROD (e.g., high and low-turnover bone disease) (Moe et al., 2006; Bover et al., 2021a). No biomarker or imaging studies can match this gold standard (Bover et al., 2021a). Tetracycline labeling allows *dynamic* quantification, e.g., by analyzing the important *bone formation rate* and the *mineral apposition rate*, among other dynamic parameters. Many other *static* evaluations, such as the *osteoid area* or the percentage of *fibrosis,* contribute to a precise diagnosis (Mazzaferro and Pasquali, 2021). Essentially, bone biopsies should evaluate bone turnover, mineralization and volume (following the useful acronym TMV) (Moe et al., 2006). Thus, different patterns of ROD are usually described (see below): high-turnover osteitis fibrosa or mild hyperparathyroidism, low-turnover adynamic bone disease (ABD) and osteomalacia, and the mixed form named uremic osteodystrophy (Moe et al., 2006).

Bone biopsies are regaining importance in nephrology, especially with the paradigm changes that have appeared in recent CKD-MBD guidelines (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group,

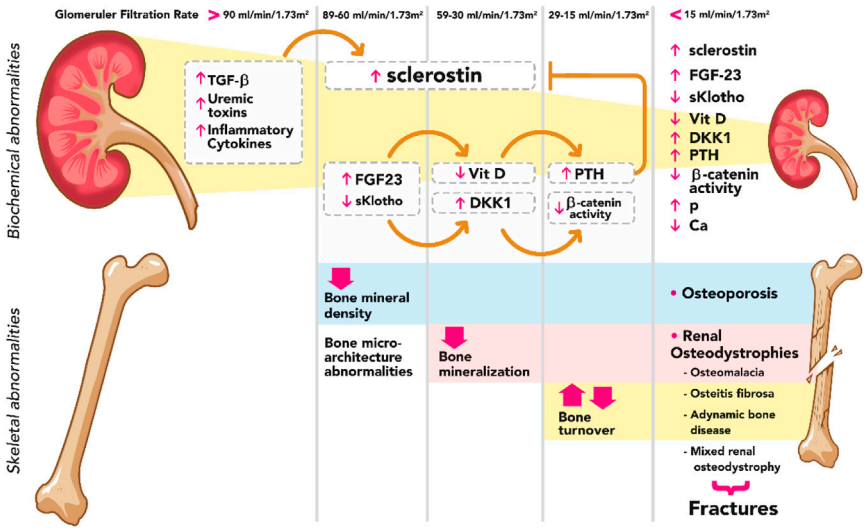

FIGURE 1
Summary diagram of biochemical and skeletal abnormalities in CKD. Sclerostin rises from very early stages of CKD with TGF-β, uremic toxins, and inflammatory cytokines as main known stimuli. Elevated FG23, in the presence of sKlotho, increases the expression of Dkk1, a Wnt inhibitor; consequently, Wnt-β catenin signaling remains suppressed during the course of CKD. FGF23 decreases the levels of calcitriol, resulting in a stimulus for PTH synthesis and secretion which, on the other hand, decreases the effects of sclerostin. TGF-β, transforming growth factor beta; FGF-23, fibroblast growth factor-23; Vit D, vitamin D; DKK1, Dickkopf 1; PTH, parathyroid hormone; P phosphorus; Ca calcium.

2017; Torregrosa et al., 2022). The potential need for better understanding of the consequences of the current more aggressive use of anti-OP treatments in patients with CKD represents an additional reason for this trend (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022). Thus, it is currently considered reasonable to perform a bone biopsy if knowledge of the type of ROD will impact treatment decisions in patients with CKD G3a-G5D (not graded) (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022). It was previously considered reasonable to perform a bone biopsy "in various settings including, but not limited to: unexplained fractures, persistent bone pain, unexplained hypercalcemia, unexplained hypophosphatemia, possible aluminum toxicity, and prior to therapy with bisphosphonates in patients with CKD-MBD" (not graded either) (Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009). A wider description of indications and the technical procedure is beyond the scope of this review and readers are referred to literature elsewhere (Salusky et al., 1988; Torres et al., 2014; Evenepoel et al., 2017). However, it should be emphasized that efforts are currently being made to standardize the variable "normality" values used in different laboratories when performing histomorphometric analysis (Sprague et al., 2016a). It is also being suggested that bone mineral density (BMD) testing should be used to assess fracture risk "if results will impact treatment decisions" in patients with CKD G3a-G5D with evidence of CKD-MBD and/or risk factors for OP (see later) (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022), since bone biopsy is not useful for fracture risk prediction.

Effects of parathyroid hormone on bone tissue

PTH plays a very important role in the dynamics of bone tissue. Several unanswered but important questions remain about the skeletal actions of PTH, with differences between intermittent administration and constant exposure to high levels (Hock and Gera, 1992; Rendina-Ruedy and Rosen, 2022). Thus, constant high PTH levels can increase bone remodelling to exert a catabolic effect on cortical and, to some extent, trabecular bone (Goltzman, 2018). On the other hand, intermittent administration of PTH can exert an anabolic effect on bone; this is especially the case for trabecular bone but also to some extent for cortical bone (Goltzman, 2018). We describe below some of the effects of PTH on bone cells and the remodelling stages.

PTH and osteoblasts

PTH administration enhances bone formation by inducing transcriptional changes in several OB pathways, being the via adenyl cyclase and protein kinase A (PKA) the most prominent (Rendina-Ruedy and Rosen, 2022) (Figure 1). PTH also influences the entire life cycle of OBs, from their differentiation from pluripotent MSCs through to activation and even apoptosis. PTH appears to increase the amount of OB precursors in the bone marrow through a direct action. The bone marrow cells capable of differentiating to OBs are the colony-forming units-fibroblast (CFU-F) and old studies already demonstrated that the administration of PTH (Kalantar-Zadeh et al., 2021; Csaba, 2022; Alberto Ortiz, 2022; Elshahat et al., 2020; Moe et al., 2006; Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update

Work Group, 2017; Wang et al., 2018; Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009; Vervloet et al., 2014; Torregrosa et al., 2022; Llach et al., 2000; Lucas, 1883; Hruska et al., 2017; Goltzman et al., 2018; Day et al., 2005; Takada et al., 2007; Guo et al., 2010; Vasikaran et al., 2011; Tridimas et al., 2021; Bover et al., 2021a; Bover et al., 2018; Bover et al., 2021b; Wada et al., 2006; Gori et al., 2000; Luo et al., 2016; Luo et al., 2009; Filipowska et al., 2022; Luxenburg et al., 2007; Wheater et al., 2013; Rochefort et al., 2010; Orlando, 2020; Richter and Faul, 2018; Vervloet, 2020; Gutiérrez et al., 2008) to rats for 1 week resulted in the doubling of CFU-F compared with placebo-treated rats (Nishida et al., 1994).

It is well known that PTH cross-talks with the cellular Wnt signaling pathway, stimulating bone formation by increasing the number of OBs. PTH also reduces the levels of sclerostin (downregulating the SOST gene), thus providing another paracrine mechanism through which PTH can stimulate the differentiation of OBs (Bellido et al., 2005; Loots et al., 2005; Drake et al., 2010; Nagata et al., 2022). Within OBs, PTH stimulates the formation of a tertiary complex PTH/PTHrp receptor and the Wnt co-receptor LRP6 (Wan et al., 2008), highlighting the importance of this complex since mice lacking LRP6 in OBs do not respond to iPTH (Wan et al., 2008). This signaling link between PTH and Wnt has also been strengthened by the observation that PTH reduces other Wnt inhibitors [such as Dickkopf 1 (Dkk1), secreted frizzled-related proteins (Sfrp) 1 and 4] (Carrillo-López et al., 2016), and that inhibition of Wnt signaling by Dkk1 prevents the effects of PTH on bone (Li et al., 2006). It has recently been reported that a newly identified osteogenic growth factor, osteolectin/Clec11a, is required for the maintenance of skeletal bone mass during adulthood, and that the combined administration of osteolectin and PTH, but not osteolectin and sclerostin inhibitor, additively increases bone volume (Zhang et al., 2021). These results demonstrate that PTH promotes osteolectin expression and that osteolectin mediates at least part of the effect of PTH on bone formation. (Zhang et al., 2021).

PTH also affects other important series of signaling pathways. For example, PTH stimulates Runx2, an essential transcription factor in bone required for OB differentiation (Arumugam et al., 2019). PTH stimulates the synthesis of growth factors including insulin-like growth factor (IGF)-1 and FGF, both of which are required for the anabolic effects of iPTH (Bikle et al., 2002; Hurley et al., 2006; Wang et al., 2007). Another PTH target gene that has been extensively studied in OBs is the matrix metalloproteinase 13 (MMP13) gene, the expression of which is mediated through an intricate signaling pathway involving PKA, Runx2, sirtuin-1, and others. Thus, PTH upregulation of MMP13 plays an important role in how OBs remodel old bone matrix as they synthesize new type I collagen. (Shimizu et al., 2010; Shimizu et al., 2014; Fei et al., 2015). PTH has also been shown to induce T lymphocytes in the bone marrow microenvironment to produce cytokines that stimulate the differentiation of OBs (Terauchi et al., 2009). Finally, one of the most important effects of PTH in OBs is inhibition of OB apoptosis (Allan et al., 2008; De Pasquale et al., 2008). All these positive actions of PTH on OBs and bone formation represent the basis on which today recombinant PTH (teriparatide) constitutes an alternative in the treatment of OP (Neer et al., 2001). Abaloparatide (an analog of human PTH-related protein) has also recently been approved for OP treatment (Paul et al., 2016). Occasionally, teriparatide has also been used in the treatment of ABD in CKD patients (Cejka et al., 2010; Sumida et al., 2016).

PTH and bone lining cells and osteocytes

Bone lining cells and osteocytes have properties which suggest that they belong to the OB lineage, expressing many of their genes. It has been shown that PTH activates these lining cells, inhibits osteocyte apoptosis, delays the differentiation of OB to lining cells, and increases the conversion of lining cells to OBs (Jang et al., 2016). Osteocytes express receptors for PTH on their surface, in such a way that their morphology and function, including cell retraction, mitochondrial congestion, and cell death, seem PTH regulated (Heller et al., 1950; Cameron et al., 1967; O'Brien et al., 2008; Rhee et al., 2011). On the other hand, PTH upregulates osteocytic RANKL, and RANKL plays a critical role in the PTH-induced increases in bone resorption (see below) (Nakashima and Takayanagi, 2011; Xiong et al., 2011; Ben-awadh et al., 2014; Xiong et al., 2014).

PTH and osteoclasts

OCs do not express receptors for PTH; therefore, PTH action is indirectly mediated through OBs. M-CSF and RANKL are the two main cytokines that drive OC differentiation and function (Feng and Teitelbaum, 2013), and PTH has been shown to increase the expression of these molecules (Itoh et al., 2000). In fact, there are multiple cellular sources of these two cytokines in bone (hypertrophic chondrocytes, marrow stromal cells, osteoblasts, resident marrow lymphocytes, and osteocytes) (O'Brien et al., 2013), and RANKL is a well-studied PTH target gene in multiple cell types (Fu et al., 2002; Fu et al., 2006; Kim et al., 2006; Kim et al., 2007). During OC-mediated bone resorption, growth factors such as TGF-ß1 and IGF-1 are released. IGF-1 is maintained in the bone matrix in complex with binding proteins (IGFBP) and OC bone remodelling leads to IGFBP cleavage and subsequent IGF-1 release (Crane and Cao, 2014). Finally, recent translation studies highlight the potent amplificatory action of T-cell on PTH-induced bone resorption in parathyroid disease (Neale, 2017). PTH acts on $CD4^+$ T-cell to drive up TNFα and IL-17, further amplifying osteoblastic RANKL production and down-regulating OPG, establishing favorable conditions for osteoclastic bone resorption (Neale, 2017).

Disorders of bone remodelling in CKD

Despite previous descriptions of "late rickets associated with albuminuria" by Lucas in The Lancet in 1883 and "tumor of the parathyroid gland" by MacCallum in 1905 (Llach et al., 2000), it was not until 1924 that a study of a patient with severe bone demineralization and multiple fractures led to the discovery that the disease resided in the parathyroid gland (Albright and Reifenstein, 1948). In 1925 the first resection of a parathyroid adenoma was performed (Albright and Reifenstein, 1948). It

1933 Langmead suggested for the first time that parathyroid hyperplasia was secondary to advanced CKD (Llach et al., 2000). Other patients began to be diagnosed with this new disease and there was a need to study derangements in divalent ion metabolism, vitamin D, and the molecule produced by the parathyroid glands that caused so much damage to the bone tissue (Albright and Reifenstein, 1948; Handler et al., 1954; Llach et al., 2000). In the 1960s, Stanbury and Lamb as well as Dent and co-workers (Llach et al., 2000) linked abnormalities of divalent ion metabolism, PTH, and vitamin D with the bone abnormalities observed in CKD. With the advent of radioimmunoassays for PTH (Brewer and Ronan, 1970; Niall et al., 1970; Llach et al., 2000), high circulating levels of PTH were detected at earlier stages of CKD (Llach et al., 2000); however, it was not until 1970 that characterization of the PTH molecule was completed, which helped to its cloning in 1983 (Brewer and Ronan, 1970; Niall et al., 1970; Vasicek et al., 1983). In the 1990s, highly sensitive immunoassays were developed and its receptor was finally cloned in 1991 (Jüppner et al., 1991; Abou-Samra et al., 1992). We now know that at least very low or very high levels of PTH (i.e., less than 2X or more than 9X the upper normal limit for the used assay) are associated with low or high-turnover bone disease (ABD or osteitis fibrosa, respectively) in dialysis patients; both extremes increase not only the risk of fractures but also mortality by different means (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009; Torregrosa et al., 2022).

It was considered previously that the elevation of PTH was the main responsible for skeletal abnormalities in CKD; however, recent evidence has shown that changes in bone tissue occur from early stages (Sabbagh et al., 2012; Baron and Kneissel, 2013a). The increase in sclerostin and FGF23 levels, two molecules secreted by osteocytes, and the consequent repression of Wnt-β catenin signaling pathway represent a clear mechanistic example explaining the impairment of bone health from the onset of CKD (Figure 1) (Cejka et al., 2012; Moysés and Schiavi, 2015; Drüeke and Massy, 2016). Increasing PTH, to a certain extent, may thus appear as an adaptive mechanism to maintain not only normal serum calcium, phosphate and/or calcitriol levels but also a normal bone remodeling (Torregrosa et al., 2022). Another molecule that is elevated in the early stages of CKD and causes changes in bone dynamics is activin A (ActA), a member of the TGF-β superfamily that is secreted by renal fibroblasts. ActA activates receptors on the surface of OCs, leading to the activation of the intracellular protein Smad2. ActA also increases the expression of RANKL by OBs which binds to RANK on the surface of OCs, stimulating the expression and phosphorylation of c-Fos. Together with smad2, these molecules form a complex that enters the nucleus of OCs generating osteoclastogenesis and bone resorption (AgapovaFangSugatani et al., 2016; Sugatani et al., 2017; Cianciolo et al., 2021).

PTH also alters metabolic homeostasis through its actions in other cells and tissues, and myriad non-skeletal metabolic or anabolic effects have been attributed to PTH (Rendina-Ruedy and Rosen, 2022). However, in addition PTH has been considered a uremic toxin in CKD patients with secondary hyperparathyroidism because of many pleiotropic detrimental effects that can be attributed to this molecule (Ureña-Torres et al., 2018). Surgical parathyroidectomy and calcimimetics seem to reverse some of these untoward effects (Massy et al., 2014; Komaba et al., 2015; McMahon et al., 2015; Komaba et al., 2022).

Regarding bone, PTH levels are actually only indirectly associated with bone formation (a secondary impact), and they probably represent parathyroid activity at a certain time point much better than bone dynamics (Pablo and Covic, 2020). Moreover, unlike most other biomarkers or regulators of bone turnover, such as APs or P1NP, PTH secretion is not dictated by local bone demands (triggered on osteocytes via mechanical stimuli) (Bover et al., 2018). Actually, the major determinants of PTH synthesis and secretion are calcium, phosphate, vitamin D, and FGF23 levels (Levin et al., 2007; Sturgeon et al., 2017). In fact, according to current guidelines, the measurement of both serum PTH and bone AP can be used to evaluate bone disease in CKD patients because markedly high or low values predict underlying bone turnover (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Jørgensen et al., 2022a). Combination of PTH and APs significantly increases both the sensitivity and the specificity of the histopathological diagnosis (Ureña et al., 1996; Bover et al., 2018). These biomarkers offer the possibility of more frequent serial measurements that can guide us on therapeutic decision-making and follow-up. However, despite it has been recently recognized that diagnostic performance of biochemical markers of bone turnover is acceptable, with clinical utility in ruling out both high and low turnover bone disease, biomarkers do have some limitations such as the scarcity of data available to consider precise target figures, their biological variability and the inherent disparity of trials leading to different reference ranges and cut-offs (Jørgensen et al., 2022a; Jørgensen et al., 2022b).

While the parathyroid glands can be affected by a primary disease, such as parathyroid adenoma or the rare parathyroid neoplasms, *secondary* (or even tertiary) hyperparathyroidism is frequently observed during the course of CKD (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022). Below we describe the two most common histologic patterns, which derive from very high (osteitis fibrosa) and relatively low levels of PTH (ABD).

Osteitis fibrosa

The first report of this disease derived from very high PTH levels was made by von Recklinghausen in 1891 (Von Recklinghausen, 1891), but the full report of *"osteitis fibrosa cystica"* was not provided until 1936, by Albright and co-workers (Llach et al., 2000). CKD-associated hyperparathyroidism was considered by far the most prevalent form of ROD until recently, especially among advanced CKD and dialysis patients. However, current patterns show a lower prevalence ranging from 20% to 40% (Bover et al., 2014). The pathophysiology of secondary/tertiary hyperparathyroidism is beyond the scope of this review and interested readers are referred elsewhere (Llach et al., 2000; Cunningham et al., 2011; Lunyera and Scialla, 2018). After increased synthesis and secretion of PTH by multiple stimuli (hypocalcemia, hyperphosphatemia, decreased calcitriol, *etc.*), PTH binds to its receptors in bone tissue (mostly PTHR1), which are located

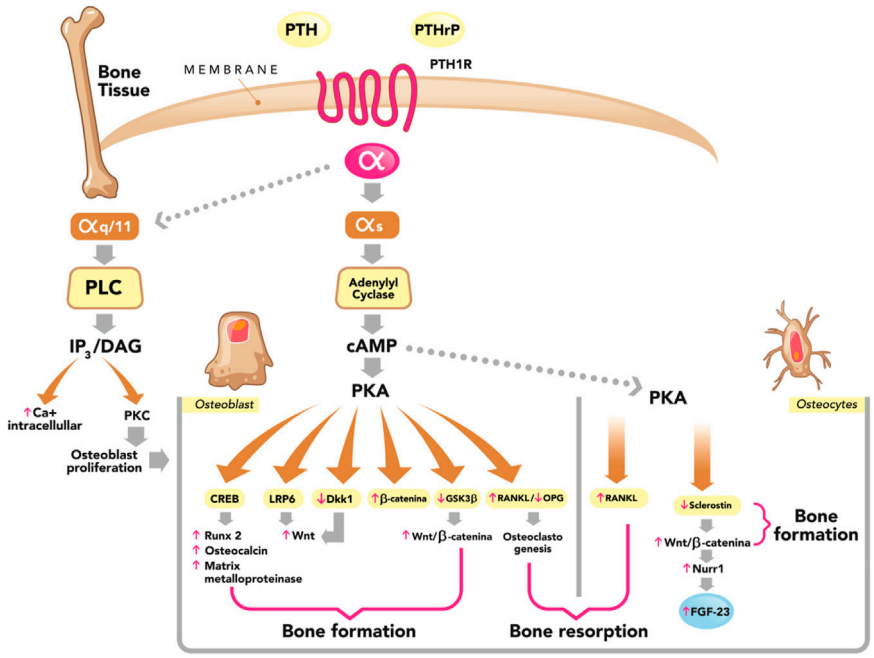

FIGURE 2
Dual effect of PTH on bone (formation and resorption). Parathyroid hormone (PTH) and PTH-related peptide (PTHrP) bind to the PTH/PTHrP type 1 receptor (PTH1R) with similar effects. This G protein-coupled receptor generates a dissociation of the α subunit. The most studied is the αs subunit and its main effector is adenylyl cyclase, which catalyzes the synthesis of the second messenger cyclic adenosine monophosphate (cAMP), which activates the cAMP-dependent protein kinase (PKA) pathway, phosphorylating several proteins that have diverse effects on bone and that we describe below: phosphorylation of cAMP response element-binding protein (CREB), increasing the expression of osteoblast-specific genes such as Runx 2, Osteocalcin and matrix metalloproteinase; stimulation of the Wnt-signaling pathway Wnt by means of the low-density lipoprotein receptor-related protein 6 (LRP6); phosphorylation and stabilization of β-catenin, suppression of Dickkopf 1 (DKK1) and glycogen synthase kinase 3 beta (GSK3β). Both are Wnt inhibitors, leading to the stimulation of the Wnt/B-catenin signaling pathway. All the aforementioned actions of PTH represent stimuli for bone formation (osteoanabolic effect). On the other hand, PTH in osteoblasts and osteocytes stimulates the production of receptor activator of nuclear factor Kβ ligand (RANKL) and decreases the production of osteoprotegerin (OPG), generating osteoclastogenesis and thereby stimulating bone resorption. In osteocytes, PTH suppresses the expression of sclerostin and this stimulates the Wnt/B-catenin signaling pathway, stimulating bone formation. PTH in osteocytes also stimulates the expression of nuclear receptor-related 1 protein (Nurr1), leading to an increase in the production of fibroblast growth factor-23 (FGF-23). PTH1R, in addition to coupling to αs, can also couple to αq/11, which activates the phospholipase C (PLC) by catalyzing the synthesis of its second messengers, inositol 1,4,5-trisphosphate (IP3); and diacylglycerol (DAG). In turn, PTH increases intracellular Ca2+ and activates protein kinase C (PKC) isozymes, PLC–PKC signaling pathway which is essential for osteoblast proliferation and for bone modeling and remodeling. Adapted from M. Bastepe, S. Turan and Q He (J Molec Endocrinol 2017; 58, R203-224).

on the surface of OBs and osteocytes. PTHR1 belongs to the B-family of G protein-coupled receptors, and once the PTH molecule has bound to its receptor, it triggers a series of intracellular signaling events (Figure 2).

In OBs, binding of PTH to its receptor induces the production of M-CSF and RANKL, responsible for the differentiation and activation of OCs (Kondo et al., 2002; Feng and Teitelbaum, 2013). PTH also induces the production of osteocytic RANKL, potentiating the differentiation and activation of OCs (Nakashima and Takayanagi, 2011; O'Brien et al., 2013; Benawadh et al., 2014; Xiong et al., 2014). The persistent and constant elevated PTH increases the bone resorption units, resulting in a considerable increase in resorptive areas and leading to negative bone balance (Malluche et al., 1982; Ma et al., 2001; Silva and Bilezikian, 2015). Increased osteoblastic activity leads to an unparalleled increase in the production of osteoid by OBs. Nevertheless, this osteoid does not have an orderly and laminar disposition, as in normal bone. This is known as *woven* bone, and this increase in osteoid is known as *hyperosteoidosis*, which occurs as a consequence of increased remodelling and not due to a delay in mineralization, as is the case with osteomalacia (Malluche et al., 1982; Feng and McDonald, 2011; Hruska et al., 2017).

Furthermore, in hyperparathyroidism there is activation of fibroblast-type mesenchymal cells that give rise to peritrabecular fibrosis (Lotinun et al., 2005; Lowry et al., 2008). The overall results is a loss of cortical bone secondary to the accelerated resorption, which far exceeds bone formation, and in the place of a laminar osteoid, a fibrous tissue even containing cysts is present (Naji Rad and Deluxe, 2022).

Adynamic bone disease

In the 1980s the term "aplastic" or "adynamic" bone disease was introduced (Malluche and Faugere, 1986). Aluminum intoxication was previously the most frequent cause of low-turnover bone disease (aluminum-induced osteomalacia) since aluminum decreases the activation of OB and OC and causes an important defective mineralization (Malluche and Faugere, 1986; Nebeker and Coburn, 1986). ABD is characterized by suppressed bone

formation, low cellularity, and thin osteoid seams, the last-mentioned being the most important difference with osteomalacia (wide osteoid volume) since mineralization is normal in ABD (Parfitt et al., 1987; Moe et al., 2006; Nagy et al., 2022). Minimodeling has been shown to potentially contribute to bone formation in dialysis patients with ABD, in the absence of remodelling stimulated by PTH, and this is especially the case in young patients with positive activities of daily living (Ubara et al., 2005). Minimodeling refers to the formation of bone by the action of OBs without prior resorption by OCs. Bone modeling can be divided into macromodeling or minimodeling depending on whether it is developed in cortical or cancellous bone, respectively. This process, which is very important during fetal and neonatal life, generates convex bone formation on smooth cement lines which do not express tartrate-resistant acid phosphatase, the hallmark of OC activity when bone formation is preceded by resorption. The first to use this term was Frost (Frost, 1966), and it was postulated later that minimodeling may continue in trabeculae throughout life (Frost, 1990). It seems to contribute to a significant percentage of bone volume in special conditions such as CKD, parathyroidectomized and/or patients with ABD, and it has been popularized with the advent of new dual medications against OP, such as romosozumab (Yamamoto et al., 2021). Increased mineralization of the minimodelling surface at the endocortical surface has also been observed in dialysis patients with ABD treated with the non-calcium-based phosphate binder lanthanum carbonate (Yajima et al., 2013).

The prevalence of ABD used to be much lower than that of osteitis fibrosa, but this pattern of bone damage has increased significantly, and in most recent studies it is described as the most prevalent (Frazão and Martins, 2009; Malluche et al., 2011; Nagy et al., 2022). It has even been proposed that ABD may be the predominant bone pattern in early stages of CKD (Massy and Drueke, 2017). The rising prevalence is probably due to patient's increasing age and a higher prevalence of diabetes mellitus (relative hypoparathyroidism) (Jara et al., 1995; Brandenburg and Floege, 2008). In fact, however, the etiology of ABD is multifactorial and there are many other potential causes, including iatrogenic factors, malnutrition-inflammation syndrome, gonadal dysfunction, and the antagonistic effect of retained PTH fragments, among many others (Brandenburg and Floege, 2008; Bover et al., 2014). Calcium overload (oral or contained in the dialysate bath) and an excessive use of antiparathyroid (vitamin D or calcimimetics) agents have also been closely associated with ABD due to excessive suppression or inadequate normalization of PTH levels in CKD (Bover et al., 2014). The combination of relatively low levels of PTH and APs currently represents the best clinical basis for assessment of potential ABD beyond the gold standard bone biopsy (Couttenye et al., 1996; Moore et al., 2009; Sprague et al., 2016b; Bover et al., 2018). In fact, multifactorial hyporesponsiveness to PTH is a well-documented consequence of CKD (Evenepoel et al., 2016; Bover et al., 2021c) and a certain degree of secondary hyperparathyroidism is beneficial in CKD patients, not only because of the positive PTH phosphaturic effect but also in order to maintain a normal bone formation rate (Ketteler et al., 2022; Torregrosa et al., 2022). ABD has also been associated with a higher mortality, occasionally attributed to an increased number of fractures and accelerated vascular calcification (Bover et al., 2014).

A crucial aspect of low remodelling is that it promotes longer secondary mineralization, which may lead to brittle bones. It should be known that the osteoid mineralization process is carried out in two stages: primary mineralization, over the course of days, during which 50%–79% of the maximum mineralization is reached, and afterwards secondary mineralization begins (Ruffoni et al., 2007; Bala et al., 2010). Secondary mineralization is a slow process and develops in the course of months, contributing to the maximum mineralization and to an increase in the quantity and size of the crystals (Boivin and Meunier, 2003). Secondary mineralization occurs inversely to bone turnover. Thus, the greater the turnover the shorter the time in which secondary mineralization develops, and the lower the turnover the longer the duration of secondary mineralization (Boivin et al., 2009). In addition, the suppression of bone turnover can cause micro fissures that are difficult to repair in the presence of a low bone formation rate (Ng et al., 2016; Dong et al., 2019). In this context, the 2009 KDIGO CKD-MBD guideline recommended a bone biopsy prior to antiresorptive therapy in patients with CKD G4 to G5D, low BMD, and/or fragility fractures (Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009). The rationale was that low BMD may be due to CKD-MBD (e.g., high PTH) and that lowering PTH is a safer and more appropriate therapy than an antiresorptive (Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009). Moreover, there was concern that bisphosphonates could induce ABD, although this hypothesis was based upon a single cross-sectional study (Amerling et al., 2010). In the intervening period, studies in patients with CKD have not definitely demonstrated that bisphosphonates are a direct cause of ABD (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022). Suppression of bone turnover is inherent to bisphosphonates and most treated patients develop a low bone formation rate (Evenepoel et al., 2021a), yet this treatment prevents fractures (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017). Suppression of bone turnover by bisphosphonates occurs even in the absence of CKD and there is no evidence that the level of remodelling suppression in CKD is greater than that in non-CKD counterparts (Allen and Aref, 2017). In any case, the implications of drug-induced suppression of bone turnover for bone strength are intensely debated (Evenepoel et al., 2021a). As we mentioned previously, low PTH levels as a proxy of low-bone turnover in CKD patients have been associated with increased fracture risk (Coco and Rush, 2000; Nitta et al., 2004) but it remains a matter of debate whether low bone turnover *per se* or the disease causing low bone turnover accounts for the perceived ABD-induced risk of fracture or adverse outcomes (Evenepoel et al., 2021a; Nagy et al., 2022).

It is noteworthy that in ABD there is a state of imbalance between the low circulating levels of bone anabolic factors such as IGF-1 and the increased expression of inhibitors of bone turnover such as sclerostin and Dkk1. This imbalance favors suppression of bone formation through inhibition of the Wnt-catenin pathway (Tanaka et al., 2015; Massy and Drueke, 2017). New treatments for OP with a dual action (anti-sclerostin antibodies with anabolic and antiresorptive properties) were shown to be promising in a rat model of progressive ROD (Moe et al., 2015). It is noteworthy that these

authors found efficacy in improving rat bone properties only when the PTH levels were low, also preventing calcium-induced vascular calcification, while no significant effect was observed in animals with high PTH levels.

Osteoporosis

OP is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to an increase in bone fragility and therefore a higher susceptibility to bone fractures (Compston et al., 2019). Consequently, the definition of OP includes not only bone *quantity* (mass) but also bone *quality* (microstructure), in addition to the important clinical outcome of fragility fractures (Compston et al., 2019). This concept was developed in 1993 by an International Consensus of experts, and the diagnostic criteria, which were also adopted by the WHO in 1994, use standard deviation (SD) scores of BMD in relation to the peak bone mass reached by young healthy women (WHO, 1994).

Briefly, in postmenopausal women, *OP* was defined as a BMD T-score lower than −2.5 SD below the average of young healthy women, and *osteopenia* as a BMD T-score between −1 and −2.5 SD below this average value (WHO, 1994). Thus, there is a significant relationship between BMD and fragility fractures, with a 1.5 to 2.6-fold increase in fracture risk for every SD decrease in BMD (Siris et al., 2004). Importantly, the diagnostic criteria recognize the importance of BMD in the pathogenesis of fragility fractures and also provide a tool to quantify the prevalence of OP (bone densitometry) (Compston et al., 2019). However, the utility of BMD as the sole clinical indicator for OP is limited since BMD is only one of multiple risk factors for fracture development. Actually, the majority of fragility fractures occur in individuals with BMD values *above* this threshold (less negative) (Siris et al., 2004; Compston et al., 2019). Accordingly, the National Bone Health Alliance Working Group published a position statement in 2014 which included not only BMD but also the presence of fragility fractures and the fracture risk assessed by the Fracture Risk Assessment Tool (FRAX) for the clinical diagnosis of OP (Siris et al., 2014). Apart from the BMD criteria, these experts also defined OP as the presence of a hip fragility fracture (with or without BMD), a non-hip fragility fracture (including vertebral, proximal humeral, pelvic, and distal forearm fractures) plus densitometric osteopenia, and a high fracture risk just based on a nationally-adapted FRAX score (Siris et al., 2014). Since bone quality is probably another important aspect to be taken into account which may be additionally affected in the presence of CKD (Tasnim et al., 2021), new tools are being developed to improve its assessment and improve fracture risk prediction both in the general population and in CKD patients (Bover et al., 2021a).

Osteoporosis and chronic kidney disease

Studies assessing the presence of densitometric OP in patients with CKD are scarce since BMD assessment was not recommended in the previous 2009 KDIGO CKD-MBD guidelines (evidence 2B) (Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009). However, as mentioned previously, an important shift occurred in the 2017 KDIGO CKD-MBD guidelines in the opposite direction (evidence 2B). Thus, it was now suggested that BMD testing could be used to assess fracture risk in patients with CKD G3a-G5D if results would impact treatment decisions (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017); the rationale being that new evidence had appeared, demonstrating that DXA does predict fractures also in patients with CKD (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Kidney Disease: Improving Global Outcomes KDIGOCKD–MBD Work Group, 2009; Pimentel et al., 2021). The same suggestion has since been adopted in other national guidelines (Torregrosa et al., 2022). In fact, at least 4 prospective cohort studies using dual-energy X-ray absorptiometry (DXA) BMD and incident fractures demonstrated that DXA BMD predicted incident fractures across the spectrum from CKD G3a to G5D (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017). Nevertheless, it is important to recognize that DXA does not distinguish among different forms of ROD and it essentially evaluates *quantity* as opposed to *quality* of bone (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Pimentel et al., 2021). Consequently, BMD does not offer information either on bone microarchitecture [the trabecular bone score (TBS) may provide some additional clues] (Silva et al., 2014; Yun et al., 2020), bone turnover or mineralization, and cannot differentiate between OP, ABD, osteomalacia or osteitis fibrosa (Ginsberg and Ix, 2022). However, as mentioned before, BMD does predict fractures and may support decision-making together with bone biomarkers (Jørgensen et al., 2022a), even in the absence of a bone biopsy, according to recent guidelines which also draw attention to the associated vital risk (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022).

Bone metabolism in CKD patients differs from that of the general population, depending on CKD stage, and type of kidney replacement therapy (i.e., hemodialysis, peritoneal dialysis, or kidney transplantation), among many other pathophysiological factors including bone location and histologic structure (Chen et al., 2014). Thus, cross-sectional analysis showed a significantly lower BMD at femoral neck and total hip and a significant higher serum PTH along with CKD stages (Cailleaux et al., 2021). Baseline age, gender, low body mass index, tobacco, and high PTH levels were significantly associated with low BMD (Cailleaux et al., 2021). Interestingly, the longitudinal bone loss observed in patients with CKD during the mean 4.3-year follow-up revealed a significant bone loss at the radius only, whereas BMD changes at the femoral neck were not associated with CKD stages or basal PTH levels (Cailleaux et al., 2021). These data invite to a better definition of the skeletal site and the monitoring schedule of serial BMD measurements in patients with CKD, and to investigate the changes of BMD and microarchitecture with high-resolution techniques, which may broaden the understanding and differential role of PTH on trabecular and cortical bone, especially in CKD patients (Cailleaux et al., 2021).

Different drugs in patients with this clinical condition (including glucocorticoids) may also affect bone metabolism, and it has to be taken into account that extensive vascular calcification or the

presence of an arteriovenous fistula may additionally affect the diagnosis of OP (Evenepoel et al., 2021a; Pimentel et al., 2021). Moreover, drugs approved for OP are not simply the subject of theoretical concerns (ABD); rather some restrictions on use may be stipulated in their summary of product characteristics (SmPC) when decreased renal function is present (an example being bisphosphonates). However, growing experience with OP medications in patients with CKD has increased the confidence in using antiresorptive therapy in patients with low BMD and a high risk of fracture (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022).

Chronic kidney disease and fractures

It seems clear that patients with CKD sustain more fragility fractures than the general population (Pimentel et al., 2021), and the risk of fragility fracture has been reported to be up to 5 times higher in individuals with an estimated glomerular filtration rate (eGFR) of less than 15 ml/min/1.73 m^2 compared to those in whom the eGFR exceeds 60 ml/min/1.73 m^2 (Naylor et al., 2015; Pimentel et al., 2021). Additionally, it should be noted that the worse the CKD stage, the higher the fracture risk (Naylor et al., 2015). For example, the Canadian Multicentre Osteoporosis Study (CaMos) reported a fracture incidence ranging from 15.0/1,000 person-years in CKD G1 up to 46.3/1,000 person-years in CKD G4 (Naylor et al., 2015). Many other factors are involved in the increased fracture risk associated (or not) with CKD such as age, gender (the risk is higher in women), history of prior hip fracture, urine albumin levels, low body mass index, long dialysis vintage, and/or high and low-turnover bone disease (Pimentel et al., 2021). On the other hand, studies assessing the prevalence of vertebral fractures among individuals with CKD are scarce and clearly seem to underestimate such fractures, as the published rates have ranged between 1% and 20% (Pimentel et al., 2021). Furthermore, it should be underlined that there is a higher mortality risk after a fragility fracture (hip and non-hip fractures) in patients with CKD G4-G5 as compared to controls with an eGFR over 60 ml/min/1.73 m^2 (de Bruin et al., 2020). Like many other authors, we have shown in a recent study that the presence of vertebral fractures is correlated with poorer survival and that these fractures are independent predictors of all-cause mortality (Castro-Alonso et al., 2020). Consequently, current nephrology guidelines have removed the requirement for bone biopsy prior to the use of antiresorptive drugs for OP because their use must be individualized in patients with CKD (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022; Ketteler et al., 2017), and the risk/benefit ratio may be favourable also in these patients (Bover et al., 2019; Casado and Neyro, 2021; Torregrosa et al., 2022). Actually, it has been proposed that it is "time for action" even in the absence of randomized clinical trials (Moe and Nickolas, 2016), although it is still prudent to use these drugs with caution (Kidney Disease: Improving Global Outcomes KDIGO CKD-MBD Update Work Group, 2017; Torregrosa et al., 2022; Bover et al., 2019; Casado and Neyro, 2021).

New bone metabolic pathways-new treatments

In recent years, we have learned more about the role of certain cell signalling pathways in the regulation of bone metabolism, examples being the OPG/RANKL (LGR4) system and the canonical Wnt-ß-catenin pathway. It is important that knowledge on these two pathophysiological pathways is further expanded, given that the latest approved antiosteoporotic treatments aim to modulate these signalling routes. Denosumab is an antibody against RANKL and romosozumab is an antibody against sclerostin. They are not specifically contraindicated in renal failure and therefore they may become a real therapeutic target for OP even in patients with CKD, also bearing the absence of chronic accumulation (Festuccia et al., 2017; Miller et al., 2022; Suzuki et al., 2022).

Wnt-β-catenin signaling

We have already mentioned that the Wnt-β-catenin is an intracellular signaling pathway that has emerged as a key regulator of osteoblastogenesis (Gordon and Nusse, 2006). Its regulation occurs mainly through its antagonists, sclerostin and Dkk-1, which are primarily expressed by osteocytes (Gordon and Nusse, 2006). In brief, when the Wnt protein binds to the dual receptor complex, which comprises frizzled (Fz) and either low-density lipoprotein receptor (LDLR)-related protein 5 (LRP5) or LRP6, ß-catenin is accumulated in the cytoplasm and enters the nucleus, where it is associated with a transcription factor, regulating the expression of canonical Wnt target genes such as *WISP1* and *RUNX2* (Gordon and Nusse, 2006) (Figure 3). Consequently, the activation of Wnt signalling finally induces the differentiation of OB precursors towards mature OBs and prevents OB (and osteocyte) apoptosis, resulting in increases in bone mass. However, when sclerostin or Dkk-1 binds to the Wnt receptors (LRP-5/6 membrane), they prevent the binding of the Wnt protein to its receptors, and the cytoplasmatic ß-catenin protein is phosphorylated and degraded. Therefore, ß-catenin cannot enter the nucleus and cannot activate osteoblastogenesis. Other inhibitors, such as the Sfrp 1and 4, (upregulated in CKD) do not specifically bind to LRPs but compete with the Wnt ligand for the binding to the receptor (Gordon and Nusse, 2006). As a consequence, the inhibition of Wnt signalling (by the absence of Wnt protein or the presence of antagonists) leads to downregulation of bone formation, probably leading to a lower bone mass (Gordon and Nusse, 2006; Baron and Kneissel, 2013b). In addition, Wnt-ß-catenin signalling is critical not only for osteoblastogenesis but also for the regulation of osteoclastogenesis (Wijenayaka et al., 2011) through the RANKL/OPG system (Figure 3).

Sclerostin

Sclerostin is a 22-kDa protein that is a member of the cystatin knot family of proteins and the product of the *SOST* gene

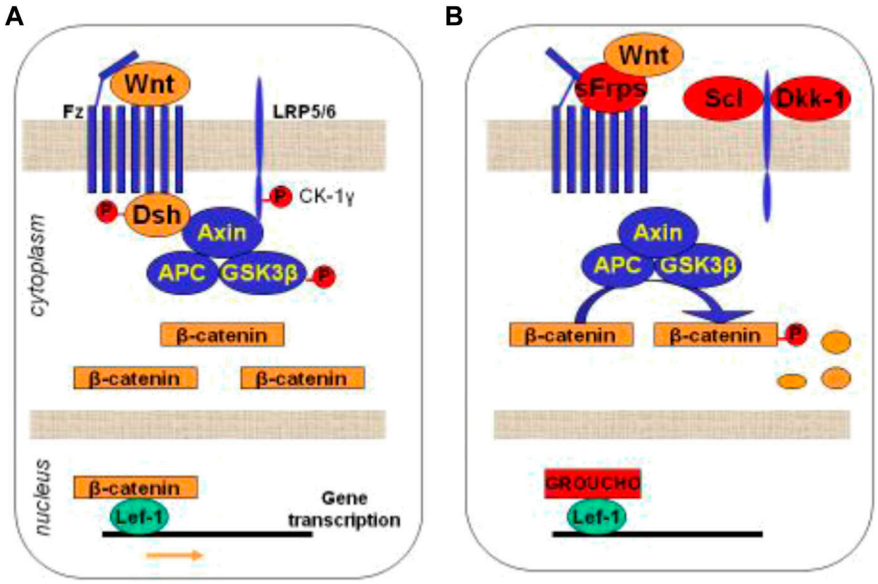

FIGURE 3
Schematic representation of the Wnt-ß-catenin signalling pathway. **(A)** Activation of the intracellular cascade depending on the union between Wnt protein and its receptors. **(B)** Inhibition of Wnt signaling by its antagonists, blocking the union between Wnt protein and its receptors. Wnt: Wnt protein; Fz: frizzled; LRP5: low-density lipoprotein receptor (LDLR)-related protein 5 or LRP6; GSK-3b: glycogen synthetase kinase 3; APC: tumor suppressor adenomatous polyposis coli; Lef-1: Tcf/lymphoid enhancer-binding factor; Sfrp: secreted Fz-related-proteins; Scl: Sclerostin; Dkk1: dickkopf–related protein 1. Adapted from [Guañabens N, Curr Osteoporos Rep 2014].

(Brandenburg et al., 2019). Loss of function mutations of the *SOST* gene have been reported in van Buchem disease (sclerosteosis; a hereditary sclerosing bone dysplasia) (), and this represented the starting point for development of an anti-sclerostin antibody which has recently been approved for the treatment of OP (Baron and Kneissel, 2013b). As mentioned above, sclerostin downregulates osteoblastogenesis but also promotes osteoclastogenesis through activation of the RANKL/OPG system; thus the antibody offers a unique dual positive action (Wijenayaka et al., 2011). Sclerostin is also expressed by osteoclast precursors, hepatocytes, and renal and vascular cells, but little is known about the systemic effects of this molecule [186].

In postmenopausal women, increased circulating sclerostin values have been associated with OP and an increased risk of fragility fractures (Clarke and Drake, 2013). It has also been described an increase of circulating sclerostin values in some clinical conditions associated with OP such as type 2 diabetes mellitus, glucocorticoid treatment, multiple myeloma or CKD; whereas decreased values of sclerostin have been described in patients with hyperparathyroidism and under osteoanabolic treatment with teriparatide (Moysés and Schiavi, 2015).

Sclerostin in chronic kidney disease

In CKD, circulating levels of sclerostin increase as kidney function declines (Moysés and Schiavi, 2015; Massy and Drueke, 2017; Figurek et al., 2020). Thus, the level has been reported to be 5 times higher in patients with CKD G5D (Figurek et al., 2020). Additionally, increased sclerostin values have been described in individuals undergoing hemodialysis (Moysés and Schiavi, 2015). Curiously, it seems that peritoneal dialysis decreases sclerostin circulating levels (as 2.5 times higher values have been found in the dialysate compared to urine), whereas after a conventional hemodialysis session the circulating levels of sclerostin remain unaltered (Figurek et al., 2020). In this context, a small prospective study has shown suppression of the increase in sclerostin level in hemodialysis using a medium cut-off dialysis filter (Ahn et al., 2021).

The pathophysiology of sclerostin in CKD and its consequences are not clear yet. On the one side, sclerostin is overexpressed by osteocytes and by injured kidney cells (Moysés and Schiavi, 2015), while on the other hand there is an increased tubular excretion of sclerostin with a reduction in renal function (Moysés and Schiavi, 2015; Figurek et al., 2020). It is well known that after kidney transplantation serum sclerostin values are rapidly restored to the normal range (Moysés and Schiavi, 2015). Altogether suggests that a sclerostin accumulation occurs in CKD, and it has been reported that it may be present even at early CKD stages in an attempt to prevent bone loss (Massy and Drueke, 2017) as a result of both a multifactorial increase in expression and a decrease in elimination (Moysés and Schiavi, 2015). Moreover, it seems that sclerostin also intervenes in the relationship among phosphate, FGF23 and bone in CKD. Thus, a positive correlation has been described between sclerostin, serum phosphate, and FGF23, while, conversely, a negative relationship with PTH has been reported (Moysés and Schiavi, 2015; Figurek et al., 2020). Additionally, high sclerostin levels have been associated with PTH resistance or hyporesponsiveness to PTH in CKD (Massy and Drueke, 2017; Figurek et al., 2020; Bover et al., 2021c). Other molecules beyond

PTH and phosphate (and probably FGF23) also seem to regulate the expression of sclerostin, such as calcitriol, BMPs, TNF, and prednisone (Moysés and Schiavi, 2015), suppressing sclerostin expression from osteocytes, and increasing the rate of bone remodelling (Figurek et al., 2020).

Finally, an inverse relationship has been described between sclerostin and bone AP (Figurek et al., 2020). A recent study assessed the relationship between bone histomorphometric parameters from patients with CKD G3-G4 and circulating levels of the Wnt antagonists sclerostin and Dkk-1 (Neto et al., 2021). It was observed that individuals with low-turnover bone disease (diagnosed by bone biopsy) had higher circulating sclerostin levels and lower DKK-1 and RANKL levels as compared to individuals with high-turnover bone disease or normal bone histology (Neto et al., 2021), demonstrating an association between higher circulating sclerostin values and lower bone remodelling (low bone turnover). Additionally, in a CKD animal model of ABD, high dietary phosphate intake was associated with high osteocyte *SOST* expression (Antoine et al., 2020). While more studies are needed to assess the usefulness of the circulating values of sclerostin as a clinical tool to identify patients with low-turnover bone disease in CKD, taking into account all the data it may be concluded that blocking sclerostin could be an interesting treatment target for those patients with low-bone turnover or at least some CKD patients with OP due its dual positive action (Antoine et al., 2020). Romosozumab has recently been approved for the treatment of postmenopausal OP, although in Europe it carries a black box warning that it should not be initiated in patients with myocardial infarction or stroke in the preceding year (Anthony, 2019). Initial positive experiences in CKD patients are being currently published (Sato et al., 2021; Miller et al., 2022; Suzuki et al., 2022).

Denosumab and romosozumab

We have already briefly discussed both the OPG-RANKL-RANK system and the Wnt-β-catenin signaling pathway, and that these monoclonal antibodies (anti-RANKL and antisclerostin, respectively) are not contraindicated in CKD or dialysis patients with OP. Nevertheless, their use in CKD may be associated with specific problems (at least in advanced stages), which are described elsewhere (Festuccia et al., 2017; Bover et al., 2019; Miller et al., 2022; Suzuki et al., 2022)". Briefly, hypocalcemia is more frequently observed in CKD patients and therefore an adequate repletion of calcium and vitamin D is recommended (Miller et al., 2022), especially in dialysis patients. Most importantly, the favorable skeletal effects of these monoclonal antibodies may reverse quickly upon discontinuation, especially after denosumab withdrawal, because of a vast increase of OC number and activity, which may lead to a subsequent profound increase of bone turnover to pre-treatment or even above pre-treatment values, a phenomenon commonly described as "rebound phenomenon" (Casado and Neyro, 2021).

In the case of denosumab, subsequent multiple vertebral fractures have been described upon discontinuation (Tsourdi et al., 2017; McClung et al., 2020). Risk was similar to that observed in the placebo group in the randomized clinical trials (Kim et al., 2022). A recent new hypothesis for the rebound effect after denosumab cessation is by the phenomenon to OB recicling from osteomorphs, a newly described cell state (Kim et al., 2022). Consequently, in case that a cessation of treatment is deemed necessary either by patient's decision, or medical reasons such as low adherence, or after reaching a determined T-score at BMD, or after completing the 12-month treatment (in the case of romosozumab), guidelines recommend different approaches and/or sequential treatments which are more detailed and specific after denosumab suppression (Tsourdi et al., 2017; McClung et al., 2020; Kendler et al., 2022; Kim et al., 2022).

In summary, CKD is a highly prevalent condition and recent years have witnessed important advances in the understanding of the associated mineral metabolism disorders. The *systemic* complex CKD-MBD has already been universally accepted because of its clear association with cardiovascular disease and extremely high mortality rates. CKD-MBD includes a bone component which is no longer just represented by the classical ROD or disorders of bone remodelling. In fact, it is now very well known that CKD is associated with a higher risk of falls and fractures, and higher mortality rates when a fracture occurs. New guidelines suggest that BMD should also be assessed in CKD patients if results will impact clinical decisions (i.e., individualized prescription of anti-OP drugs). Several illustrative algorithms for CKD patients have already been published, broadly calling for a shift from nihilism to pragmatism (Pimentel et al., 2017; Evenepoel et al., 2021b; Evenepoel et al., 2021c; Pimentel et al., 2021; Casado et al., 2022; Ginsberg and Ix, 2022; Haarhaus et al., 2022). However, it is still prudent to use these drugs (bisphosphonates, denosumab, recombinant PTH and romosozumab) with caution, especially in advanced kidney disease, balancing the risk/benefit ratio, since, as documented in this article, pathophysiological pathways are extremely intricate and not completely unraveled yet.

Author contributions

AA Preparation of the draft (first version). LG Preparation of the draft (first version). PU-T, NC-L, MR-G, EM, IdS, VL-B, MS-B, AP-E, MU, JP, MB, AV, IG-L, JN-G, and LM, Critical review of the article with important contributions to its intellectual content and approval of the final version to be published. JB Preparation of the draft (first version), Critical review of the article with important contributions to its intellectual content and approval of the final version to be published. All authors contributed to the article and approved the submitted version.

Acknowledgments

Some authors belong to the SGR program of "Generalitat de Catalunya" (2021-SGR-00187 REMAR Group), and the RICORS 2040 (kidney Disease) (Redes de Investigación Cooperativa Orientadas a Resultados en Salud), IDI-2021-000080 or RD21/0005/0009 and RD21/0005/0013, ISCIII, Instituto de Salud Carlos III, Spain.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abou-Samra, A-B., Juppner, H., Force, T., Freeman, M. W., Kong, X-F., Schipani, E., et al. (1992). Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: A single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium. *Proc. Natl. 5. Acad. Sci. U. S. A.* 89, 2732–2736. doi:10.1073/pnas.89.7.2732

AgapovaFangSugatani, O. A. Y. T., Seifert, M. E., and Hruska, K. A. (2016). Ligand trap for the activin type IIA receptor protects against vascular disease and renal fibrosis in mice with chronic kidney disease. *Kidney Int.* 89, 1231–1243. doi:10.1016/j.kint.2016.02.002

Ahn, S. H., Ko, M. M., Song, J. H., and Jung, J. H. (2021). Changes in plasma sclerostin level associated with use of a medium cut-off dialyzer in end-stage renal disease. *Kidney Res. Clin. Pract.* 40 (1), 120–134. doi:10.23876/j.krcp.20.173

Alberto Ortiz (2022). Ckd: The burden of disease invisible to research funders. *Nefrología* 42 (1), 65–84. doi:10.1016/j.nefroe.2021.09.005

Albright, F., and Reifenstein, E. C. (1948). *The parathyroid glands and metabolic bone disease. Selected studies*. Baltimore: Williams & Wilkins Co.

Allan, E. H., Häusler, K. D., Wei, T., Gooi, J. H., Quinn, J. M. W., Crimeen-Irwin, B., et al. (2008). EphrinB2 regulation by PTH and PTHrP revealed by molecular profiling in differentiating osteoblasts. *J. Bone Min. Res.* 23 (8), 1170–1181. doi:10.1359/jbmr.080324

Allen, M. R., and Aref, M. W. (2017). What animal models have taught us about the safety and efficacy of bisphosphonates in chronic kidney disease. *Curr. Osteoporos. Rep.* 15 (3), 171–177. PMID: 28432595; PMCID: PMC9055792. doi:10.1007/s11914-017-0361-4

Amerling, R., Harbord, N. B., James, P., and Feinfeld, D. A. (2010). Bisphosphonate use in chronic kidney disease: Association with adynamic bone disease in a bone histology series. *Blood Purif.* 29, 293–299. doi:10.1159/000276666

Anthony, M. (2019). Romosozumab: First global approval. *Drugs* 79, 471–476. doi:10.1007/s40265-019-01072-6

Antoine, B., Evenepoel, P., Paquot, F., Malaisec, O., Cavalier, E., and Delanaye, P. (2020). Sclerostin within the chronic kidney disease spectrum. *Clin. Chim. Acta* 502, 84–90. doi:10.1016/j.cca.2019.12.008

Arumugam, B., Vishal, M., Shreya, S., Malavika, D., Rajpriya, V., He, Z., et al. (2019). Parathyroid hormone-stimulation of Runx2 during osteoblast differentiation via the regulation of lnc-SUPT3H-1:16 (RUNX2-AS1:32) and miR-6797-5p. *Biochimie* 158, 43–52. Epub 2018 Dec 15. doi:10.1016/j.biochi.2018.12.006

Bala, Y., Farlay, D., Delmas, P. D., Meunier, P. J., and Boivin, G. (2010). Time sequence of secondary mineralization and microhardness in cortical and cancellous bone from ewes. *Bone* 46 (4), 1204–1212. Epub 2009 Dec 5. PMID: 19969115. doi:10.1016/j.bone.2009.11.032

Baron, R., and Kneissel, M. (2013). WNT signaling in bone homeostasis and disease: From human mutations to treatments. *Nat. Med.* 19 (2), 179–192. Epub 2013 Feb 6. PMID: 23389618. doi:10.1038/nm.3074

Baron, R., and Kneissel, M. (2013). WNT signaling in bone homeostasis and disease: From human mutations to treatments. *Nat. Med.* 19 (2), 179–192. doi:10.1038/nm.3074

Bellido, T., Ali, A. A., Gubrij, I., Plotkin, L. I., Fu, Q., O'Brien, C. A., et al. (2005). Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: A novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* 146 (11), 4577–4583. doi:10.1210/en.2005-0239

Ben-awadh, A. N., Delgado-Calle, J., Tu, X., Kuhlenschmidt, K., Allen, M. R., Plotkin, L. I., et al. (2014). Parathyroid hormone receptor signaling induces bone resorption in the adult skeleton by directly regulating the RANKL gene in osteocytes. *Endocrinology* 155 (8), 2797–2809. doi:10.1210/en.2014-1046

Bikle, D. D., Sakata, T., Leary, C., Elalieh, H., Ginzinger, D., Rosen, C. J., et al. (2002). Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. *J. Bone Min. Res. [Internet]* 17 (9), 1570–1578. doi:10.1359/jbmr.2002.17.9.1570

Boivin, G., Farlay, D., Bala, Y., Doublier, A., Meunier, P. J., and Delmas, P. D. (2009). Influence of remodeling on the mineralization of bone tissue. *Osteoporos. Int.* 20, 1023–1026. doi:10.1007/s00198-009-0861-x

Boivin, G., and Meunier, P. J. (2003). Methodological considerations in measurement of bone mineral content. *Osteoporos. Int.* 14 (5), 22–S27. doi:10.1007/s00198-003-1469-1

Bonewald, L. F., and Johnson, M. L. (2008). Osteocytes, mechanosensing and Wnt signaling. *Bone* 42 (4), 606–615. doi:10.1016/j.bone.2007.12.224

Bonewald, L. F. (2007). Osteocytes as dynamic multifunctional cells. *Ann. N. Y. Acad. Sci.* 1116 (1), 281–290. doi:10.1196/annals.1402.018

Bover, J., Ureña -Torres, P., Laiz Alonso, A. M., Torregrosa, J. V., Rodríguez-García, M., Castro-Alonso, C., et al. (2019). Osteoporosis, bone mineral density and CKD-MBD (II): Therapeutic implications. *Nefrologia* 39, 227–242. doi:10.1016/j.nefro.2018.10.009

Bover, J., Ureña, P., Brandenburg, V., Goldsmith, D., Ruiz, C., DaSilva, I., et al. (2014). Adynamic bone disease: From bone to vessels in chronic kidney disease. *Semin. Nephrol.* 34 (6), 626–640. PMID: 25498381. doi:10.1016/j.semnephrol.2014.09.008

Bover, J., Ureña-Torres, P., Cozzolino, M., Rodríguez-García, M., and Gómez-Alonso, C. (2021). The non-invasive diagnosis of bone; disorders in CKD. *Calcif. Tissue Int.* 108, 512–527. doi:10.1007/s00223-020-00781-5

Bover, J., Aguilar, A., Arana, C., Molina, P., Jackson, O., Berna, G., et al. (2021). Clinical approach to vascular calcification in patients with non-dialysis dependent chronic kidney disease: Mineral-bone disorder-related aspects. *Front. Med.* 8, 642718. doi:10.3389/fmed.2021.642718

Bover, J., Arana, C., Ureña, P., Torres, A., Martín-Malo, A., Fayos, L., et al. (2021). Hyporesponsiveness or resistance to the action of parathyroid hormone in chronic kidney disease. *Nefrología.* 41, 514–528. doi:10.1016/j.nefroe.2021.11.014

Bover, J., Ureña, P., Aguilar, A., Mazzaferro, S., Benito, S., López-Báez, V., et al. (2018). Alkaline phosphatases in the complex chronic kidney disease-mineral and bone disorders. *Calcif. Tissue Int.* 103 (2), 111–124. doi:10.1007/s00223-018-0399-z

Brandenburg, V. M., and Floege, J. (2008). Adynamic bone disease-bone and beyond. *NDT Plus* 1 (3), 135–147. doi:10.1093/ndtplus/sfn040

Brandenburg, V. M., Verhulst, A., Babler, A., D'Haese, P. C., Evenepoel, P., and Kaesler, N. (2019). Sclerostin in chronic kidney disease–mineral bone disorder think first before you block it. *Nephrol. Dial. Transpl.* 34, 408–414. doi:10.1093/ndt/gfy129

Brewer, H. B., Jr, and Ronan, R. (1970). Bovine parathyroid hormone: Amino acid sequence. *Proc. Natl. Acad. Sci. U. S. A.* 67 (4), 1862–1869. doi:10.1073/pnas.67.4.1862

Burger, E. H., Klein-Nulend, J., and Smit, T. H. (2003). Strain-derived canalicular fluid flow regulates osteoclast activity in a remodelling osteon- a proposal. *J. Biomech.* 36 (10), 1453–1459. doi:10.1016/s0021-9290(03)00126-x

Cailleaux, P-E., Ostertag, A., Metzger, M., Stengel, B., Boucquemont, J., Houillier, P., et al. (2021). Longitudinal bone loss occurs at the radius in CKD. *Kidney Int. Rep.* 6 (6), 1525–1536. doi:10.1016/j.ekir.2021.03.874

Cameron, D. A., Paschall, H. A., and Robinson, R. A. (1967). Changes in the fine structure of bone cells after the administration of parathyroid extract. *J. Cell Biol.* 33 (1), 1–14. doi:10.1083/jcb.33.1.1

Carrillo-López, N., Panizo, S., Alonso-Montes, C., Román-García, P., Rodríguez, I., Martínez-Salgado, C., et al. (2016). Direct inhibition of osteoblastic Wnt pathway by fibroblast growth factor 23 contributes to bone loss in chronic kidney disease. *Kidney Int.* 90 (1), 77–89. doi:10.1016/j.kint.2016.01.024

Casado, E., Bover, J., Gómez-Alonso, C., and Navarro-González, J. F. (2022). Osteoporosis in chronic kidney disease: A essential challenge. *Med. Clin. Barc.* 158 (1), 27–34. Epub 2021 Jun 18. PMID: 34154811. doi:10.1016/j.medcli.2021.05.007

Casado, E., and Neyro, J. L. (2021). Tratamiento secuencial en osteoporosis. Nuevas tendencias. *Rev. Osteoporos. Metab. Min.* 13 (4), 107–116. doi:10.4321/s1889-836x2021000400002

Castro-Alonso, C., D'Marco, L., Pomes, J., Del Amo Conill, M., García-Diez, A. I., Molina, P., et al. (2020). Prevalence of vertebral fractures and their prognostic significance in the survival in patients with chronic kidney disease stages 3–5 not on dialysis. *J. Clin. Med.* 9 (5), 1604. doi:10.3390/jcm9051604

Cejka, D., Jager-Lansky, A., Kieweg, H., Weber, M., Bieglmayer, C., Haider, D. G., et al. (2012). Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients. *Nephrol. Dial. Trans. plant.* 27 (1), 226–230. doi:10.1093/ndt/gfr270

Cejka, D., Kodras, K., Bader, T., and Haas, M. (2010). Treatment of hemodialysis-associated adynamic bone disease with teriparatide (PTH1-34): A pilot study. *Kidney Blood Press Res.* 33 (3), 221–226. Epub 2010 Jun 24. PMID: 20588059. doi:10.1159/000316708

Chen, Y. J., Kung, P. T., Wang, Y. H., Huang, C. C., Hsu, S. C., Tsai, W. C., et al. (2014). Greater risk of hip fracture in hemodialysis than in peritoneal dialysis. *Osteoporos. Int.* 25, 1513–1518. doi:10.1007/s00198-014-2632-6

Cianciolo, G., La Manna, G., Capelli, I., Gasperoni, L., Galassi, A., Ciceri, P., et al. (2021). The role of activin: The other side of chronic kidney disease-mineral bone disorder? *Nephrol. Dial. Transpl.* 36 (6), 966–974. doi:10.1093/ndt/gfaa203

Clarke, B. L., and Drake, M. T. (2013). Clinical utility of serum sclerostin measurements. *Bonekey Rep.* 2, 361. doi:10.1038/bonekey.2013.95

Coco, M., and Rush, H. (2000). Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. *Am. J. Kidney Dis.* 36 (6), 1115–1121. doi:10.1053/ajkd.2000.19812

Compston, J. E., McClung, M. R., and Leslie, W. D. (2019). *Osteoporos. Lancet* 393, 364–376. doi:10.1016/S0140-6736(18)32112-3

Couttenye, M. M., D'Haese, P. C., Van Hoof, V. O., Lemoniatou, E., Goodman, W., Verpooten, G. A., et al. (1996). Low serum levels of alkaline phosphatase of bone origin: A good marker of adynamic bone disease in haemodialysis patients. *Nephrol. Dial. Transpl.* 11 (6), 1065–1072. doi:10.1093/oxfordjournals.ndt.a027457

Crane, J. L., and Cao, X. (2014). Function of matrix IGF-1 in coupling bone resorption and formation. *J. Mol. Med.* 92 (2), 107–115. doi:10.1007/s00109-013-1084-3

Csaba, P. (2022). Epidemiology of chronic kidney disease: An update 2022. *Kidney Int. Suppl.* 12, 7–11. doi:10.1016/j.kisu.2021.11.003

Cunningham, J., Locatelli, F., and Rodriguez, M. (2011). Secondary hyperparathyroidism. Pathogenesis, disease progression, and therapeutic options. *Clin. J. Am. Soc. Nephrol.* 6 (4), 913–921. doi:10.2215/CJN.06040710

Day, T. F., Guo, X., Garrett-Beal, L., and Yang, Y. (2005). Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev. Cell* 8 (5), 739–750. doi:10.1016/j.devcel.2005.03.016

de Bruin, I. J. A., Wyers, C. E., Souverein, P. C., van Staa, T. P., Geusens, P. P. M. M., van den Bergh, J. P. W., et al. (2020). The risk of new fragility fractures in patients with chronic kidney disease and hip fracture-a population-based cohort study in the UK. *Osteoporos. Int.* 31 (8), 1487–1497. doi:10.1007/s00198-020-05351-x

De Pasquale, L., Gobatti, D., Ravini, M. L., Barassi, A., Porreca, W., Melzi d'Eril, G. V., et al. (2008). Intra-operative testing for parathyroid hormone: The central laboratory option. *J. Endocrinol. Invest.* 31 (1), 62–67. doi:10.1007/BF03345568

Dong, B., Endo, I., Ohnishi, Y., Mitsui, Y., Kurahashi, K., Kanai, M., et al. (2019). Persistent activation of calcium-sensing receptor suppresses bone turnover, increases microcracks, and decreases bone strength: Increased microcracks with reduced bone strength in adh1 mice. *JBMR Plus* 3 (7), e10182. doi:10.1002/jbm4.10182

Drake, M. T., Srinivasan, B., Modder, U. I., Peterson, J. M., McCready, L. K., Lawrence Riggs, B., et al. (2010). Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J. Clin. Endocrinol. Metab. Novemb.* 95 (11), 5056–5062. doi:10.1210/jc.2010-0720

Drüeke, T. B., and Massy, Z. A. (2016). Changing bone patterns with progression of chronic kidney disease. *Kidney Int.* 89 (2), 289–302. PMID: 26806832. doi:10.1016/j.kint.2015.12.004

Elshahat, S., Paul, C., Maxwell, A. P., Griffin, M., O'Brien, T., and O'Neill, C. (2020). The impact of chronic kidney disease on developed countries from a health economics perspective: A systematic scoping review. *PLoS One* 15 (3), e0230512. doi:10.1371/journal.pone.0230512

Evenepoel, P., Bover, J., and Ureña Torres, P. (2016). Parathyroid hormone metabolism and signaling in health and chronic kidney disease. *Kidney Int.* 90, 1184–1190. doi:10.1016/j.kint.2016.06.041

Evenepoel, P., Cunningham, J., Ferrari, S., Haarhaus, M., Javaid, M. K., Lafage-Proust, M. H., et al. (2021). European Consensus Statement on the diagnosis and management of osteoporosis in chronic kidney disease stages G4-G5D. *Nephrol. Dial. Transpl.* 36, 42–59. doi:10.1093/ndt/gfaa192

Evenepoel, P., Cunningham, J., Ferrari, S., Haarhaus, M., Javaid, M. K., Lafage-Proust, M. H., et al. (2021). Diagnosis and management of osteoporosis in chronic kidney disease stages 4 to 5D: A call for a shift from nihilism to pragmatism. *Osteoporos. Int.* 32 (12), 2397–2405. Epub 2021 Jun 15. PMID: 34129059. doi:10.1007/s00198-021-05975-7

Evenepoel, P., Cunningham, J., Ferrari, S., Haarhaus, M., Javaid, M. K., Lafage-Proust, M. H., et al. (2021). European Consensus Statement on the diagnosis and management of osteoporosis in chronic kidney disease stages G4-G5D. *Nephrol. Dial. Transpl.* 36 (1), 42–59. doi:10.1093/ndt/gfaa192

Evenepoel, P., D'Haese, P., Bacchetta, J., Cannata-Andia, J., Ferreira, A., Haarhaus, M., et al. (2017). Bone biopsy practice patterns across Europe: The European renal osteodystrophy initiative-a position paper. *Nephrol. Dial. Transpl.* 32, 1608–1613. doi:10.1093/ndt/gfw468

Fei, Y., Shimizu, E., McBurney, M. W., and Partridge, N. C. (2015). Sirtuin 1 is a negative regulator of parathyroid hormone stimulation of matrix metalloproteinase 13 expression in osteoblastic cells: Role of sirtuin 1 in the action of PTH on osteoblasts. *J. Biol. Chem.* 290 (13), 8373–8382. doi:10.1074/jbc.M114.602763

Feng, X., and McDonald, J. M. (2011). Disorders of bone remodeling. *Annu. Rev. Pathol.* 6, 121–145. PMID: 20936937; PMCID: PMC3571087. doi:10.1146/annurev-pathol-011110-130203

Feng, X., and Teitelbaum, S. L. (2013). Osteoclasts: New insights. *Bone Res.* 1 (1), 11–26. doi:10.4248/BR201301003

Festuccia, F., Jafari, M. T., Moioli, A., Fofi, C., Barberi, S., Amendola, S., et al. (2017). Safety and efficacy of denosumab in osteoporotic hemodialysed patients. *J. Nephrol.* 30, 271–279. doi:10.1007/s40620-016-0334-1

Figurek, A., Rroji, M., and Spasovski, G. (2020). Sclerostin: A new biomarker of CKD-MBD. *Int. Urol. Nephrol.* 52 (1), 107–113. Epub 2019 Oct 14. PMID: 31612420. doi:10.1007/s11255-019-02290-3

Filipowska, J., Kondegowda, N. G., Leon-Rivera, N., Dhawan, S., and Vasavada, R. C. (2022). LGR4, a G Protein-Coupled receptor with a systemic role: From development to metabolic regulation. *Front. Endocrinol. (Lausanne)* 13, 867001. doi:10.3389/fendo.2022.867001

Frazão, J. M., and Martins, P. (2009). Adynamic bone disease: Clinical and therapeutic implications. *Curr. Opin. Nephrol. Hypertens.* 18 (4), 303–307. PMID: 19424062. doi:10.1097/MNH.0b013e32832c4df0

Frost, H. M. (1966). Bone dynamics in metabolic bone disease. *J. Bone Jt. Surg. Am.* 48, 1192–1203. doi:10.2106/00004623-196648060-00018

Frost, H. M. (1990). Skeletal structural adaptations to mechanical usage (SATMU):1. Redefining wolff's law: The bone modeling problem. *Anat. Rec.* 226, 403–413. doi:10.1002/ar.1092260402

Fu, Q., Jilka, R. L., Manolagas, S. C., and O'Brien, C. A. (2002). Parathyroid hormone stimulates receptor activator of NFkappa B ligand and inhibits osteoprotegerin expression via protein kinase A activation of cAMP-response element-binding protein. *J. Biol. Chem.* 277 (50), 48868–48875. doi:10.1074/jbc.M208494200

Fu, Q., Manolagas, S. C., and O'Brien, C. A. (2006). Parathyroid hormone controls receptor activator of NF-kappaB ligand gene expression via a distant transcriptional enhancer. *Mol. Cell Biol.* 26 (17), 6453–6468. doi:10.1128/MCB.00356-06

Ginsberg, C., and Ix, J. H. (2022). Diagnosis and management of osteoporosis in advanced kidney disease: A review. *Am. J. Kidney Dis.* 79 (3), 427–436. Epub 2021 Aug 20. PMID: 34419519. doi:10.1053/j.ajkd.2021.06.031

Goltzman, David (2018). Physiology of parathyroid hormone. *Endocrinol. Metab. Clin. North Am.* 47 (4), 743–758. Epub 2018 Oct 11. doi:10.1016/j.ecl.2018.07.003

Goltzman, D., Mannstadt, M., and Marcocci, C. (2018). "Physiology of the calcium-parathyroid hormone-vitamin D Axis," in *Vitamin D in clinical medicine*. Editors A. Giustina and J. P. Bilezikian (Front Horm Res. Basel, Karger), 1–13. doi:10.1159/000486060

Gordon, M. D., and Nusse, R. (2006). Wnt signaling: Multiple pathways, multiple receptors, and múltiple transcription factors. *J. Biol. Chem.* 281 (32), 22429–22433. doi:10.1074/jbc.R600015200

Gori, F., Hofbauer, L. C., Dunstan, C. R., Spelsberg, T. C., Khosla, S., and Riggs, B. L. (2000). The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. *Endocrinology* 141 (12), 4768–4776. doi:10.1210/endo.141.12.7840

Guo, J., Liu, M., Yang, D., Bouxsein, M. L., Saito, H., Galvin, R. J. S., et al. (2010). Suppression of wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metab.* 11 (2), 161–171. doi:10.1016/j.cmet.2009.12.007

Gutiérrez, O. M., Mannstadt, M., Isakova, T., Rauh-Hain, J. A., Tamez, H., Shah, A., et al. (2008). Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N. Engl. J. Med.* 359, 584–592. doi:10.1056/NEJMoa0706130

Haarhaus, M., Aaltonen, L., Cejka, D., Cozzolino, M., de Jong, R. T., D'Haese, P., et al. (2022). Management of fracture risk in CKD-traditional and novel approaches. *Clin. Kidney J.* 16 (3), 456–472. PMID: 36865010; PMCID: PMC9972845. doi:10.1093/ckj/sfac230

Handler, P., Cohn, D. V., and Dratz, A. F. (1954). "Effect of parathyroid extract on renal function," in *En: Metabolic interrelations.* (New York: Progress Associates), 320–332.

Heller, M., Mclean, F. C., and Bloom, W. (1950). Cellular transformations in mammalian bones induced by parathyroid extract. *Am. J. Anat.* 87 (3), 315–345. doi:10.1002/aja.1000870302

Hock, J. M., and Gera, I. (1992). Effects of continuous and intermittent administration and inhibition of resorption on the anabolic response of bone to parathyroid hormone. *J. Bone Min. Res.* 7 (1), 65–72. doi:10.1002/jbmr.5650070110

Hruska, K. A., Sugatani, T., Agapova, O., and Fang, Y. (2017). The chronic kidney disease — mineral bone disorder (CKD-MBD): Advances in pathophysiology. *Bone* 100, 80–86. doi:10.1016/j.bone.2017.01.023

Hurley, M. M., Okada, Y., Xiao, L., Tanaka, Y., Ito, M., Okimoto, N., et al. (2006). Impaired bone anabolic response to parathyroid hormone in Fgf2-/and Fgf2+/mice. *Biochem. Biophys. Res. Commun.*341 (4), 989–994. doi:10.1016/j.bbrc.2006.01.044

Itoh, K., Udagawa, N., Matsuzaki, K., Takami, M., Amano, H., Shinki, T., et al. (2000). Importance of membrane- or matrix-associated forms of M-CSF and RANKL/ODF in osteoclastogenesis supported by SaOS-4/3 cells expressing recombinant PTH/PTHrP receptors. *J. bone mineral Res.* 15 (9), 1766–1775. doi:10.1359/jbmr.2000.15.9.1766

Jang, M-G., Lee, J. Y., Yang, J-Y., Park, H., Kim, J. H., Kim, J-E., et al. (2016). Intermittent PTH treatment can delay the transformation of mature osteoblasts into lining cells on the periosteal surfaces. *J. Bone Min. Metab.* 34 (5), 532–539. doi:10.1007/s00774-015-0707-x

Jara, A., Bover, J., and Arnold, J. (1995). Development of secondary hyperparathyroidism and bone disease in diabetic rats with renal failure. *Kidney Int.* 47, 1746–1751. doi:10.1038/ki.1995.241

Jørgensen, H. S., Behets, G., Viaene, L., Bammens, B., Claes, K., Meijers, B., et al. (2022). Diagnostic accuracy of noninvasive bone turnover markers in renal osteodystrophy. *Am. J. Kidney Dis.* 79 (5), 667–676.e1. Epub 2021 Oct 26. PMID: 34710517. doi:10.1053/j.ajkd.2021.07.027

Jørgensen, H. S., Ferreira, A. C., D'Haese, P., Haarhaus, M., Vervloet, M., Lafage-Proust, M. H., et al. (2022). Bone histomorphometry for the diagnosis of renal osteodystrophy: A call for harmonization of reference ranges. *Kidney Int.* 102 (2), 431–434. Epub 2022 May 25. PMID: 35643374. doi:10.1016/j.kint.2022.04.030

Jüppner, H., Abou-Samra, A. B., Freeman, M., Kong, X. F., Schipani, E., Richards, J., et al. (1991). A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. *Science* 254 (5034), 1024–1026. doi:10.1126/science.1658941

Kalantar-Zadeh, K., Jafar, T. H., Nitsch, D., Neuen, B. L., and Perkovic, V. (2021). Chronic kidney disease. *Lancet* 398 (10302), 786–802. Epub 2021 Jun 24. doi:10.1016/S0140-6736(21)00519-5

Katsimbri, P. (2017). The biology of normal bone remodelling. *Eur. J. Cancer Care (Engl).* 26 (6), e12740. doi:10.1111/ecc.12740

Kendler, D. L., Cosman, F., Stad, R. K., and Ferrari, S. (2022). Denosumab in the treatment of osteoporosis: 10 Years later: A narrative review. *Adv. Ther.* 39 (1), 58–74. Epub 2021 Nov 11. PMID: 34762286; PMCID: PMC8799550. doi:10.1007/s12325-021-01936-y

Ketteler, M., Block, G. A., Evenepoel, P., Fukagawa, M., Herzog, C. A., McCann, L., et al. (2017). Executive summary of the 2017 KDIGO chronic kidney disease–mineral and bone disorder (CKD-MBD) guideline update: what's changed and why it matters. *Kidney Int.* 92 (6), 26–36. doi:10.1016/j.kint.2017.04.006

Ketteler, M., Bover, J., and Mazzaferro, S.ERA CKD-MBD Working Groups (2022). Treatment of secondary hyperparathyroidism in non-dialysis CKD: An appraisal 2022s. *Nephrol. Dial. Transplant.* 2022, gfac236–7. doi:10.1093/ndt/gfac236

Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group (2017). KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease–mineral and bone disorder (CKD-MBD). *Kidney Int. Suppl.* 7, 1–59. doi:10.1016/j.kisu.2017.04.001

Kidney Disease: Improving Global Outcomes (KDIGO)CKD–MBD Work Group (2009). KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease–mineral and bone disorder (CKD–MBD). *Kidney Int.* 76, S1–S130. doi:10.1038/ki.2009.188

Kim, A. S., Girgis, C. M., and McDonald, M. M. (2022). Osteoclast recycling and the rebound phenomenon following denosumab discontinuation. *Curr. Osteoporos. Rep.* 20 (6), 505–515. Epub 2022 Oct 6. PMID: 36201122; PMCID: PMC9718877. doi:10.1007/s11914-022-00756-5

Kim, J-B., Leucht, P., Luppen, C. A., Park, Y. J., Beggs, H. E., Damsky, C. H., et al. (2007). Reconciling the roles of FAK in osteoblast differentiation, osteoclast remodeling, and bone regeneration. *Bone* 41 (1), 39–51. doi:10.1016/j.bone.2007.01.024

Kim, M. S., Magno, C. L., Day, C. J., and Morrison, N. A. (2006). Induction of chemokines and chemokine receptors CCR2b and CCR4 in authentic human osteoclasts differentiated with RANKL and osteoclast like cells differentiated by MCP-1 and RANTES. *J. Cell Biochem.* 97 (3), 512–518. doi:10.1002/jcb.20649

Komaba, H., Hamano, T., Fujii, N., Moriwaki, K., Wada, A., Masakane, I., et al. (2022). Parathyroidectomy vs cinacalcet among patients undergoing hemodialysis. *J. Clin. Endocrinol. Metab.* 107 (7), 2016–2025. doi:10.1210/clinem/dgac142

Komaba, H., Taniguchi, M., Wada, A., Iseki, K., Tsubakihara, Y., and Fukagawa, M. (2015). Parathyroidectomy and survival among Japanese hemodialysis patients with secondary hyperparathyroidism. *Kidney Int.* 88, 350–359. doi:10.1038/ki.2015.72

Kondo, H., Guo, J., and Bringhurst, F. R. (2002). Cyclic adenosine monophosphate/protein kinase A mediates parathyroid hormone/parathyroid hormone-related protein receptor regulation of osteoclastogenesis and expression of RANKL and osteoprotegerin mRNAs by marrow stromal cells. *J. Bone Min. Res.* 17 (9), 1667–1679. doi:10.1359/jbmr.2002.17.9.1667

Levin, A., Bakris, G. L., Molitch, M., Smulders, M., Tian, J., Williams, L. A., et al. (2007). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney Int.* 71, 31–38. doi:10.1038/sj.ki.5002009

Li, J., Sarosi, I., Cattley, R. C., Pretorius, J., Asuncion, F., Grisanti, M., et al. (2006). Dkk1-mediated inhibition of Wnt signaling in bone results in osteopenia. *Bone* 39, 754–766. doi:10.1016/j.bone.2006.03.017

Llach, F., and Bover, J. (2000). "Renal osteodystrophies," in *The kidney*. Editor B. M. Brenner (Philadelphia: W.B. Saunders Company).

Loots, G. G., Kneissel, M., Keller, H., Baptist, M., Chang, J., Collette, N. M., et al. (2005). Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res.* 15 (7), 928–935. doi:10.1101/gr.3437105

Lotinun, S., Sibonga, J. D., and Turner, R. T. (2005). Evidence that the cells responsible for marrow fibrosis in a rat model for hyperparathyroidism are preosteoblasts. *Endocrinology* 146 (9), 4074–4081. Epub 2005 Jun 9. PMID: 15947001. doi:10.1210/en.2005-0480

Lowry, M. B., Lotinun, S., Leontovich, A. A., Zhang, M., Maran, A., Shogren, K. L., et al. (2008). Osteitis fibrosa is mediated by Platelet-Derived Growth Factor-A via a phosphoinositide 3-kinase-dependent signaling pathway in a rat model for chronic hyperparathyroidism. *Endocrinology* 149 (11), 5735–5746. doi:10.1210/en.2008-0134

Lucas, R. C. (1883). On a form of late rickets associated with albuminuria, rickets of adolescents. *Lancet* 121 (3119), 993–994. doi:10.1016/s0140-6736(02)37965-0

Lunyera, J., and Scialla, J. J. (2018). Update on chronic kidney disease mineral and bone disorder in cardiovascular disease. *Semin. Nephrol.* 38 (6), 542–558. PMID: 30413250; PMCID: PMC6372293. doi:10.1016/j.semnephrol.2018.08.001

Luo, J., Yang, Z., Ma, Y., Yue, Z., Lin, H., Qu, G., et al. (2016). LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. *Nat. Med.* 22 (5), 539–546. doi:10.1038/nm.4076

Luo, J., Zhou, W., Zhou, X., Li, D., Weng, J., Yi, Z., et al. (2009). Regulation of bone formation and remodeling by G-protein-coupled receptor 48. *Development* 136 (16), 2747–2756. doi:10.1242/dev.033571

Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B., et al. (2007). The architecture of the adhesive apparatus of cultured osteoclasts: From podosome formation to sealing zone assembly. *PLoS One* 2 (1), e179. doi:10.1371/journal.pone.0000179

Ma, Y. L., Cain, R. L., Halladay, D. L., Yang, X., Zeng, Q., Miles, R. R., et al. (2001). Catabolic effects of continuous human PTH (1–38) *in vivo* is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology* 142 (9), 4047–4054. doi:10.1210/endo.142.9.8356

Malluche, H. H., and Faugere, M-C. (1986). *Atlas of mineralized bone histology*. Basilea, Suiza: S Karger AG.

Malluche, H. H., Mawad, H. W., and Monier-Faugere, M-C. (2011). Renal osteodystrophy in the first decade of the new millennium: Analysis of 630 bone biopsies in black and white patients. *J. Bone Min. Res.* 26 (6), 1368–1376. doi:10.1002/jbmr.309

Malluche, H. H., Sherman, D., Meyer, W., Ritz, E., Norman, A. W., and Massry, S. G. (1982). Effects of long-term infusion of physiologic doses of 1-34 PTH on bone. *Am. J. Physiol.* 242 (2), F197–F201. PMID: 7065136. doi:10.1152/ajprenal.1982.242.2.F197

Martin, T. J., and Sims, N. A. (2005). Osteoclast-derived activity in the coupling of bone formation to resorption. *Trends Mol. Med.* 11 (2), 76–81. doi:10.1016/j.molmed.2004.12.004

Massy, Z., and Drueke, T. (2017). Adynamic bone disease is a predominant bone pattern in early stages of chronic kidney disease. *J. Nephrol.* 30 (5), 629–634. doi:10.1007/s40620-017-0397-7

Massy, Z. A., Hénaut, L., Larsson, T. E., and Vervloet, M. G. (2014). Calcium-sensing receptor activation in chronic kidney disease: Effects beyond parathyroid hormone control. *Semin. Nephrol.* 34 (6), 648–659. doi:10.1016/j.semnephrol.2014.10.001

Mazzaferro, S., and Pasquali, M. (2021). Bone biopsy in chronic kidney disease: Still neglected and in need of revitalization. *Nephrol. Dial. Transpl.* 36, 202–204. doi:10.1093/ndt/gfaa269

McClung, M. R., Bolognese, M. A., Brown, J. P., Reginster, J. Y., Langdahl, B. L., Maddox, J., et al. (2020). A single dose of zoledronate preserves bone mineral density for up to 2 years after a second course of romosozumab. *Osteoporos. Int.* 31 (11), 2231–2241. Epub 2020 Jul 4. PMID: 32623487; PMCID: PMC7560921. doi:10.1007/s00198-020-05502-0

McMahon, D. J., Carrelli, A., Palmeri, N., Zhang, C., DiTullio, M., Silverberg, S. J., et al. (2015). Effect of parathyroidectomy upon left ventricular mass in primary hyperparathyroidism: A meta-analysis. *J. Clin. Endocrinol. Metab.* 100, 4399–4407. doi:10.1210/jc.2015-3202

Miller, P. D., Adachi, J. D., Albergaria, B.-H., Cheung, A. M., Chines, A. A., Gielen, E., et al. (2022). Efficacy and safety of romosozumab among postmenopausal women with osteoporosis and mild-to-moderate chronic kidney disease. *J. Bone Mineral Res.* 37 (8), 1437–1445. doi:10.1002/jbmr.4563

Moe, S., Drüeke, T., Cunningham, J., Goodman, W., Martin, K., Olgaard, K., et al. (2006). Definition, evaluation, and classification of renal osteodystrophy: A position statement from kidney disease: Improving global outcomes (KDIGO). *Kidney Int.* 69 (11), 1945–1953. doi:10.1038/sj.ki.5000414

Moe, S. M., Chen, N. X., Newman, C. L., Organ, J. M., Kneissel, M., Kramer, I., et al. (2015). Anti-sclerostin antibody treatment in a rat model of progressive renal osteodystrophy. *J. Bone Mineral Res.* 30 (3), 499–509. doi:10.1002/jbmr.2372

Moe, S. M., and Nickolas, T. L. (2016). Fractures in patients with CKD: Time for action. *Clin. J. Am. Soc. Nephrol.* 11 (11), 1929–1931. Epub 2016 Oct 24. PMID: 27797903; PMCID: PMC5108207. doi:10.2215/CJN.09500916

Moore, C., Yee, J., Malluche, H., Rao, D. S., Monier-Faugere, M. C., Adams, E., et al. (2009). Relationship between bone histology and markers of bone and mineral metabolism in african-American hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* 4 (9), 1484–1493. doi:10.2215/CJN.01770408

Moysés, R. M., and Schiavi, S. C. (2015). Sclerostin, osteocytes, and chronic kidney disease mineral bone disorder. *Semin. Dial.* 28 (6), 578–586. Epub 2015 Aug 19. PMID: 26288182. doi:10.1111/sdi.12415

Nagata, Y., Imanishi, Y., Tateishi, T., Miyaoka, D., Kurajoh, M., Arnold, A., et al. (2022). Parathyroid hormone regulates circulating levels of sclerostin and FGF23 in a primary hyperparathyroidism model. *J. Endocr. Soc.* 6, bvac027–10. doi:10.1210/jendso/bvac027

Nagy, E., Sobh, M., Abdalbary, M., Elnagar, S., Elrefaey, R., Shabaka, S., et al. (2022). Is adynamic bone always a disease? Lessons from patients with chronic kidney disease. *J. Clin. Med.* 11, 7130. doi:10.3390/jcm11237130

Naji Rad, S., and Deluxe, L. (2022). "Osteitis fibrosa cystica," in *StatPearls* (Treasure Island: StatPearls Publishing).

Nakashima, T., and Takayanagi, H. (2011). RANKL signal and osteoimmunology. *Clin. Calcium* 21 (8), 1131–1140.

Naylor, K. L., Garg, A. X., Zou, G., Langsetmo, L., Leslie, W. D., Fraser, L. A., et al. (2015). Comparison of fracture risk prediction among individuals with reduced and normal kidney function. *Clin. J. Am. Soc. Nephrol.* 10 (4), 646–653. doi:10.2215/CJN.06040614

Neale, M. (2017). Weitzmann; session: Bone as an endocrine organ; bone and the immune system. *Toxicol. Pathol.* 45 (7), 911–924. doi:10.1177/0192623317735316

Nebeker, H. G., and Coburn, J. W. (1986). Aluminum and renal osteodystrophy. *Annu. Rev. Med.* 37 (1), 79–95. doi:10.1146/annurev.me.37.020186.000455

Neer, R. M., Arnaud, C. D., Zanchetta, J. R., Prince, R., Gaich, G. A., Reginster, J. Y., et al. (2001). Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N. Engl. J. Med.* 344 (19), 1434–1441. doi:10.1056/NEJM200105103441904

Neto, R., Pereira, L., Magalhães, J., Quelhas-Santos, J., Martins, S., Carvalho, C., et al. (2021). Sclerostin and DKK1 circulating levels associate with low bone turnover in patients with chronic kidney disease Stages 3 and 4. *Clin. Kidney J.* 14 (11), 2401–2408. doi:10.1093/ckj/sfab081

Ng, A. H., Omelon, S., Variola, F., Allo, B., Willett, T. L., Alman, B. A., et al. (2016). Adynamic bone decreases bone toughness during aging by affecting mineral and matrix: Adynamic bone decreases bone toughness during aging. *J. Bone Min. Res.* 31 (2), 369–379. doi:10.1002/jbmr.2702

Niall, H. D., Keutmann, H., Sauer, R., Hogan, M., Dawson, B., Aurbach, G., et al. (1970). The amino acid sequence of bovine parathyroid hormone I. *Hoppe Seylers Z Physiol. Chem.* 351 (12), 1586–1588.

Nishida, S., Yamaguchi, A., Tanizawa, T., Endo, N., Mashiba, T., Uchiyama, Y., et al. (1994). Increased bone formation by intermittent parathyroid hormone administration is due to the stimulation of proliferation and differentiation of osteoprogenitor cells in bone marrow. *Bone* 15 (6), 717–723. doi:10.1016/8756-3282(94)90322-0

Nitta, K., Akiba, T., Suzuki, K., Uchida, K., Watanabe, R., Majima, K., et al. (2004). Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. *Am. J. Kidney Dis.* 44 (4), 680–688. doi:10.1016/s0272-6386(04)00937-0

O'Brien, C. A., Nakashima, T., and Takayanagi, H. (2013). Osteocyte control of osteoclastogenesis. *Bone* 54 (2), 258–263. doi:10.1016/j.bone.2012.08.121

O'Brien, C. A., Plotkin, L. I., Galli, C., Goellner, J. J., Gortazar, A. R., Allen, M. R., et al. (2008). Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PLoS One* 3 (8), e2942. doi:10.1371/journal.pone.0002942

Orlando, M. (2020). Fibroblast growth factor 23 and the last mile. *Clin. J. Am. Soc. Nephrol.* 15, 1355–1357. doi:10.2215/CJN.13631119

Pablo, A. (2020). "Ureña-torres, jordi bover and martine cohen-solal. Relation between PTH and biochemical markers of MBD," in *Parathyroid glands in chronic kidney disease*. Editor A. Covic (Berlin, Germany: Researchgate). doi:10.1007/978-3-030-43769-5_7

Parfitt, A. M., Drezner, M. K., Glorieux, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., et al. (1987). Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR histomorphometry nomenclature committee. *J. Bone Min. Res.* 2 (6), 595–610. doi:10.1002/jbmr.5650020617

Paul, D., Miller, M. D., Gary Hattersley, PhD., Bente Juel Riis, M. D., GregoryWilliams, C. PhD., Edith Lau, M. D., et al. (2016). Effect of abaloparatide vs placebo on new vertebral fractures in postmenopausal women with osteoporosis: A randomized clinical trial. *JAMA* 316 (7), 722–733. doi:10.1001/jama.2016.11136

Pimentel, A., Ureña-Torres, P., Bover, J., Luis Fernandez-Martín, J., and Cohen-Solal, M. (2021). Bone fragility fractures in CKD patients. *Calcif. Tissue Int.* 108 (4), 539–550. doi:10.1007/s00223-020-00779-z

Pimentel, A., Ureña-Torres, P., Zillikens, M. C., Bover, J., and Cohen-Solal, M. (2017). Fractures in patients with CKD-diagnosis, treatment, and prevention: A review by members of the European calcified tissue society and the European renal association of nephrology dialysis and transplantation. *Kidney Int.* 92 (6), 1343–1355. Epub 2017 Sep 28. PMID: 28964571. doi:10.1016/j.kint.2017.07.021

Rendina-Ruedy, E., and Rosen, C. J. (2022). Parathyroid hormone (PTH) regulation of metabolic homeostasis: An old dog teaches us new tricks. *Mol. Metab.* 60, 101480. doi:10.1016/j.molmet.2022.101480

Rhee, Y., Bivi, N., Farrow, E., Lezcano, V., Plotkin, L. I., White, K. E., et al. (2011). Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 *in vitro* and *in vivo*. *Bone* 49 (4), 636–643. Epub 2011 Jun 25. doi:10.1016/j.bone.2011.06.025

Richter, B., and Faul, C. (2018). FGF23 actions on target tissues-with and without klotho. *Front. Endocrinol. (Lausanne)* 9, 189. doi:10.3389/fendo.2018.00189

Rochefort, G. Y., Pallu, S., and Benhamou, C. L. (2010). Osteocyte: The unrecognized side of bone tissue. *Osteoporos. Int.* 21 (9), 1457–1469. doi:10.1007/s00198-010-1194-5

Ruffoni, D., Fratzl, P., Roschger, P., Klaushofer, K., and Weinkamer, R. (2007). The bone mineralization density distribution as a fingerprint of the mineralization process. *Bone* 40, 1308–1319. doi:10.1016/j.bone.2007.01.012

Sabbagh, Y., Graciolli, F. G., O'Brien, S., Tang, W., dos Reis, L. M., Ryan, S., et al. (2012). Repression of osteocyte Wnt/β-catenin signaling is an early event in the progression of renal osteodystrophy. *J. Bone Min. Res.* 27 (8), 1757–1772. doi:10.1002/jbmr.1630

Salusky, I. B., Coburn, J. W., Brill, J., Foley, J., Slatopolsky, E., Fine, R. N., et al. (1988). Bone disease in pediatric patients undergoing dialysis with CAPD or CCPD. *Kidney Int.* 33 (5), 975–982. doi:10.1038/ki.1988.96

Sato, M., Inaba, M., Yamada, S., Emoto, M., Ohno, Y., and Tsujimoto, Y. (2021). Efficacy of romosozumab in patients with osteoporosis on maintenance hemodialysis in Japan; an observational study. *J. Bone Min. Metab.* 39 (6), 1082–1090. doi:10.1007/s00774-021-01253-y

Shimizu, E., Nakatani, T., He, Z., and Partridge, N. C. (2014). Parathyroid hormone regulates histone deacetylase (HDAC) 4 through protein kinase A-mediated phosphorylation and dephosphorylation in osteoblastic cells. *J. Biol. Chem.* 289 (31), 21340–21350. doi:10.1074/jbc.M114.550699

Shimizu, E., Selvamurugan, N., Westendorf, J. J., Olson, E. N., and Partridge, N. C. (2010). HDAC4 represses matrix metalloproteinase-13 transcription in osteoblastic cells, and parathyroid hormone controls this repression. *J. Biol. Chem.* 285 (13), 9616–9626. doi:10.1074/jbc.M109.094862

Silva, B. C., and Bilezikian, J. P. (2015). Parathyroid hormone: Anabolic and catabolic actions on the skeleton. *Curr. Opin. Pharmacol.* 22, 41–50. Epub 2015 Apr 5. PMID: 25854704; PMCID: PMC5407089. doi:10.1016/j.coph.2015.03.005

Silva, B. C., Leslie, W. D., Resch, H., Lamy, O., Lesnyak, O., Binkley, N., et al. (2014). Trabecular bone score: A noninvasive analytical method based upon the DXA image. *J. Bone Min. Res.* 29 (3), 518–530. doi:10.1002/jbmr.2176

Siris, E. S., Chen, Y. T., Abbott, T. A., Barrett-Connor, E., Miller, P. D., Wehren, L. E., et al. (2004). Bone mineral density thresholds for pharmacological intervention to prevent fractures. *Arch. Intern Med.* 164, 1108–1112. doi:10.1001/archinte.164.10.1108

Siris, E. S., Adler, R., Bilezikian, J., Bolognese, M., Dawson-Hughes, B., Favus, M. J., et al. (2014). The clinical diagnosis of osteoporosis: A position statement from the national bone health alliance working group. *Osteoporos. Int.* 25, 1439–1443. doi:10.1007/s00198-014-2655-z

Sprague, S. M., Bellorin-Font, E., Jorgetti, V., Carvalho, A. B., Malluche, H. H., Ferreira, A., et al. (2016). Diagnostic accuracy of bone turnover markers and bone histology in patients with CKD treated by dialysis. *Am. J. Kidney Dis.* 67, 559–566. doi:10.1053/j.ajkd.2015.06.023

Sprague, S. M., Bellorin-Font, E., Jorgetti, V., Carvalho, A. B., Malluche, H. H., Ferreira, A., et al. (2016). Diagnostic accuracy of bone turnover markers and bone histology in patients with CKD treated by dialysis. *Am. J. Kidney Dis.* 67 (4), 559–566. doi:10.1053/j.ajkd.2015.06.023

Sturgeon, C. M., Sprague, S., Almond, A., Cavalier, E., Fraser, W. D., Algeciras-Schimnich, A., et al. (2017). Perspective and priorities for improvement of parathyroid hormone (PTH) measurement A view from the IFCC Working Group for PTH. *Clin. Chim. Acta* 467, 42–47. doi:10.1016/j.cca.2016.10.016

Sugatani, T., Agapova, O. A., Fang, Y., Berman, A. G., Wallace, J. M., Malluche, H. H., et al. (2017). Ligand trap of the activin receptor type IIA inhibits osteoclast stimulation of bone remodeling in diabetic mice with chronic kidney disease. *Kidney Int.* 91, 86–95. doi:10.1016/j.kint.2016.07.039

Sumida, K., Ubara, Y., Hoshino, J., Mise, K., Hayami, N., Suwabe, T., et al. (2016). Once-weekly teriparatide in hemodialysis patients with hypoparathyroidism and low bone mass: A prospective study. *Osteoporos. Int.* 27 (4), 1441–1450. Epub 2015 Nov 2. PMID: 26525045. doi:10.1007/s00198-015-3377-6

Suzuki, T., Mizobuchi, M., Yoshida, S., Terado, N., Aoki, S., Sato, N., et al. (2022). Romosozumab successfully regulated progressive osteoporosis in a patient with autosomal dominant polycystic kidney disease undergoing hemodialysis. *Osteoporos. Int.* 33, 2649–2652. doi:10.1007/s00198-022-06534-4

Takada, I., Mihara, M., Suzawa, M., Ohtake, F., Kobayashi, S., Igarashi, M., et al. (2007). A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat. Cell Biol.* 9 (11), 1273–1285. doi:10.1038/ncb1647

Tanaka, K-I., Yamaguchi, T., Kanazawa, I., and Sugimoto, T. (2015). Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells. *Biochem. Biophys. Res. Commun.* 461 (2), 193–199. doi:10.1016/j.bbrc.2015.02.091

Tasnim, N., Dutta, P., Nayeem, J., Masud, P., Ferdousi, A., Ghosh, A. S., et al. (2021). Osteoporosis, an inevitable circumstance of chronic kidney disease: A systematic review.

Cureus 13 (10), e18488. PMID: 34692259; PMCID: PMC8526087. doi:10.7759/cureus.18488

Terauchi, M., Li, J. Y., Bedi, B., Baek, K. H., Tawfeek, H., Galley, S., et al. (2009). T lymphocytes amplify the anabolic activity of parathyroid hormone through Wnt10b signaling. *Cell Metab.* 10 (3), 229–240. doi:10.1016/j.cmet.2009.07.010

Torregrosa, J.-V., Bover, J., Portillo, M. R., Parra, E. G., Caravaca, Francisco, CasausLorenzo, M-L. G. Víctor, et al. (2022). Recomendaciones de la Sociedad española de Nefrología para el manejo de las alteraciones del metabolismo óseo-mineral en los pacientes con enfermedad renal crónica: 2021 (SEN-MM). *Nefrología* 42 (3), 1–37. doi:10.1016/j.nefro.2022.03.007

Torres, P. U., Bover, J., Mazzaferro, S., de Vernejoul, M. C., and Cohen-Solal, M. (2014). When, how, and why a bone biopsy should Be performed in patients with chronic kidney disease. *Seminars Nephrol.* 34 (6), 612–625. doi:10.1016/j.semnephrol.2014.09.004

Tridimas, A., Anna, M., and Marks, E. (2021). Assessing bone formation in patients with chronic kidney disease using procollagen type I N-terminal propeptide (PINP): The choice of assay makes a difference. *Ann. Clin. Biochem.* 58 (5), 528–536. doi:10.1177/00045632211025567

Tsourdi, E., Langdahl, B., Cohen-Solal, M., Aubry-Rozier, B., Eriksen, E. F., Guañabens, N., et al. (2017). Discontinuation of denosumab therapy for osteoporosis: A systematic review and position statement by ects. *Bone* 105, 11–17. Epub 2017 Aug 5. PMID: 28789921. doi:10.1016/j.bone.2017.08.003

Ubara, Y., Tagami, T., Nakanishi, S., Sawa, N., Hoshino, J., Suwabe, T., et al. (2005). Significance of minimodeling in dialysis patients with adynamic bone disease. *Kidney Int.* 68 (2), 833–839. doi:10.1111/j.1523-1755.2005.00464.x

Ureña, P., Hruby, M., Ferreira, A., Ang, K. S., and de Vernejoul, M. (1996). Plasma total versus bone alkaline phosphatase as markers of bone turnover in hemodialysis patients. *J. Am. Soc. Nephrol.* 7 (3), 506–512. doi:10.1681/ASN.V73506

Ureña-Torres, P. A., Vervloet, M., Mazzaferro, S., Oury, F., Brandenburg, V., Bover, J., et al. (2018). Novel insights into parathyroid hormone: Report of the parathyroid day in chronic kidney disease. *Clin. Kidney J.* 12 (2), 269–280. doi:10.1093/ckj/sfy061

Vasicek, T. J., McDevitt, B. E., Freeman, M. W., Fennick, B. J., Hendy, G. N., Potts, J. T., Jr, et al. (1983). Nucleotide sequence of the human parathyroid hormone gene. *Proc. Natl. Acad. Sci. U. S. A.* 80 (8), 2127–2131. doi:10.1073/pnas.80.8.2127

Vasikaran, S., Cooper, C., Eastell, R., Griesmacher, A., Morris, H. A., Trenti, T., et al. (2011). International osteoporosis foundation and international federation of clinical Chemistry and laboratory medicine position on bone marker standards in osteoporosis. *Clin. Chem. Lab. Med.* 49 (8), 1271–1274. doi:10.1515/CCLM.2011.602

Vervloet, M. G. (2020). FGF23 measurement in chronic kidney disease: What is it really reflecting? *Clin. Chim. Acta* 505, 160–166. doi:10.1016/j.cca.2020.03.013

Vervloet, M. G., Massy, Z. A., Vincent, M. B., Mazzaferro, S., Cozzolino, M., Ureña-Torres, P., et al. (2014). Bone: A new endocrine organ at the heart of chronic kidney disease and mineral and bone disorders. *Lancet Diabetes Endocrinol.* 2, 427–436. doi:10.1016/s2213-8587(14)70059-2

Von Recklinghausen, F. D. (1891). "Die fibröse oder deformirende Ostitis, die Osteomalacie und die osteoplastische Carcinose in ihren gegenseitigen Beziehungen," in *Festschrift R virchow* (Berlin: G. Reimer).

Wada, T., Nakashima, T., Hiroshi, N., and Penninger, J. M. (2006). RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Mol. Med.* 12 (1), 17–25. doi:10.1016/j.molmed.2005.11.007

Wan, M., Yang, C., Li, J., Wu, X., Yuan, H., Ma, H., et al. (2008). Parathyroid hormone signaling through low-density lipoprotein-related protein 6. *Genes Dev.* 22 (21), 2968–2979. doi:10.1101/gad.1702708

Wang, Y., Nishida, S., Boudignon, B. M., Burghardt, A., Elalieh, H. Z., Hamilton, M. M., et al. (2007). IGF-I receptor is required for the anabolic actions of parathyroid hormone on bone. *J. Bone Min. Res.* 22 (9), 1329–1337. doi:10.1359/jbmr.070517

Wang, Z., JiangFang, A. W., and Chen, H. (2018). Cardiac valve calcification and risk of cardiovascular or all-cause mortality in dialysis patients: A meta-analysis. *BMC Cardiovasc. Disord.* 18, 12. doi:10.1186/s12872-018-0747-y

Wheater, G., Elshahaly, M., Tuck, S. P., Datta, H. K., and van Laar, J. M. (2013). The clinical utility of bone marker measurements in osteoporosis. *J. Transl. Med.* 11, 201. doi:10.1186/1479-5876-11-201

WHO (1994). Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech. Rep. Ser.* 843, 1–129.

Wijenayaka, A. R., Kogawa, M., Lim, H. P., Bonewald, L. F., Findlay, D. M., and Atkins, G. J. (2011). Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS ONE* 6 (10), e25900. doi:10.1371/journal.pone.0025900

Xiong, J., Onal, M., Jilka, R. L., Weinstein, R. S., Manolagas, S. C., and O'Brien, C. A. (2011). Matrix-embedded cells control osteoclast formation. *Nat. Med.* 17 (10), 1235–1241. doi:10.1038/nm.2448

Xiong, J., Piemontese, M., Thostenson, J. D., Weinstein, R. S., Manolagas, S. C., and O'Brien, C. A. (2014). Osteocyte-derived RANKL is a critical mediator of the increased bone resorption caused by dietary calcium deficiency. *Bone* 66, 146–154. doi:10.1016/j.bone.2014.06.006

Yajima, A., Inaba, M., Tominaga, Y., Tanaka, M., Otsubo, S., Nitta, K., et al. (2013). Impact of lanthanum carbonate on cortical bone in dialysis patients with adynamic bone disease. *Ther. Apher. Dialysis* 17 (1), 41–48. doi:10.1111/1744-9987.12038

Yamamoto, T., Hasegawa, T., Fraitas, P. H. L., Hongo, H., Zhao, S., Yamamoto, T., et al. (2021). Histochemical characteristics on minimodeling-based bone formation induced by anabolic drugs for osteoporotic treatment. *Biomed. Res.* 42 (5), 161–171. doi:10.2220/biomedres.42.161

Yun, H. J., Ryoo, S. R., Kim, J. E., Choi, Y. J., Park, I., Shin, G. T., et al. (2020). Trabecular bone score may indicate chronic kidney disease-mineral and bone disorder (CKD-MBD) phenotypes in hemodialysis patients: A prospective observational study. *BMC Nephrol.* 21 (1), 299. PMID: 32711466; PMCID: PMC7382149. doi:10.1186/s12882-020-01944-0

Zhang, J., Cohen, A., Shen, B., Du, L., Tasdogan, A., Zhao, Z., et al. (2021). The effect of parathyroid hormone on osteogenesis is mediated partly by osteolectin. *Proc. Natl. Acad. Sci. U. S. A.* 118 (25), e2026176118. doi:10.1073/pnas.2026176118

frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 20 July 2023
DOI 10.3389/fendo.2023.1168687

OPEN ACCESS

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RECEIVED 17 February 2023
ACCEPTED 05 July 2023
PUBLISHED 20 July 2023

CITATION
Bathina S and Armamento-Villareal R (2023) The complex pathophysiology of bone fragility in obesity and type 2 diabetes mellitus: therapeutic targets to promote osteogenesis. *Front. Endocrinol.* 14:1168687. doi: 10.3389/fendo.2023.1168687

The complex pathophysiology of bone fragility in obesity and type 2 diabetes mellitus: therapeutic targets to promote osteogenesis

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Fractures associated with Type2 diabetes (T2DM) are major public health concerns in an increasingly obese and aging population. Patients with obesity or T2DM have normal or better than normal bone mineral density but at an increased risk for fractures. Hence it is crucial to understand the pathophysiology and mechanism of how T2DM and obesity result in altered bone physiology leading to increased fracture risk. Although enhanced osteoclast mediated bone resorption has been reported for these patients, the most notable observation among patients with T2DM is the reduction in bone formation from mostly dysfunction in osteoblast differentiation and survival. Studies have shown that obesity and T2DM are associated with increased adipogenesis which is most likely at the expense of reduced osteogenesis and myogenesis considering that adipocytes, osteoblasts, and myoblasts originate from the same progenitor cells. Furthermore, emerging data point to an inter-relationship between bone and metabolic homeostasis suggesting that these physiologic processes could be under the control of common regulatory pathways. Thus, this review aims to explore the complex mechanisms involved in lineage differentiation and their effect on bone pathophysiology in patients with obesity and T2DM along with an examination of potential novel pharmacological targets or a re-evaluation of existing drugs to improve bone homeostasis.

KEYWORDS

obesity, diabetes, adipogenesis, myogenesis, osteogenesis

1 Introduction

1.1 Obesity type 2 diabetes and bone

Obesity is associated with increased risk of T2DM (1), Cardiovascular diseases (2) and Cancer (3). The World Health Organization (WHO) defined overweight as a BMI of 25 to 29.9 kg/m2 and obesity as a BMI greater than or equal to 30 kg/m2 (4). According to new world health Atlas 2022, by 2030, 20% of women and 14% of men and over 1 billion people

will be living with obesity globally (https://www.worldobesityday.org) and nearly 1 in 4 adults will have severe obesity with prevalence of more than 25% higher in 25 states in US (5). Obesity may lead to T2DM and by 2035, the global prevalence of T2DM is likely to be 592 million (6). The duo (obesity and T2DM) increases as the population ages. Both conditions are associated with normal or better than normal bone mineral density (BMD) but paradoxically increase in the risk for fractures. Obesity is a risk factor for T2DM such that the bone phenotype in the two conditions likely overlap in a major way. Thus, this review aims to examine, the complicated underlying molecular mechanisms involved in the alteration in lineage differentiation and identify pharmacological targets that redirect cell differentiation from the adipogenic to the osteogenic/myogenic pathways.

1.2 Pathophysiology of skeletal fragility in obesity and T2DM

Increase in bone marrow adipose tissue volume has been reported both in diabetes and obesity (7). Earlier studies confirmed an increased risk for hip fracture in both male and female patients with type1 diabetes(T1DM) (8). Osteoporotic fractures especially on the hip, are increased in both T1DM and T2DM, but the risk is 7 fold for those with T1DM compared to 1.38 fold increase in hip fractures of T2DM (9). The increased risk in T1DM is due to lack of anabolic effects of insulin which may contribute to lower peak bone mass while bone mass seems to be preserved in the T2DM (10). Regardless, studies have shown that both T1DM and T2DM is associated with a switch from osteogenesis to adipogenesis, increase in bone marrow adiposity leading to cellular marrow replacement with fat (11). The higher BMD in obesity is believed to be due to skeletal adaptation to accommodate mechanical load and strain (12, 13). However, visceral and total adiposity was not associated with vertebral fractures in men (14). Some studies reported negative correlation between BMD (15, 16). Obesity, is associated with increased secretion of pro-inflammatory factors (as described in Figure 1)) that may be harmful to bone and activation peroxisome proliferator-activated receptor-γ (*PPARγ*) and CCAAT/enhancer-binding protein alpha (CEBPa), nuclear factor kappa light chain enhancer of activated B cells (NF-Kb) pathway (17, 18). Adipokines produced in the adipocytes have inverse relationship to fat mass (19, 20), variably effects bone mass (21). Cao et al, found reduced serum bone formation marker osteocalcin (OCN) and increased bone resorption markers, serum C-telopeptide of type I collagen (CTx) and Tartrate-resistant acid phosphatase 5b (TRAP5b) in diet-induced obese mice (22). Furthermore, Jain et al., studies confirmed that visceral adipose tissue (VAT) is negatively associated with bone mineral density (23). On the other hand, in T2D BMD is normal or above normal,likely protective against vertebral fractures (24), but some studies show reduced BMD (25, 26) due to accumulation of advanced glycation end products (AGEs) (27, 28) increased proinflammatory cytokines such as TNF-a, IL-6 (28, 29) high sclerostin levels (30) leading to reduction in bone formation, OCN (31) and (Procollagen I N-terminal propeptide) P1NP levels in T2DM (31, 32) and impairment in osteoblastogenesis (33–35). There is also reduction in bone resorption markers (CTX and TRAP5b) (28) though bone turnover markers are not as predictive of fractures compared to BMD and maybe difficult to interpret,. Mesenchymal stem cells residents in the bone marrow (BMSCs) are endowed with plasticity and can differentiate into the osteogenic, myogenic or adipogenic lineages depending on the predominant transcription factors present. The enhanced potential of skeletal muscle satellite cells or SMSCs for adipogenic differentiation was observed in diabetic rats using a 3-dimensional matrices *in vitro* model (36) and from in genetically obese Zucker rats (37). Furthermore, myoblasts isolated from Wnt10b (wingless-type mouse mammary tumor virus integration site) null mice showed increased adipogenic potential (38). Jiang et al. found that PRDM16 (Positive Regulatory Domain Motif -16) over expression could partially reverse the effect of mir-499 on adipogenic differentiation of SMSCs and maybe a target for obesity treatment (39). Therefore, there is a need to fully understand the molecular mechanisms behind this shift along with investigations on common regulatory pathways.

Despite the high BMD in obese subjects, these individuals are at increased risk of fractures at nonvertebral skeletal sites (i.e. lower extremities and humerus) (40–42). There are several mechanisms proposed to explain the increased skeletal fragility in obese individuals such as low vitamin D with consequent secondary hyperparathyroidism (43, 44), increased levels of proinflammatory cytokine release from the expanded adipose tissue volume and possibly the high levels of leptin and reduced adiponectin though both have variable effects on the skeleton (44) Low vitamin D is easily corrected clinically but the increase in adipose tissue volume and subsequent proinflammatory state requires more effort (45). Likewise, studies (46–52) have shown that BMD is also higher in patients with T2DM compared to nondiabetic subjects but associated with an increased fracture risk affecting any skeletal site (52, 53). Given that obesity is a risk for T2DM, it would be hard to separate out the effect of obesity from diabetes on the bone. Clinical studies (54–57), including from our group (58, 59) demonstrated suppressed bone formation maker OCN, P1NP (48) and bone resorption marker (CTX) in patients with T2DM. Additionally, Vigevano et al. showed that among obese men, those with concurrent T2DM had higher bone density but reduced bone turnover markers (CTx and OCN) (60) and lower bone strength suggesting that if obesity has a negative effect on the bone, T2DM further adds to the skeletal compromise from obesity alone or that diabetes is the driver for the skeletal phenotype in those who have both. In a study of older women, relative to nonobese without diabetes, those with diabetes but nonobese had a 1.9 risk for vertebral or hip fracture and 1.4 for nonvertebral and non-hip fractures. The corresponding numbers for nondiabetic but obese were 1.2 and 1.1, respectively, while they were 1.5 and 1.8, respectively, for those with both diabetes and obesity (61). Meanwhile, given the clinical observation of increased in bone marrow fat in obesity and diabetes, it is likely that MSCs are involved in the pathology of skeletal fragility seen in in patients with obesity, diabetes or both. This hypothesis was supported by a study from Tencerova et al., which showed that an increase in adipocyte differentiation along with accelerated senescence in BMSCs lead to bone fragility in obese men (62). Thus, pathophysiology of brittle bone in both obesity and T2DM may be attributed as due to the mechanisms discussed below.

1.3 Lack of regulation of brown fat synthesis and/or enhancement of adipogenesis

Differentiation of fat and its control is regulated by transcriptional cascade which can affect the physiological functioning of white and brown adipocytes (63). Normally, the conversion of pre-adipocytes to mature lipid containing adipocytes is a multi-step complex process regulated by transcription factors which can be altered by inflammatory signaling pathways of obesity (64). Of all transcription factors, (*PPARg and CEBPa*) are the key regulators in driving fat cell differentiation (65, 66). Crucially, *PPARγ* which is the driving factor for adipogenesis needs co-activation by *CEBPa* to promote myogenesis (67–69) Cohen et al. (70) found that knock out of PRDM16 resulted in obesity and severe insulin resistance mice fed a high-fat diet (70). Several pre-clinical experiments have confirmed the association of PRDM16 with PGC 1α (71) and *PPARγ* (72) resulting in activation of the myogenic cascade (73) and BAT formation. Recent human studies have shown positive correlations between BAT volume and bone density (74–76). Nevertheless, *PPARy* remains the novel target because of its dual role in MSC-derived adipogenesis.as well as HSC-derived osteoclastogenesis (67). Studies of Beekman et al. (77) showed that *PPARγ* inhibitor, GW9962 has no direct impact on bone marrow adipose tissue (BMAT) in C3H/HeJ mice (77) suggesting that BMAT accumulation might be regulated by a different mechanism. In contrast, another study demonstrated upregulation of sphingosine-1-phosphate (S1P) by S1P lyase, mediated *PPARg* suppression resulting in enhanced bone formation (78). Similarly, Wnt cascade also plays a significant role in the initiation of adipogenesis in obese people (79, 80). Normally, Wnt ligands bind to one of the frizzled family receptors (FZD) and to a co-receptor low-density lipoprotein receptor-related protein (LRP) to activate β-catenin dependent pathway (canonical signaling) and subsequent bone formation (81, 82). Conversely, Wnt signal transduction seems to be redundant in both obesity (83) and T2DM (84). Previous studies showed a close relationship between upregulation of classical Wnt signaling and enhanced myogenesis and/or osteogenesis (85, 86). In humans, subcutaneous injection of Romosozumab which targets sclerostin (an inhibitor of the Wnt pathway), reduced the risk of vertebral and clinical fractures in women with postmenopausal osteoporosis and hence this drug was approved to treat osteoporosis (87). Thus, attractive therapeutic targets using Wnt-targets, acting on obesity associated genes such as secreted frizzled receptor(Srfp1) and Wnt inhibitory factor (WIF-1) acting on classical Wnt-β catenin pathway are undergoing pre-clinical and clinical trials (81).

1.4 Effect of T2DM and obesity on satellite cells and bone senescence

Sarcopenia which is defined as low muscle mass and function is common in the elderly and is associated with increased falls and fractures (88–93). It can accompany obesity in a significant number of older adults for a condition called sarcopenic obesity resulting in frailty (94). Exercise improved muscle strength and physical function in older adults (95–99) and mice (100). For instance, the Lifestyle Interventions and Independence for Elders (LIFE) study (101) in 424 sedentary older persons showed that engaging in moderate-intensity physical activity (combination of aerobic and resistance) intervention reduced the incidence of major mobility disability with an increase in the Short Physical Performance Battery (SPPB) (102). Similarly weight loss from lifestyle intervention by a combination diet and exercise improves physical function, and ameliorates frailty in obese older adults (103–105). In addition, these studies exercise added to diet resulted in amelioration of muscle and bone loss experienced by those who were on diet alone. Since obesity is a risk factor for T2DM, it is expected that a significant number of obese patients with T2DM also have sarcopenic obesity (106). It is likely that skeletal muscle mass and function relies on muscle progenitor cells cascade including satellite cells, interstitial progenitor cells and hence discovery of novel therapeutic targets to improve muscle mass and function are of utmost importance (107). Although the mechanism leading to impairment of muscle dysfunction in obesity remains unclear, the proinflammatory cytokines present in the muscles such *TNFα, IL-6* which are elevated in obesity has been found to be reduced by exercise (108).

Verpoorten et al., showed that cluster of differentiation (CD36) deficient mice although protected from diet-induced obesity, developed impaired satellite cell function and muscle regeneration (109). Apart from adipogenic and inflammatory markers, impairment in fatty acid uptake *via* CD36 can also affect bone integrity (110). Our recent studies showed that in patients with poorly-controlled T2DM had significantly higher circulating osteogenic precursor cells (COPs) compared to well-controlled diabetics. This could mean that COPs are markers of poor metabolic control or the possibility for uncontrolled hyperglycemia results in retardation of differentiation of COPs into mature osteoblasts (59). Studies from our lab also confirmed, that poor glycemic control over 1year is associated with poor bone microarchitecture and strength in men with T2DM (59, 111). On the other hand, alteration in crucial genes of myogenesis can promote development of osteoprogenitor cells. Studies from Hashimoto et al. (112), showed both primary and immortalized progenitor cells derived from muscle of healthy non-dystrophic woman expressed two osteoblastic specific bone proteins, alkaline phosphatase and Runt-related transcription factor 2 (*Runx2)* (112). Studies in knock-out mice (113) and other aging studies (114) also showed that Runx2 deficiency resulted in impairment in osteoblastogenesis and depletion for satellite cells. Thus, it is likely that satellite cells and its gene machinery, play significant role on mediating the process of bone repair and thus, can be used as strategy in treatment (115). The next section discusses on the targets to minimize/nullify the inflammatory oxidative stress and enhance osteogenesis.

2 Emerging therapeutic treatment in bone loss of obese and T2DM patients

Currently, there are numerous medications and therapeutic options for the treatment of osteoporosis but not for bone fragility in diabetic or obese patients in particular (116–120).

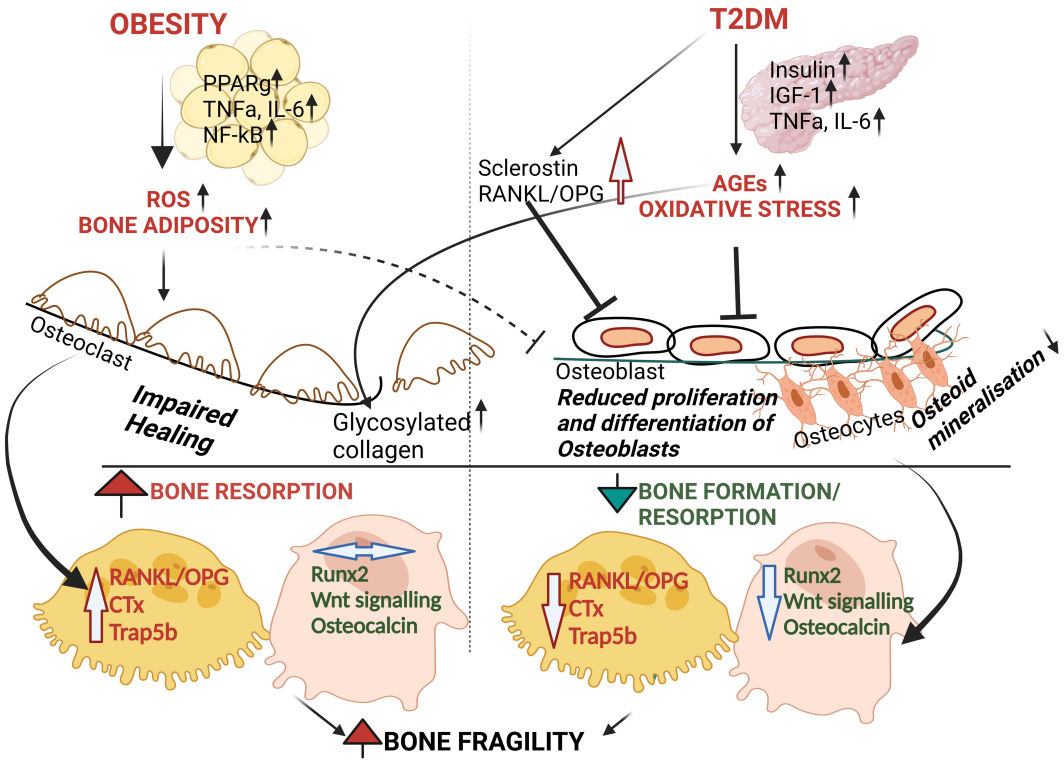

FIGURE 1
Schematic proposal of pathophysiology of bone fragility in Obesity and T2DM. Even though underlying mechanisms are unclear, both T2DM and Obesity causes oxidative stress and inflammation. T2DM causes the accumulation of AGEs with increased oxidative stress leading to osteoblast dysfunction and reduced bone formation (i.e. reduced Runx2 and OCN) followed by increasing bone fragility. On the other hand, obesity is associated with increased pro-inflammatory markers and pro-resorptive factors such as RANKL, TRAP5b. However, whether obesity by itself has any effect on osteoblastic function is unclear. Altogether, the above contribute to simultaneous effect on bone tissue homeostasis leading to bone fragility[15–21].

Given this unmet need, understanding the pathways involved in bone disease in these patients will potentially lead to future strategies to prevent fractures.

2.1 Novel therapies -targeting bone formation

2.1.1 Role of PRDM16 in adipo-myogenic shift and osteogenesis

The novel therapeutic strategies that suppress bone marrow adipogenesis and bone resorption and enhanced bone formation deserve further research. Human PRDM16 located on chromosome 1p36 with 370kb, a zinc finger containing transcriptional regulator protein (121), was recently reported to interact with *PPARg* (122), *CEBPa* (123) and/or *Pgc-1a* (124) promote browning of fat. Additionally, *Prdm16* represses adipogenesis mediated through its association with C-terminal binding proteins (CtBP-1 and -2) suggesting that *PPARγ* can act as bi-directional switch between adipogenesis and myogenesis through its interaction with multiple proteins (125). Apart from *Prdm16* and *PPARγ*, *Pgc1a* might act as co-activator and play critical role from adipogenic to myogenic shift. This was suggested by studies from Seo et al., showing reduction in obesity among mice fed a high-fat diet through suppression of adipogenesis by upregulation of *Prdm16*, *Pgc1a* and uncoupling protein 1 (UCP1) (126). Furthermore, Kaneda et al., found a synergistic association between *Prdm16* and Osteogenic *Runx2* gene in Mel1/*Prdm16*-deficient mice (127). They observed that BMP2 stimulated osteoblasts isolated from Mel1/Prdm16$^{+/-}$ mice are highly stained with alizarin due to extensive calcification and enhanced expression of osteogenic markers such as osteopontin (OPN), OCN when compared to control mice (127). Thus, any ligand inducing a confirmational change in *PPARg* promoting the dissociation of transcriptional repressors and intake of co-activators (*Pgc1a*) leading to activation of the myogenic cascade (as described in Figure 2) along with promotion of the osteogenic *Runx2* gene might be a novel therapeutic targets. The research on these transcriptional activators needs to be investigated. In the next section, we explore the targets involved in myogenesis and osteogenesis and blocking of adipogenesis.

2.1.2 Stem cell therapy

Obesity and T2DM enhance the recruitment of adipocyte precursors, resulting in fat deposition in the viscera, muscles, and other organs and bone fragility. Hence, it is critical to develop therapies to prevent adipocyte differentiation. Stem cell therapy remains an attractive candidate for tissue engineering (128).

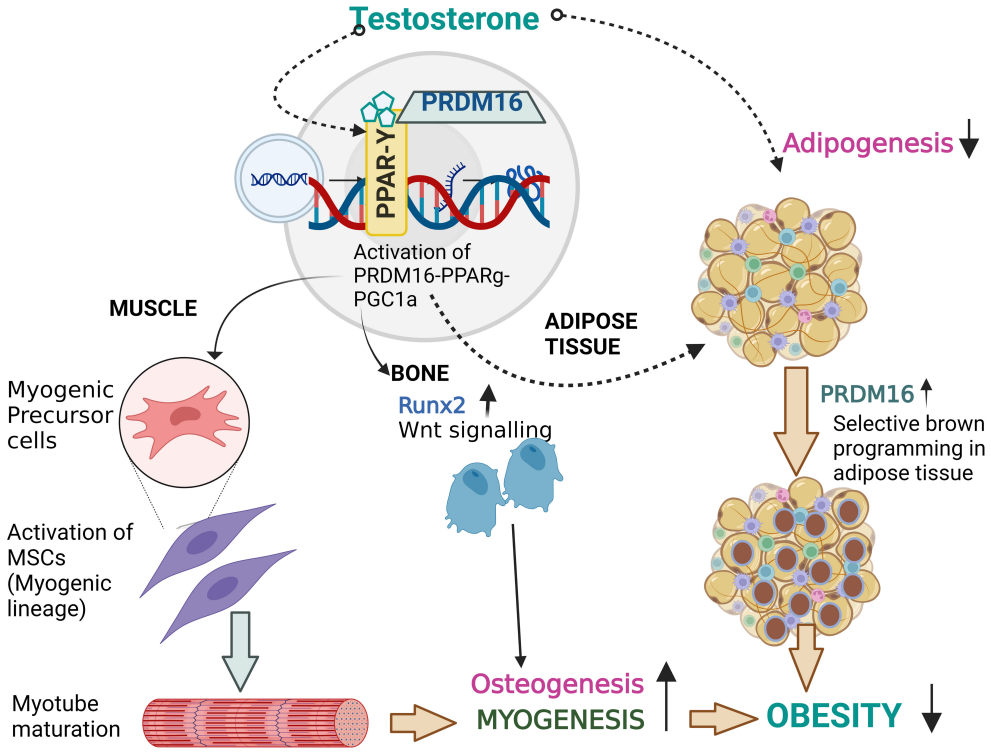

FIGURE 2
Proposed effect of T therapy in Obesity. Binding of testosterone might initiate the *PPARγ-Prdm16-Pgc1a* complex with retinoid X receptor (RXR) which not only activates the browning of adipose tissue by re-programming but may also activate the myogenic cascade and runx2 gene involved osteogenesis. Thus, T therapy with its activator *Prdm16* might be a novel therapeutic target and research on this transcriptional activator needs to be consider.

Adipose-derived mesenchymal stem cells (AD-MSCs) can exhibit various phenotypes of ecto and endodermal hematopoietic stem cells (HSCs) and mesodermal adipocytes, myocytes and osteocytes (129). Louwen et al. showed that human ASCs from obese patients had reduced capacity for osteogenic lineage differentiation (130, 131). Furthermore, Lee et al., reported that intra-articular injection of adipose derived AD-MSCs in patients with knee osteoarthritis, resulted in functional improvements for 6 months without major adverse effect (131). Thus, AD-MSC transplantation is feasible and can possibly be used to repair areas where osteoblastogenesis and subsequent endogenous bone formation is necessary (131, 132). This therapeutic potential of AD-MSCs depends on understanding the mechanism of differentiation capacity in the BM. In line with this, are over 1000 clinical trials registered with the Clinicaltrials.gov (http://www.Clinicaltrials.gov) which may demonstrate the clinical applications of AD-MSCs against bone fragility (133).

2.1.3 Si RNA and other inhibitors

Targeted drug delivery strategies with reliable, efficient delivery remain crucial for cell-based therapy. Even though the delivery of siRNA to bone is challenging due to limited drug penetration and poor vascular perfusion, siRNAs (Short interfering RNA) play pivotal role than chemical-based studies (134), Previous studies targeting *Shn3* (adaptor protein Schnurri-3) gene silencing by genetically engineered BT-Exo-si*Shn3* novel MSC-derived exosome as carrier, resulted in osteogenesis along with blocking of Receptor activator of nuclear factor kappa-B ligand/Dickkopf WNT Signaling Pathway Inhibitor 1 (RANKL/DKK-1), thereby inhibiting osteoclastogenesis in mouse MC3T3-E1 pre-osteoblast cell line (135). Liang et al, developed CH6 aptamer–functionalized lipid nanoparticles (LNPs), specifically targeting both rat and human osteoblasts, was found to promote bone formation (136). Due to high stability and non-immunogenicity aptamers, small single stranded oligonucleotides which can form 3D structure, are used in the ongoing clinical trials for their potential use as novel drug therapy targets for osteoporosis (137). Furthermore, in order to overcome the limitations of direct drug delivery, the combination of nanotechnology with bone target agents can provide more effective therapeutic approach in the near future (138).

2.1.4 Testosterone therapy

Testosterone which is an old drug used for treatment of hypogonadism, has been found in recent years to have beneficial effects in both myogenesis and osteogenesis (139). T is well-known to improve BMD and bone quality in men (140–145). Various studies demonstrated that T therapy increased, levels of OCN (146, 147) and reduced levels of CTx (144, 145) (148) *In vitro* studies showed that 5α-dihydrotestosterone (a potent agonist of androgen receptor synthesized from T by the enzyme 5α-reductase) treatment of bone forming MC3T3-E1 cells not only enhanced

osteoblast differentiation (149) but also downregulated bone resorption promoter RANKL (150) in human osteoblastic cells. Furthermore, testosterone administration increased the width of epiphyseal growth plate of growing rats directly (151, 152). Similarly, Chin et al, showed decreased trabecular bone volume and increased trabecular porosity in orchiectomized (ORX) rats when compared to sham-untreated (SH) group. Conversely, T treatment (7mg/kg) for 8weeks in ORX-TE group prevented these changes and decreased expression of RANKL significantly when compared to SH group (153).

Muscle function contributes in some measure to bone mass and testosterone increases muscle mass and function (154). Preclinical studies suggest a critical role of the adipogenic/myogenic/osteogenic switch on the observed effects of T therapy. Using mouse C3H 10T1/2 pluripotent cells, Singh et al, evaluated the effect of T treatment (0-300 nM) on the myogenic/adipogenic conversion by immunocytochemical staining for MyoD and PPARy (155). They found that T not only promotes commitment of SMSCs into the myogenic lineage but also inhibits adipogenic lineage. Apart from the myogenic machinery, Gao et al., further reported that osteoblast differentiation was activated by T therapy in MC3T3-E1 cells through ERK-1/2, activated *Runx2* pathway (156). Changes in body composition and bone density with T therapy from our lab and other investigators support the above findings from *in-vitro* and animal studies (146, 157, 158). Hence, we hypothesize that the reciprocal effect of T therapy on fat mass, lean mass and bone mass is due to the shift in lineage differentiation from adipogenesis to both myogenesis and osteogenesis. Thus, this concept provided a unifying mechanism for the observed effect of T in hypogonadal men. Roles of other gene machinery such as *Prdm16. Pgc1a* on the adipogenic/myogenic cascade need to be explored. We hypothesize that T therapy activates the trio cascade *PPARγ-Prdm16-Pgc1a* leading to initiation of the switch from adipogenesis to myogenesis along with promotion of osteogenesis (Figure 2) responsible for the observed positive effect on fat mass, lean mass and bone mass in hypogonadal men (146, 157, 158). The Endocrine Society has suggested the use of T to maintain or prevent loss of lean mass in men with HIV (159). Given the emergence of a substantial amount of data showing the positive effects of T on body composition and bone, it is possible that obesity may become one of the indications for T therapy.

3 Conclusion

Obesity and T2DM are increasing at an alarming rate worldwide. Despite the normal or better than normal BMD, both appear to be associated with increased fracture risk, most especially with T2DM. Though it is difficult to separate the skeletal effects of one from the other, there seems to be more data supporting the negative skeletal effects of T2DM than that of obesity, however, this is a complicated issue that needs further investigation. To date, there is no drug approved specifically to treat skeletal fragility in these patients. Since BMD cannot alone predict the risk of bone fragility, this review explores potential new methods or agents to promote the adipo-myogenic/osteogenic lineage shift which may include but not limited to targeting Prdm16, stem cell therapy, si-RNA inhibitors and repurposing of an old drug, testosterone in the general population of patients with obesity, T2DM or both. With further drug development, it is possible to prevent skeletal fragility and promote overall health in these patients.

Author contributions

SB and RA-V, conceptualization, resources and analysis, writing, reviewing, and editing. The figures in this manuscript were created with biorender software. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the US Department of Veterans Affairs Clinical Sciences Research and Development Merit Review Award 101CX001665 and National Institutes of Health R01HD093047 to RA-V.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

References

1. Maggio CA, Pi-Sunyer FX. Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am* (2003) 32(4):805–22, viii. doi: 10.1016/S0889-8529(03)00071-9

2. Hariharan R, Odjidja EN, Scott D, Shivappa N, Hebert JR, Hodge A, et al. The dietary inflammatory index, obesity, type 2 diabetes, and cardiovascular risk factors and diseases. *Obes Rev* (2022) 23(1):e13349. doi: 10.1111/obr.13349

3. Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism* (2019) 92:121–35. doi: 10.1016/j.metabol.2018.11.001

4. Schetz M, De Jong A, Deane AM, Druml W, Hemelaar P, Pelosi P, et al. Obesity in the critically ill: a narrative review. *Intensive Care Med* (2019) 45(6):757–69. doi: 10.1007/s00134-019-05594-1

5. Ward ZJ, Bleich SN, Cradock AL, Barrett JL, Giles CM, Flax C, et al. Projected U.S. State-Level Prevalence of Adult Obesity and Severe Obesity. *N Engl J Med* (2019) 381(25):2440–50. doi: 10.1007/s00223-022-00993-x

6. Forouhi NG, Wareham NJ. Epidemiology of diabetes. *Med (Abingdon)* (2014) 42 (12):698–702. doi: 10.1016/j.mpmed.2014.09.007

7. Walsh JS, Vilaca T. Obesity, Type 2 Diabetes and Bone in Adults. *Calcif Tissue Int* (2017) 100(5):528–35. doi: 10.1007/s00223-016-0229-0

8. Miao J, Brismar K, Nyren O, Ugarph-Morawski A, Ye W. Elevated hip fracture risk in type 1 diabetic patients: a population-based cohort study in Sweden. *Diabetes Care* (2005) 28(12):2850–5. doi: 10.2337/diacare.28.12.2850

9. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis. *Osteoporos Int* (2007) 18(4):427–44. doi: 10.1007/s00198-006-0253-4

10. Hofbauer LC, Busse B, Eastell R, Ferrari S, Frost M, Muller R, et al. Bone fragility in diabetes: novel concepts and clinical implications. *Lancet Diabetes Endocrinol* (2022) 10(3):207–20. doi: 10.1016/S2213-8587(21)00347-8

11. Sebo ZL, Rendina-Ruedy E, Ables GP, Lindskog DM, Rodeheffer MS, Fazeli PK, et al. Bone Marrow Adiposity: Basic and Clinical Implications. *Endocr Rev* (2019) 40 (5):1187–206. doi: 10.1210/er.2018-00138

12. Aguirre LE, Colleluori G, Dorin R, Robbins D, Chen R, Jiang B, et al. Hypogonadal Men with Higher Body Mass Index have Higher Bone Density and Better Bone Quality but Reduced Muscle Density. *CalcifTissue Int* (2017) 101(6):602–11. doi: 10.1007/s00223-017-0316-x

13. Iwaniec UT, Turner RT. Influence of body weight on bone mass, architecture and turnover. *J Endocrinol* (2016) 230(3):R115–30. doi: 10.1530/JOE-16-0089

14. Hind K, Pearce M, Birrell F. Total and Visceral Adiposity Are Associated With Prevalent Vertebral Fracture in Women but Not Men at Age 62 Years: The Newcastle Thousand Families Study. *J Bone Miner Res* (2017) 32(5):1109–15. doi: 10.1002/jbmr.3085

15. Bhupathiraju SN, Dawson-Hughes B, Hannan MT, Lichtenstein AH, Tucker KL. Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults. *Am J Clin Nutr* (2011) 94(4):1063–70. doi: 10.3945/ajcn.111.016030

16. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. *JClinEndocrinolMetab* (2007) 92(5):1640–6. doi: 10.1210/jc.2006-0572

17. Ramirez-Moreno E, Arias-Rico J, Jimenez-Sanchez RC, Estrada-Luna D, Jimenez-Osorio AS, Zafra-Rojas QY, et al. Role of Bioactive Compounds in Obesity: Metabolic Mechanism Focused on Inflammation. *Foods* (2022) 11(9):1232. doi: 10.3390/foods11091232

18. Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. *J Musculoskelet Neuronal Interact* (2020) 20 (3):372–81.

19. Vega GL, Grundy SM. Metabolic risk susceptibility in men is partially related to adiponectin/leptin ratio. *J Obes* (2013) 2013:409679. doi: 10.1155/2013/409679

20. Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C, et al. Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. *J Clin Endocrinol Metab* (2003) 88 (10):4823–31. doi: 10.1210/jc.2003-030214

21. Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, Freedman BI, et al. Adiponectin as a novel determinant of bone mineral density and visceral fat. *Bone* (2003) 33(4):646–51. doi: 10.1016/S8756-3282(03)00237-0

22. Cao JJ, Sun L, Gao H. Diet-induced obesity alters bone remodeling leading to decreased femoral trabecular bone mass in mice. *Ann N Y Acad Sci* (2010) 1192:292–7. doi: 10.1111/j.1749-6632.2009.05252.x

23. Jain RK, Vokes T. Visceral Adipose Tissue is Negatively Associated With Bone Mineral Density in NHANES 2011-2018. *J Endocr Soc* (2023) 7(4):bvad008. doi: 10.1210/jendso/bvad008

24. Dytfeld J, Michalak M. Type 2 diabetes and risk of low-energy fractures in postmenopausal women: meta-analysis of observational studies. *Aging Clin Exp Res* (2017) 29(2):301–9. doi: 10.1007/s40520-016-0562-1

25. Faienza MF, Pontrelli P, Brunetti G. Type 2 diabetes and bone fragility in children and adults. *World J Diabetes* (2022) 13(11):900–11. doi: 10.4239/wjd.v13.i11.900

26. Kurra S, Siris E. Diabetes and bone health: the relationship between diabetes and osteoporosis-associated fractures. *Diabetes Metab Res Rev* (2011) 27(5):430–5. doi: 10.1002/dmrr.1197

27. Cavati G, Pirrotta F, Merlotti D, Ceccarelli E, Calabrese M, Gennari L, et al. Role of Advanced Glycation End-Products and Oxidative Stress in Type-2-Diabetes-Induced Bone Fragility and Implications on Fracture Risk Stratification. *Antioxidants (Basel)* (2023) 12(4):928. doi: 10.3390/antiox12040928

28. Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, et al. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. *Cytokine* (2012) 57(1):136–42. doi: 10.1016/j.cyto.2011.09.029

29. Sanguineti R, Puddu A, Mach F, Montecucco F, Viviani GL. Advanced glycation end products play adverse proinflammatory activities in osteoporosis. *Mediators Inflammation* (2014) 2014:975872. doi: 10.1155/2014/975872

30. Sylvawani M, Setyohadi B, Purnamasari D, Abdullah M, Kurniawan MR. Comparison of insulin-like growth factor-1 and sclerostin levels between premenopausal women with and without diabetes mellitus. *J Taibah Univ Med Sci* (2021) 16(5):719–23. doi: 10.1016/j.jtumed.2021.05.007

31. An Y, Liu S, Wang W, Dong H, Zhao W, Ke J, et al. Low serum levels of bone turnover markers are associated with the presence and severity of diabetic retinopathy in patients with type 2 diabetes mellitus. *J Diabetes* (2021) 13(2):111–23. doi: 10.1111/1753-0407.13089

32. Hou Y, Hou X, Nie Q, Xia Q, Hu R, Yang X, et al. Association of Bone Turnover Markers with Type 2 Diabetes Mellitus and Microvascular Complications: A Matched Case-Control Study. *Diabetes Metab Syndr Obes* (2023) 16:1177–92. doi: 10.2147/DMSO.S400285

33. Sanches CP, Vianna AGD, Barreto FC. The impact of type 2 diabetes on bone metabolism. *Diabetol Metab Syndr* (2017) 9:85. doi: 10.1186/s13098-017-0278-1

34. Wu X, Zhang Y, Xing Y, Zhao B, Zhou C, Wen Y, et al. High-fat and high-glucose microenvironment decreases Runx2 and TAZ expression and inhibits bone regeneration in the mouse. *J Orthop Surg Res* (2019) 14(1):55. doi: 10.1186/s13018-019-1084-2

35. Fowlkes JL, Bunn RC, Liu L, Wahl EC, Coleman HN, Cockrell GE, et al. Runt-related transcription factor 2 (RUNX2) and RUNX2-related osteogenic genes are down-regulated throughout osteogenesis in type 1 diabetes mellitus. *Endocrinology* (2008) 149(4):1697–704. doi: 10.1210/en.2007-1408

36. Acosta FM, Jia UA, Stojkova K, Pacelli S, Brey EM, Rathbone C. Divergent effects of myogenic differentiation and diabetes on the capacity for muscle precursor cell adipogenic differentiation in a fibrin matrix. *Biochem Biophys Res Commun* (2020) 526(1):21–8. doi: 10.1016/j.bbrc.2020.03.025

37. Scarda A, Franzin C, Milan G, Sanna M, Dal Pra C, Pagano C, et al. Increased adipogenic conversion of muscle satellite cells in obese Zucker rats. *Int J Obes (Lond)* (2010) 34(8):1319–27. doi: 10.1038/ijo.2010.47

38. Vertino AM, Taylor-Jones JM, Longo KA, Bearden ED, Lane TF, McGehee REJr., et al. Wnt10b deficiency promotes coexpression of myogenic and adipogenic programs in myoblasts. *MolBiolCell* (2005) 16(4):2039–48. doi: 10.1091/mbc.e04-08-0720

39. Jiang J, Li P, Ling H, Xu Z, Yi B, Zhu S. MiR-499/PRDM16 axis modulates the adipogenic differentiation of mouse skeletal muscle satellite cells. *Hum Cell* (2018) 31 (4):282–91. doi: 10.1007/s13577-018-0210-5

40. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, et al. Obesity is not protective against fracture in postmenopausal women: GLOW. *AmJMed* (2011) 124(11):1043–50. doi: 10.1016/j.amjmed.2011.06.013

41. Prieto-Alhambra D, Premaor MO, Fina AF, Hermosilla E, Martinez-Laguna D, Carbonell-Abella C, et al. The association between fracture and obesity is site-dependent: a population-based study in postmenopausal women. *JBone MinerRes* (2012) 27(2):294–300. doi: 10.1002/jbmr.1466

42. Armstrong ME, Spencer EA, Cairns BJ, Banks E, Pirie K, Green J, et al. Body mass index and physical activity in relation to the incidence of hip fracture in postmenopausal women. *J Bone Miner Res* (2011) 26(6):1330–8. doi: 10.1002/jbmr.315

43. Cipriani C, Pepe J, Colangelo L, Minisola S. Vitamin D and Secondary Hyperparathyroid States. *Front Horm Res* (2018) 50:138–48. doi: 10.1159/000486077

44. Vilaca T, Evans A, Gossiel F, Paggiosi M, Eastell R, Walsh JS. Fat, adipokines, bone structure and bone regulatory factors associations in obesity. *Eur J Endocrinol* (2022) 187(6):743–50. doi: 10.1530/EJE-22-0530

45. Karampela I, Sakelliou A, Vallianou N, Christodoulatos GS, Magkos F, Dalamaga M. Vitamin D and Obesity: Current Evidence and Controversies. *Curr Obes Rep* (2021) 10(2):162–80. doi: 10.1007/s13679-021-00433-1

46. Oei L, Zillikens MC, Dehghan A, Buitendijk GH, Castano-Betancourt MC, Estrada K, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. *Diabetes Care* (2013) 36(6):1619–28. doi: 10.2337/dc12-1188

47. Ma L, Oei L, Jiang L, Estrada K, Chen H, Wang Z, et al. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. *Eur J Epidemiol* (2012) 27(5):319–32. doi: 10.1007/s10654-012-9674-x

48. Liu B, Liu J, Pan J, Zhao C, Wang Z, Zhang Q. The association of diabetes status and bone mineral density among US adults: evidence from NHANES 2005-2018. *BMC Endocr Disord* (2023) 23(1):27. doi: 10.1186/s12902-023-01266-w

49. Tonks KT, White CP, Center JR, Samocha-Bonet D, Greenfield JR. Bone Turnover Is Suppressed in Insulin Resistance, Independent of Adiposity. *J Clin Endocrinol Metab* (2017) 102(4):1112–21. doi: 10.1210/jc.2016-3282

50. Lee RH, Sloane R, Pieper C, Lyles KW, Adler RA, Van Houtven C, et al. Glycemic Control and Insulin Treatment Alter Fracture Risk in Older Men With Type 2 Diabetes Mellitus. *J Bone Miner Res* (2019) 34(11):2045–51. doi: 10.1002/jbmr.3826

51. Vestergaard P. Diabetes and bone fracture: risk factors for old and young. *Diabetologia* (2014) 57(10):2007–8. doi: 10.1007/s00125-014-3338-1

52. Schwartz AV. Epidemiology of fractures in type 2 diabetes. *Bone* (2016) 82:2–8. doi: 10.1016/j.bone.2015.05.032

53. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *JClinEndocrinolMetab* (2006) 91(9):3404–10. doi: 10.1210/jc.2006-0614

54. Purnamasari D, Puspitasari MD, Setiyohadi B, Nugroho P, Isbagio H. Low bone turnover in premenopausal women with type 2 diabetes mellitus as an early process of diabetes-associated bone alterations: a cross-sectional study. *BMC Endocr Disord* (2017) 17(1):72. doi: 10.1186/s12902-017-0224-0

55. Zeng H, Ge J, Xu W, Ma H, Chen L, Xia M, et al. Type 2 Diabetes Is Causally Associated With Reduced Serum Osteocalcin: A Genomewide Association and Mendelian Randomization Study. *J Bone Miner Res* (2021) 36(9):1694–707. doi: 10.1002/jbmr.4330

56. Wang Q, Zhang B, Xu Y, Xu H, Zhang N. The Relationship between Serum Osteocalcin Concentration and Glucose Metabolism in Patients with Type 2 Diabetes Mellitus. *Int J Endocrinol* (2013) 2013:842598. doi: 10.1155/2013/842598

57. Manavalan JS, Cremers S, Dempster DW, Zhou H, Dworakowski E, Kode A, et al. Circulating osteogenic precursor cells in type 2 diabetes mellitus. *JClinEndocrinolMetab* (2012) 97(9):3240–50. doi: 10.1210/jc.2012-1546

58. Colleluori G, Aguirre L, Dorin R, Robbins D, Blevins D, Barnouin Y, et al. Hypogonadal men with type 2 diabetes mellitus have smaller bone size and lower bone turnover. *Bone* (2017) 99:14–9. doi: 10.1016/j.bone.2017.03.039

59. Ballato E, Deepika F, Prado M, Russo V, Fuenmayor V, Bathina S, et al. Circulating osteogenic progenitors and osteoclast precursors are associated with long-term glycemic control, sex steroids, and visceral adipose tissue in men with type 2 diabetes mellitus. *Front Endocrinol (Lausanne)* (2022) 13:936159. doi: 10.3389/fendo.2022.936159

60. Vigevano F, Gregori G, Colleluori G, Chen R, Autemrongsawat V, Napoli N, et al. In Men With Obesity, T2DM Is Associated With Poor Trabecular Microarchitecture and Bone Strength and Low Bone Turnover. *J Clin Endocrinol Metab* (2021) 106(5):1362–76. doi: 10.1210/clinem/dgab061

61. Adami G, Gatti D, Rossini M, Orsolini G, Pollastri F, Bertoldo E, et al. Risk of fragility fractures in obesity and diabetes: a retrospective analysis on a nation-wide cohort. *Osteoporos Int* (2020) 31(11):2113–22. doi: 10.1007/s00198-020-05519-5

62. Tencerova M, Frost M, Figeac F, Nielsen TK, Ali D, Lauterlein JL, et al. Obesity-Associated Hypermetabolism and Accelerated Senescence of Bone Marrow Stromal Stem Cells Suggest a Potential Mechanism for Bone Fragility. *Cell Rep* (2019) 27(7):2050–62 e6. doi: 10.1016/j.celrep.2019.04.066

63. Mota de Sa P, Richard AJ, Hang H, Stephens JM. Transcriptional Regulation of Adipogenesis. *Compr Physiol* (2017) 7(2):635–74. doi: 10.1002/cphy.c160022

64. Sarjeant K, Stephens JM. Adipogenesis. *Cold Spring Harb Perspect Biol* (2012) 4(9):a008417. doi: 10.1101/cshperspect.a008417

65. Cereijo R, Giralt M, Villarroya F. Thermogenic brown and beige/brite adipogenesis in humans. *Ann Med* (2015) 47(2):169–77. doi: 10.3109/07853890.2014.952328

66. Madsen MS, Siersbaek R, Boergesen M, Nielsen R, Mandrup S. Peroxisome proliferator-activated receptor gamma and C/EBPalpha synergistically activate key metabolic adipocyte genes by assisted loading. *Mol Cell Biol* (2014) 34(6):939–54. doi: 10.1128/MCB.01344-13

67. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell* (1999) 4(4):585–95. doi: 10.1016/S1097-2765(00)80209-9

68. Dammone G, Karaz S, Lukjanenko L, Winkler C, Sizzano F, Jacot G, et al. PPARgamma Controls Ectopic Adipogenesis and Cross-Talks with Myogenesis During Skeletal Muscle Regeneration. *Int J Mol Sci* (2018) 19(7):2044. doi: 10.3390/ijms19072044

69. Sun W, He T, Qin C, Qiu K, Zhang X, Luo Y, et al. A potential regulatory network underlying distinct fate commitment of myogenic and adipogenic cells in skeletal muscle. *Sci Rep* (2017) 7:44133. doi: 10.1038/srep44133

70. Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, et al. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* (2014) 156(1-2):304–16. doi: 10.1016/j.cell.2013.12.021

71. Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, et al. Transcriptional control of brown fat determination by PRDM16. *Cell Metab* (2007) 6(1):38–54. doi: 10.1016/j.cmet.2007.06.001

72. Chen S, Bastarrachea RA, Shen JS, Laviada-Nagel A, Rodriguez-Ayala E, Nava-Gonzalez EJ, et al. Ectopic BAT mUCP-1 overexpression in SKM by delivering a BMP7/PRDM16/PGC-1a gene cocktail or single PRMD16 using non-viral UTMD gene therapy. *Gene Ther* (2018) 25(7):497–509. doi: 10.1038/s41434-018-0036-5

73. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* (2008) 454(7207):961–7. doi: 10.1038/nature07182

74. Bredella MA, Gill CM, Rosen CJ, Klibanski A, Torriani M. Positive effects of brown adipose tissue on femoral bone structure. *Bone* (2014) 58:55–8. doi: 10.1016/j.bone.2013.10.007

75. Lee P, Brychta RJ, Collins MT, Linderman J, Smith S, Herscovitch P, et al. Cold-activated brown adipose tissue is an independent predictor of higher bone mineral density in women. *Osteoporos Int* (2013) 24(4):1513–8. doi: 10.1007/s00198-012-2110-y

76. Ponrartana S, Aggabao PC, Hu HH, Aldrovandi GM, Wren TA, Gilsanz V. Brown adipose tissue and its relationship to bone structure in pediatric patients. *J Clin Endocrinol Metab* (2012) 97(8):2693–8. doi: 10.1210/jc.2012-1589

77. Beekman KM, Veldhuis-Vlug AG, van der Veen A, den Heijer M, Maas M, Kerckhofs G, et al. The effect of PPARgamma inhibition on bone marrow adipose tissue and bone in C3H/HeJ mice. *Am J Physiol Endocrinol Metab* (2019) 316(1):E96–E105. doi: 10.1152/ajpendo.00265.2018

78. Weske S, Vaidya M, Reese A, von Wnuck Lipinski K, Keul P, Bayer JK, et al. Targeting sphingosine-1-phosphate lyase as an anabolic therapy for bone loss. *Nat Med* (2018) 24(5):667–78. doi: 10.1038/s41591-018-0005-y

79. Prestwich TC, Macdougald OA. Wnt/beta-catenin signaling in adipogenesis and metabolism. *Curr Opin Cell Biol* (2007) 19(6):612–7. doi: 10.1016/j.ceb.2007.09.014

80. Colaianni G, Brunetti G, Faienza MF, Colucci S, Grano M. Osteoporosis and obesity: Role of Wnt pathway in human and murine models. *World J Orthop* (2014) 5(3):242–6. doi: 10.5312/wjo.v5.i3.242

81. Nie X, Wei X, Ma H, Fan L, Chen WD. The complex role of Wnt ligands in type 2 diabetes mellitus and related complications. *J Cell Mol Med* (2021) 25(14):6479–95. doi: 10.1111/jcmm.16663

82. Martinez-Gil N, Ugartondo N, Grinberg D, Balcells S. Wnt Pathway Extracellular Components and Their Essential Roles in Bone Homeostasis. *Genes (Basel)* (2022) 13(1):138. doi: 10.3390/genes13010138

83. Benzler J, Andrews ZB, Pracht C, Stohr S, Shepherd PR, Grattan DR, et al. Hypothalamic WNT signalling is impaired during obesity and reinstated by leptin treatment in male mice. *Endocrinology* (2013) 154(12):4737–45. doi: 10.1210/en.2013-1746

84. Gassel LC, Schneider S, Banke IJ, Braun KF, Volkering C, Zeeb L, et al. Dysregulation of Wnt signaling in bone of type 2 diabetes mellitus and diabetic Charcot arthropathy. *BMC Musculoskelet Disord* (2022) 23(1):365. doi: 10.1186/s12891-022-05314-9

85. Galli C, Passeri G, Macaluso GM. Osteocytes and WNT: the mechanical control of bone formation. *JDentRes* (2010) 89(4):331–43. doi: 10.1177/0022034510363963

86. Kang S, Bennett CN, Gerin I, Rapp LA, Hankenson KD, Macdougald OA. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. *J Biol Chem* (2007) 282(19):14515–24. doi: 10.1074/jbc.M700030200

87. Cosman F, Crittenden DB, Grauer A. Romosozumab Treatment in Postmenopausal Osteoporosis. *N Engl J Med* (2017) 376(4):396–7. doi: 10.1056/NEJMc1615367

88. Scott D, Seibel M, Cumming R, Naganathan V, Blyth F, Le Couteur DG, et al. Sarcopenic Obesity and Its Temporal Associations With Changes in Bone Mineral Density, Incident Falls, and Fractures in Older Men: The Concord Health and Ageing in Men Project. *J Bone Miner Res* (2017) 32(3):575–83. doi: 10.1002/jbmr.3016

89. Yeung SSY, Reijnierse EM, Pham VK, Trappenburg MC, Lim WK, Meskers CGM, et al. Sarcopenia and its association with falls and fractures in older adults: A systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle* (2019) 10(3):485–500. doi: 10.1002/jcsm.12411

90. Verschueren S, Gielen E, O'Neill TW, Pye SR, Adams JE, Ward KA, et al. Sarcopenia and its relationship with bone mineral density in middle-aged and elderly European men. *OsteoporosInt* (2012) 24(1):87–98. doi: 10.1007/s00198-012-2057-z

91. Turcotte AF, Jean S, Morin SN, Mac-Way F, Gagnon C. Relationships between Obesity and Incidence of Fractures in a Middle-Aged Population: A Study from the CARTaGENE Cohort. *JBMR Plus* (2023) 7(5):e10730. doi: 10.1002/jbm4.10730

92. Turcotte AF, O'Connor S, Morin SN, Gibbs JC, Willie BM, Jean S, et al. Association between obesity and risk of fracture, bone mineral density and bone quality in adults: A systematic review and meta-analysis. *PloS One* (2021) 16(6):e0252487. doi: 10.1371/journal.pone.0252487

93. Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, et al. A meta-analysis of the association of fracture risk and body mass index in women. *J Bone Miner Res* (2014) 29(1):223–33. doi: 10.1002/jbmr.2017

94. Batsis JA, Mackenzie TA, Lopez-Jimenez F, Bartels SJ. Sarcopenia, sarcopenic obesity, and functional impairments in older adults: National Health and Nutrition Examination Surveys 1999-2004. *NutrRes* (2015) 35(12):1031–9. doi: 10.1016/j.nutres.2015.09.003

95. Marzetti E, Calvani R, Tosato M, Cesari M, Di Bari M, Cherubini A, et al. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging Clin Exp Res* (2017) 29(1):35–42. doi: 10.1007/s40520-016-0705-4

96. Landi F, Marzetti E, Martone AM, Bernabei R, Onder G. Exercise as a remedy for sarcopenia. *Curr Opin Clin Nutr Metab Care* (2014) 17(1):25–31. doi: 10.1097/MCO.0000000000000018

97. Phu S, Boersma D, Duque G. Exercise and Sarcopenia. *J Clin Densitom* (2015) 18 (4):488–92. doi: 10.1016/j.jocd.2015.04.011

98. Montero-Fernandez N, Serra-Rexach JA. Role of exercise on sarcopenia in the elderly. *Eur J Phys Rehabil Med* (2013) 49(1):131–43.

99. Keogh J, Henwood T, Climstein M. Exercise to reduce effects of sarcopenia. *Aust Nurs J* (2012) 19(9):39–40.

100. Styner M, Pagnotti GM, McGrath C, Wu X, Sen B, Uzer G, et al. Exercise Decreases Marrow Adipose Tissue Through ss-Oxidation in Obese Running Mice. *J Bone Miner Res* (2017) 32(8):1692–702. doi: 10.1002/jbmr.3159

101. Pahor M, Guralnik JM, Ambrosius WT, Blair S, Bonds DE, Church TS, et al. Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. *JAMA* (2014) 311(23):2387–96. doi: 10.1001/jama.2014.5616

102. Custodero C, Agosti P, Anton SD, Manini TM, Lozupone M, Panza F, et al. Effect of Physical Activity Intervention on Gait Speed by Frailty Condition: A Randomized Clinical Trial. *J Am Med Dir Assoc* (2023) 24(4):489–96. doi: 10.1016/j.jamda.2023.01.023

103. Villareal DT, Aguirre L, Gurney AB, Waters DL, Sinacore DR, Colombo E, et al. Aerobic or Resistance Exercise, or Both, in Dieting Obese Older Adults. *N Engl J Med* (2017) 376(20):1943–55. doi: 10.1056/NEJMoa1616338

104. Villareal DT, Chode S, Parimi N, Sinacore DR, Hilton T, Armamento-Villareal R, et al. Weight loss, exercise, or both and physical function in obese older adults. *NEnglJMed* (2011) 364(13):1218–29. doi: 10.1056/NEJMoa1008234

105. Batsis JA, Villareal DT. Sarcopenic obesity in older adults: aetiology, epidemiology and treatment strategies. *Nat Rev Endocrinol* (2018) 14(9):513–37. doi: 10.1038/s41574-018-0062-9

106. Wang M, Tan Y, Shi Y, Wang X, Liao Z, Wei P. Diabetes and Sarcopenic Obesity: Pathogenesis, Diagnosis, and Treatments. *Front Endocrinol (Lausanne)* (2020) 11:568. doi: 10.3389/fendo.2020.00568

107. Yoshimoto Y, Oishi Y. Mechanisms of skeletal muscle-tendon development and regeneration/healing as potential therapeutic targets. *Pharmacol Ther* (2023) 243:108357. doi: 10.1016/j.pharmthera.2023.108357

108. Lambert CP, Wright NR, Finck BN, Villareal DT. Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons. *JApplPhysiol* (2008) 105(2):473–8. doi: 10.1152/japplphysiol.00006.2008

109. Verpoorten S, Sfyri P, Scully D, Mitchell R, Tzimou A, Mougios V, et al. Loss of CD36 protects against diet-induced obesity but results in impaired muscle stem cell function, delayed muscle regeneration and hepatic steatosis. *Acta Physiol (Oxf)* (2020) 228(3):e13395. doi: 10.1111/apha.13395

110. Kevorkova O, Martineau C, Martin-Falstrault L, Sanchez-Dardon J, Brissette L, Moreau R. Low-bone-mass phenotype of deficient mice for the cluster of differentiation 36 (CD36). *PloS One* (2013) 8(10):e77701. doi: 10.1371/journal.pone.0077701

111. Ballato E, Deepika FNU, Russo V, Fleires-Gutierrez A, Colleluori G, Fuenmayor V, et al. One-Year Mean A1c of > 7% is Associated with Poor Bone Microarchitecture and Strength in Men with Type 2 Diabetes Mellitus. *Calcif Tissue Int* (2022) 11(3):267–78. doi: 10.1007/s00223-022-00993-x

112. Hashimoto N, Kiyono T, Wada MR, Umeda R, Goto Y, Nonaka I, et al. Osteogenic properties of human myogenic progenitor cells. *Mech Dev* (2008) 125(3-4):257–69. doi: 10.1016/j.mod.2007.11.004

113. Qin X, Jiang Q, Komori H, Sakane C, Fukuyama R, Matsuo Y, et al. Runt-related transcription factor-2 (Runx2) is required for bone matrix protein gene expression in committed osteoblasts in mice. *J Bone Miner Res* (2021) 36(10):2081–95. doi: 10.1002/jbmr.4386

114. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. *Aging Cell* (2004) 3(6):379–89. doi: 10.1111/j.1474-9728.2004.00127.x

115. Xu R, Zeng Q, Xia C, Chen J, Wang P, Zhao S, et al. Fractions of Shen-Sui-Tong-Zhi Formula Enhance Osteogenesis V*ia* Activation of beta-Catenin Signaling in Growth Plate Chondrocytes. *Front Pharmacol* (2021) 12:711004. doi: 10.3389/fphar.2021.711004

116. Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development* (2011) 138(17):3625–37. doi: 10.1242/dev.064162

117. Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, et al. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* (2011) 138(17):3647–56. doi: 10.1242/dev.067587

118. Kim MJ, Kim WS, Byun JE, Choi JH, Yoon SR, Choi I, et al. Inhibition of Osteoclastogenesis by Thioredoxin-Interacting Protein-Derived Peptide (TN13). *J Clin Med* (2019) 8(4):431. doi: 10.3390/jcm8040431

119. Luo J, Yang Z, Ma Y, Yue Z, Lin H, Qu G, et al. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. *Nat Med* (2016) 22(5):539–46. doi: 10.1038/nm.4076

120. Nagaoka M, Maeda T, Moriwaki S, Nomura A, Kato Y, Niida S, et al. Petunidin, a B-ring 5'-O-Methylated Derivative of Delphinidin, Stimulates Osteoblastogenesis and Reduces sRANKL-Induced Bone Loss. *Int J Mol Sci* (2019) 20(11):2795. doi: 10.3390/ijms20112795

121. Mochizuki N, Shimizu S, Nagasawa T, Tanaka H, Taniwaki M, Yokota J, et al. A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. *Blood* (2000) 96(9):3209–14.

122. Hondares E, Rosell M, Diaz-Delfin J, Olmos Y, Monsalve M, Iglesias R, et al. Peroxisome proliferator-activated receptor alpha (PPARalpha) induces PPARgamma coactivator 1alpha (PGC-1alpha) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. *J Biol Chem* (2011) 286(50):43112–22. doi: 10.1074/jbc.M111.252775

123. He L, Tang M, Xiao T, Liu H, Liu W, Li G, et al. Obesity-Associated miR-199a/214 Cluster Inhibits Adipose Browning *via* PRDM16-PGC-1alpha Transcriptional Network. *Diabetes* (2018) 67(12):2585–600. doi: 10.2337/db18-0626

124. Pradhan RN, Zachara M, Deplancke B. A systems perspective on brown adipogenesis and metabolic activation. *Obes Rev* (2017) 18 Suppl 1:65–81. doi: 10.1111/obr.12512

125. Jiang N, Yang M, Han Y, Zhao H, Sun L. PRDM16 Regulating Adipocyte Transformation and Thermogenesis: A Promising Therapeutic Target for Obesity and Diabetes. *Front Pharmacol* (2022) 13:870250. doi: 10.3389/fphar.2022.870250

126. Seo YJ, Kim KJ, Choi J, Koh EJ, Lee BY. Spirulina maxima Extract Reduces Obesity through Suppression of Adipogenesis and Activation of Browning in 3T3-L1 Cells and High-Fat Diet-Induced Obese Mice. *Nutrients* (2018) 10(6):712. doi: 10.3390/nu10060712

127. Kaneda-Nakashima K, Igawa K, Suwanruengsri M, Naoyuki F, Ichikawa T, Funamoto T, et al. Role of Mel1/Prdm16 in bone differentiation and morphology. *Exp Cell Res* (2022) 410(2):112969. doi: 10.1016/j.yexcr.2021.112969

128. Minteer D, Marra KG, Rubin JP. Adipose-derived mesenchymal stem cells: biology and potential applications. *Adv Biochem Eng Biotechnol* (2013) 129:59–71. doi: 10.1007/10_2012_146

129. Song Y, Du H, Dai C, Zhang L, Li S, Hunter DJ, et al. Human adipose-derived mesenchymal stem cells for osteoarthritis: a pilot study with long-term follow-up and repeated injections. *Regener Med* (2018) 13(3):295–307. doi: 10.2217/rme-2017-0152

130. Louwen F, Ritter A, Kreis NN, Yuan J. Insight into the development of obesity: functional alterations of adipose-derived mesenchymal stem cells. *Obes Rev* (2018) 19 (7):888–904. doi: 10.1111/obr.12679

131. Lee WS, Kim HJ, Kim KI, Kim GB, Jin W. Intra-Articular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Phase IIb, Randomized, Placebo-Controlled Clinical Trial. *Stem Cells Transl Med* (2019) 8(6):504–11. doi: 10.1002/sctm.18-0122

132. Ye X, Zhang P, Xue S, Xu Y, Tan J, Liu G. Adipose-derived stem cells alleviate osteoporosis by enhancing osteogenesis and inhibiting adipogenesis in a rabbit model. *Cytotherapy* (2014) 16(12):1643–55. doi: 10.1016/j.jcyt.2014.07.009

133. Arjmand B, Sarvari M, Alavi-Moghadam S, Payab M, Goodarzi P, Gilany K, et al. Prospect of Stem Cell Therapy and Regenerative Medicine in Osteoporosis. *Front Endocrinol (Lausanne)* (2020) 11:430. doi: 10.3389/fendo.2020.00430

134. Wang Y, Grainger DW. Developing siRNA therapies to address osteoporosis. *Ther Delivery* (2013) 4(10):1239–46. doi: 10.4155/tde.13.85

135. Cui Y, Guo Y, Kong L, Shi J, Liu P, Li R, et al. A bone-targeted engineered exosome platform delivering siRNA to treat osteoporosis. *Bioact Mater* (2022) 10:207–21. doi: 10.1016/j.bioactmat.2021.09.015

136. Liang C, Guo B, Wu H, Shao N, Li D, Liu J, et al. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. *Nat Med* (2015) 21(3):288–94. doi: 10.1038/nm.3791

137. He F, Wen N, Xiao D, Yan J, Xiong H, Cai S, et al. Aptamer-Based Targeted Drug Delivery Systems: Current Potential and Challenges. *Curr Med Chem* (2020) 27 (13):2189–219. doi: 10.2174/0929867325666181008142831

138. Chen Y, Wu X, Li J, Jiang Y, Xu K, Su J. Bone-Targeted Nanoparticle Drug Delivery System: An Emerging Strategy for Bone-Related Disease. *Front Pharmacol* (2022) 13:909408. doi: 10.3389/fphar.2022.909408

139. Mohamad NV, Soelaiman IN, Chin KY. A concise review of testosterone and bone health. *Clin Interv Aging* (2016) 11:1317–24. doi: 10.2147/CIA.S115472

140. Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E. Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. *JClinEndocrinolMetab* (1997) 82(8):2386–90. doi: 10.1210/jcem.82.8.4163

141. Benito M, Vasilic B, Wehrli FW, Bunker B, Wald M, Gomberg B, et al. Effect of testosterone replacement on trabecular architecture in hypogonadal men. *JBone MinerRes* (2005) 20(10):1785–91. doi: 10.1359/JBMR.050606

142. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH, et al. Effect of testosterone treatment on bone mineral density in men over 65 years of age. *JClinEndocrinolMetab* (1999) 84(6):1966–72. doi: 10.1210/jc.84.6.1966

143. Snyder PJ, Kopperdahl DL, Stephens-Shields AJ, Ellenberg SS, Cauley JA, Ensrud KE, et al. Effect of Testosterone Treatment on Volumetric Bone Density and Strength in Older Men With Low Testosterone: A Controlled Clinical Trial. *JAMA InternMed* (2017) 177(4):471–9. doi: 10.1001/jamainternmed.2016.9539

144. Ng Tang Fui M, Hoermann R, Bracken K, Handelsman DJ, Inder WJ, Stuckey BGA, et al. Effect of Testosterone Treatment on Bone Microarchitecture and Bone Mineral Density in Men: A 2-Year RCT. *J Clin Endocrinol Metab* (2021) 106(8):e3143–e58. doi: 10.1210/clinem/dgab149

145. Ng Tang Fui M, Hoermann R, Nolan B, Clarke M, Zajac JD, Grossmann M. Effect of testosterone treatment on bone remodelling markers and mineral density in obese dieting men in a randomized clinical trial. *Sci Rep* (2018) 8(1):9099. doi: 10.1038/s41598-018-27481-3

146. Colleluori G, Aguirre L, Napoli N, Qualls C, Villareal DT, Armamento-Villareal R. Testosterone Therapy Effects on Bone Mass and Turnover in Hypogonadal Men with Type 2 Diabetes. *J Clin Endocrinol Metab* (2021) 106(8): e3058–e68. doi: 10.1210/clinem/dgab181

147. Ghanim H, Dhindsa S, Green K, Abuaysheh S, Batra M, Makdissi A, et al. Increase in Osteocalcin Following Testosterone Therapy in Men With Type 2 Diabetes and Subnormal Free Testosterone. *J Endocr Soc* (2019) 3(8):1617–30. doi: 10.1210/js.2018-00426

148. Groti Antonic K. Impact of testosterone therapy on bone turnover markers in obese males with type 2 diabetes and functional hypogonadism. *Aging male* (2022) 25 (1):269–77. doi: 10.1080/13685538.2022.2134338

149. Thu HE, Mohamed IN, Hussain Z, Shuid AN. Dihydrotestosterone, a robust promoter of osteoblastic proliferation and differentiation: understanding of time-mannered and dose-dependent control of bone forming cells. *Iran J Basic Med Sci* (2017) 20(8):894–904. doi: 10.22038/IJBMS.2017.9111

150. Huber DM, Bendixen AC, Pathrose P, Srivastava S, Dienger KM, Shevde NK, et al. Androgens suppress osteoclast formation induced by RANKL and macrophage-colony stimulating factor. *Endocrinology* (2001) 142(9):3800–8. doi: 10.1210/endo.142.9.8402

151. Phillip M, Maor G, Assa S, Silbergeld A, Segev Y. Testosterone stimulates growth of tibial epiphyseal growth plate and insulin-like growth factor-1 receptor abundance in hypophysectomized and castrated rats. *Endocrine* (2001) 16(1):1–6. doi: 10.1385/ENDO:16:1:01

152. Ren SG, Malozowski S, Sanchez P, Sweet DE, Loriaux DL, Cassorla F. Direct administration of testosterone increases rat tibial epiphyseal growth plate width. *Acta Endocrinol (Copenh)* (1989) 121(3):401–5. doi: 10.1530/acta.0.1210401

153. Chin KY, Ima-Nirwana S. The effects of orchidectomy and supraphysiological testosterone administration on trabecular bone structure and gene expression in rats. *Aging male* (2015) 18(1):60–6. doi: 10.3109/13685538.2014.954995

154. Sattler F, Bhasin S, He J, Chou CP, Castaneda-Sceppa C, Yarasheski K, et al. Testosterone threshold levels and lean tissue mass targets needed to enhance skeletal muscle strength and function: the HORMA trial. *J Gerontol A Biol Sci Med Sci* (2011) 66 (1):122–9. doi: 10.1093/gerona/glq183

155. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology* (2003) 144 (11):5081–8. doi: 10.1210/en.2003-0741

156. Gao K, Wang X, Liu Q, Chen W, Wang G, Zhang D, et al. Evaluation of osteoblast differentiation and function when cultured on mesoporous bioactive glass adsorbed with testosterone. *J Cell Biochem* (2018) 119(7):5222–32. doi: 10.1002/jcb.26566

157. Deepika F, Ballato E, Colleluori G, Aguirre L, Chen R, Qualls C, et al. Baseline Testosterone Predicts Body Composition and Metabolic Response to Testosterone Therapy. *Front Endocrinol (Lausanne)* (2022) 13:915309. doi: 10.3389/fendo.2022.915309

158. Aguirre LE, Colleluori G, Robbins D, Dorin R, Shah VO, Chen R, et al. Bone and body composition response to testosterone therapy vary according to polymorphisms in the CYP19A1 gene. *Endocrine* (2019) 65(3):692–706. doi: 10.1007/s12020-019-02008-6

159. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, et al. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2018) 103(5):1715–44. doi: 10.1210/jc.2018-00229

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 15 February 2024
DOI 10.3389/fendo.2024.1227196

OPEN ACCESS

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RECEIVED 22 May 2023
ACCEPTED 08 January 2024
PUBLISHED 15 February 2024

CITATION
Gómez-García I, Ladehesa-Pineda ML, Diaz-Tocados JM, López-Medina C, Abalos-Aguilera MC, Ruiz-Vilches D, Paz-Lopez G, Gonzalez-Jimenez A, Ranea JAG, Escudero-Contreras A, Moreno-Indias I, Tinahones FJ, Collantes-Estévez E and Ruiz-Limón P (2024) Bone metabolism and inflammatory biomarkers in radiographic and non-radiographic axial spondyloarthritis patients: a comprehensive evaluation.
Front. Endocrinol. 15:1227196.
doi: 10.3389/fendo.2024.1227196

Bone metabolism and inflammatory biomarkers in radiographic and non-radiographic axial spondyloarthritis patients: a comprehensive evaluation

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Introduction: Axial spondyloarthritis (axSpA) is a heterogeneous disease that can be represented by radiographic axSpA (r-axSpA) and non-radiographic axSpA (nr-axSpA). This study aimed to evaluate the relationship between the markers of inflammation and bone turnover in r-axSpA patients and nr-axSpA patients.

Methods: A cross-sectional study included 29 r-axSpA patients, 10 nr-axSpA patients, and 20 controls matched for age and sex. Plasma markers related to bone remodeling such as human procollagen type 1 N-terminal propeptide (P1NP), sclerostin, tartrate-resistant acid phosphatase 5b (TRACP5b), receptor activator of nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) were measured by an ELISA kit. A panel of 92 inflammatory molecules was analyzed by proximity extension assay.

Results: R-axSpA patients had decreased plasma levels of P1NP, a marker of bone formation, compared to controls. In addition, r-axSpA patients exhibited decreased plasma levels of sclerostin, an anti-anabolic bone hormone, which would not explain the co-existence of decreased plasma P1NP concentration;

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however, sclerostin levels could also be influenced by inflammatory processes. Plasma markers of osteoclast activity were similar in all groups. Regarding inflammation-related molecules, nr-axSpA patients showed increased levels of serum interleukin 13 (IL13) as compared with both r-axSpA patients and controls, which may participate in the prevention of inflammation. On the other hand, r-axSpA patients had higher levels of pro-inflammatory molecules compared to controls (i.e., IL6, Oncostatin M, and TNF receptor superfamily member 9). Correlation analysis showed that sclerostin was inversely associated with IL6 and Oncostatin M among others.

Conclusion: Altogether, different inflammatory profiles may play a role in the development of the skeletal features in axSpA patients particularly related to decreased bone formation. The relationship between sclerostin and inflammation and the protective actions of IL13 could be of relevance in the axSpA pathology, which is a topic for further investigation.

KEYWORDS

radiographic axial spondyloarthritis, bone metabolism, inflammation, biomarkers, Interleukin 13, sclerostin

Introduction

Spondyloarthritis (SpA) is a heterogeneous group of chronic inflammatory rheumatic diseases that share common characteristics (1). This group includes several entities, such as the prototype axial spondyloarthritis (axSpA), which is the most frequent phenotype of axSpA, and the radiographic axial SpA (r-axSpA), which affects the axial skeleton and the sacroiliac joints with radiographic changes (1). On the other hand, the non-radiographic axSpA (nr-axSpA) includes patients with suggestive clinical characteristics of axSpA and the presence of bone marrow edema in the sacroiliac joint in magnetic resonance but no radiographic sacroiliitis (2).

In r-axSpA, the pathological process is characterized by disruption of the normal bone homeostasis, i.e., aberrant new bone formation and concomitant bone loss (3). Pathological bone formation, also known as osteoproliferation, produces structural damage to the spine in the form of syndesmophytes. As the disease progresses, these syndesmophytes can fuse the vertebrae, leading to a condition called ankylosis (4).

In contrast, excessive bone loss can result in osteopenia and osteoporosis, which can already be observed at the early stages of the disease. Severe loss of vertebral bone mineral density increased the risk of vertebral fractures, resulting in spinal deformities (5). Bone loss in r-axSpA may be influenced by several factors, including age, disease duration, gender, inflammation, new bone formation, diet, vitamin D levels, and immobility (6).

Inflammation is a key feature of r-axSpA, and it is thought to precede structural damage in affected joints. Therefore, several markers related to inflammation have been evaluated as prognostic biomarkers in axSpA. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are two commonly used markers of inflammation in clinical practice. High levels of CRP and ESR have been associated with increased disease activity and also with early radiographic progression in axSpA patients of the German Spondyloarthritis Inception Cohort (GESPIC) (7). Additionally, interleukin 6 (IL6) is a major pro-inflammatory cytokine that stimulates the formation and activity of bone-resorbing osteoclasts *in vitro* (8), playing a key role in bone disorders such as Paget´s disease (9).

Identifying biomarkers involved in bone metabolism and turnover could improve the management of r-axSpA. Previous studies conducted in axSpA have suggested that those patients who have syndesmophytes exhibited elevated levels of certain bone resorption markers, such as the C-terminal telopeptide fragments of type I collagen (CTX), as well as bone formation markers, such as osteocalcin (OC) and procollagen type I N-terminal peptide (P1NP) (3, 10). In a study conducted by Ruiz Heiland et al., lower serum levels of sclerostin and dickkopf-1 were found to be significantly associated with 2-year radiographic progression of the spine in patients with AS (11). On the other hand, higher serum levels of these Wnt pathway inhibitors were associated with no new syndesmophyte formation in AS (11, 12).

While there is a growing body of evidence indicating abnormal bone metabolism in patients with axSpA, the impact of inflammation on bone metabolism is still largely unknown. Therefore, this study aimed to evaluate the relationship between the markers of inflammation and bone turnover in r-axSpA patients and nr-axSpA patients.

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Materials and methods

Population and study design

In this cross-sectional study, 29 r-axSpA patients, 10 nr-axSpA patients, and 20 controls matched for age and sex were included. Eligible patients had to fulfill the Assessment of SpondyloArthritis International Society (ASAS) classification criteria for the classification of axSpA (13). Patients who fulfilled the radiographic criteria established by the Modified New York Criteria were classified as r-axSpA, while those who did not fulfill these criteria were categorized as nr-axSpA (14). The patients belonged to the CASTRO cohort (Cordoba Ankylosing Spondylitis Task Registry and Outcomes) of the Reina Sofia University Hospital (Cordoba, Spain). Controls did not have a clinical history of axSpA or inflammatory diseases, or the presence of symptoms that could be related to an undiagnosed axSpA. The exclusion criteria were pregnancy, malignancies, chronic infections, other rheumatology diseases, extra-articular manifestations [psoriasis, inflammatory bowel disease (IBD), and uveitis], receiving biological disease-modifying antirheumatic drugs or treatment with drugs that could interfere with bone metabolism (bisphosphonates, strontium ranelate, selective estrogen receptor modulators, calcitonin, hormone therapy, denosumab, or teriparatide), and inability to understand the procedures to the protocol. All participants underwent a complete medical history physical examination and clinical chemistry analysis before enrolment. A case report form was used to collect the following clinical data: age, gender, disease duration, human leukocyte antigen (HLA)-B27 status, and current medications.

Disease activity was evaluated by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (15), Ankylosing Spondylitis Disease Activity Score (ASDAS; calculated with the CRP) (16), CRP (mg/L), and ESR (mm/h). Spinal mobility and functionality of patients were measured by the Bath Ankylosing Spondylitis Metrology Index (BASMI) (17) and Bath Ankylosing Spondylitis Functionality Index (BASFI) (15), respectively. Structural damage was assessed by the modified stoke Ankylosing Spondylitis Spine Score (mSASSS), which was evaluated by two trained readers (18). Bone mineral density (BMD) was evaluated by dual x-ray absorptiometry (DXA) BMD measurement of the total hip, lumbar, and femoral neck using a DXA LUNAR DPX 8548 BX-1 L densitometer (coefficient of variation < 1%). Exercise was measured using a questionnaire.

All participants provided written informed consent before being included. The study was conducted according to the principles of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Reina Sofia University Hospital, Córdoba, Spain.

Blood sample collection and assessment of biochemical parameters

Peripheral venous blood samples were collected and processed as previously described (19). Plasma and serum were aliquoted and stored at −80°C for subsequent analysis. Laboratory markers of inflammation (ESR and CRP), the genetic factor human leukocyte antigen (HLA)-B27, lipid profile [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides], serum glucose, ferritin, calcium, phosphate, 25-hydroxyvitamin D^3 (25OH vit D), and parathyroid hormone (PTH) were quantified as part of routine patient management.

Markers related to bone remodeling

To estimate the osteoblastic activity, also referred to as bone formation, human procollagen type 1 N-terminal propeptide (P1NP) quantification was carried out in plasma samples (1:200 dilution) using a commercial ELISA Kit (Cusabio Technology LLC, Houston, TX, USA) following the manufacturer's instructions. In addition, the plasma levels of sclerostin, an inhibitor of bone formation that blocks the Wnt/β-catenin pathway (20), were also measured with a commercial ELISA kit (Cusabio Technology LLC) as indicated by the manufacturer.

The osteoclastic activity was determined by measuring plasma levels of tartrate-resistant acid phosphatase 5b (TRACP5b) and receptor activator of nuclear factor kappa B ligand (RANKL), both biomarkers of bone resorption. Plasma TRACP5b concentration was determined using ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions. Circulating levels of RANKL were determined in plasma, diluted at 1:5, and then the quantification was performed with a commercial ELISA kit (Cusabio Technology LLC) as indicated by the manufacturer's instructions. Additionally, the plasma concentration of osteoprotegerin (OPG), an inhibitor of RANKL that prevents osteoclast activity (21), was measured with an ELISA kit (RayBiotech, Peachtree Corners, GA, USA).

Inflammation-related proteins measurement

A panel of 92 inflammatory mediators (Olink target 96 inflammation panel) was evaluated in the serum of all the participants included in the study using the highly specific and sensitive technology proximity extension assay (PEA) (Cobiomic Bioscience, Cordoba, Spain). PEA utilizes a dual-recognition immunoassay in which two matched antibodies labeled with distinct DNA oligonucleotides bind to a target protein in solution. This results in the proximity of the two antibodies, allowing their DNA oligonucleotides to hybridize. Following that, the hybridized oligonucleotides serve as a template for a DNA polymerase-dependent extension step, leading to the creation of a double-stranded DNA "barcode" unique to the specific antigen and quantitatively proportional to the initial concentration of the target protein. The hybridization and extension steps are followed by PCR amplification.

Statistical analysis

A descriptive analysis of the variables was performed and expressed as percentages or as mean ± standard deviation (SD) or median [interquartile range (IQR)], as applicable. The normality of

the distribution was assessed using the Kolmogorov–Smirnov test. Multiple comparisons were analyzed by one-way analysis of variance (ANOVA) with the Tukey test as *post-hoc* analysis for continuous data or by the Kruskal–Wallis test with Dunn multiple-comparison test for non-normally distributed data. Categorical data were analyzed using Pearson's chi-square test. Student's *t*-test or Mann–Whitney *U* test was used to calculate differences between groups for numerical variables, and Pearson's chi-square test was used for categorical variables. Differential protein analysis was performed using R programming language. Data normality and homoscedasticity were checked using base statistical tests from R. Shapiro–Wilk test was used to check the normality of the distribution of values of each molecule per group. Likewise, Levene's test was used to assess homoscedasticity. The analysis of variance (ANOVA) test was applied to each molecule to detect differential abundance between groups. *Post-hoc* pairwise *t*-tests were applied subsequently on each pair of groups to check differences. Afterward, *p*-values were corrected using the FDR method to reduce the probability of observing type I errors, and fold change was calculated for each molecule. The sensitivity and specificity were evaluated for the most significant biomarkers using Receiver Operating Characteristic (ROC) curves that determine the sensitivity, specificity, and cutoff values. The effect sizes were analyzed using Cohen's *d* test. Finally, volcano plots were made using the EnhancedVolcano package (version 1.12.0). The correlations were assessed by Spearman's rank correlation. Molecules with at least one significative correlation value, depending on the p-value, were selected. Heatmaps were made using the pheatmap package (version 1.0.12). Statistical significance was set at $p < 0.05$. Statistical analyses and graph editions were performed with SPSS (15.0 version for Windows: SPSS, Chicago, IL, USA), GraphPad Prism 8.0.2 (GraphPad Software Boston, MA, USA), and R (version 4.1.3).

Results

Characteristics of the study population

Table 1 shows the baseline characteristics of r-axSpA and nr-axSpA patients, and controls. Patients with r-axSpA had a larger disease duration compared to nr-axSpA patients ($p = 0.024$). Moreover, compared with the nr-axSpA and control groups, r-axSpA patients had higher levels of serum CRP ($p = 0.003$) and a greater frequency of arterial hypertension ($p = 0.001$). Furthermore, six r-axSpA patients were diagnosed with hyperlipidemia ($p = 0.002$) and received treatment to control lipid levels; therefore, lipid profiles were similar across the three groups. Regarding the BMD values, nr-axSpA patients displayed a significant decrease in the total hip BMD and femoral neck BMD compared to r-axSpA patients ($p = 0.010$ and $p = 0.012$, respectively). Moreover, compared to controls, r-axSpA patients showed a significant decrease in the femoral neck BMD ($p = 0.046$). No differences were found in terms of disease activity (evaluated with BASDAI and ASDAS), spinal mobility and functionality (measured by BASFI and BASMI), structural damage (mSASSS), HLA-B27 antigen, or treatments received [neither non-steroidal anti-inflammatory drugs (NSAIDs) nor sulfasalazine]. Plasma levels of calcium, phosphate, PTH, and 25-OH-VitD remained similar among all groups. The exercise was similar in these three groups.

Plasma biomarkers of bone turnover in radiographic and non-radiographic axial SpA patients

To investigate whether bone turnover was altered differently in both axSpA patient groups and controls, we measured the levels of well-known bone biomarkers in plasma samples. We found that the circulating levels of the P1NP were significantly decreased (effect size, 0.376) in r-axSpA patients compared to the control group (Figure 1A), indicating lower bone formation; however, no significant differences were observed in the plasma levels of TRACP5b and RANKL, both markers of osteoclast activity (Figures 1B, C, respectively). We also measured OPG plasma concentration (Figure 1D), a decoy receptor that binds to RANKL and prevents interaction with its receptor RANK, and similarly, we did not observe significant differences among the three groups, consistently with similar levels of the RANKL/OPG ratio (Figure 1E). Interestingly, we found a significant reduction in the plasma concentration of sclerostin in r-axSpA patients as compared with controls (Figure 1F), which was not associated with the decrease in plasma P1NP concentration, suggesting that other factors would be involved.

Analysis of inflammatory-related proteins in patients and controls

The levels of 92 serum inflammatory-related proteins were evaluated in this study. Nr-axSpA patients exhibited significantly increased IL13 levels compared to controls and r-axSpA patients with effect sizes of 0.371 and 0.646, respectively (Figures 2A, C). Compared to r-axSpA patients, controls exhibited significantly decreased levels of IL6, Oncostatin M (OSM), and TNF-receptor named tumor necrosis factor receptor superfamily member 9 (TNFRSF9), with effect sizes of 0.911, 0.629, and 0.266, respectively (Figure 2B).

Inflammatory-related proteins were divided into five groups based on their functions: CC Chemokines, CXC Chemokines, interleukins (ILs), cell surface molecules and receptors, and other cytokines and proteins. Notably, patients with r-axSpA showed elevated plasma levels of IL6 compared to the control group (4.38 ±0.876 vs 3.58±0.469, $p = 0.003$), while nr-axSpA patients did not exhibit a significant increase (Figure 3A). Plasma levels of IL18, IL8, IL33, IL17-A and IL10 remained similar among all groups (Figures 3B–F). Interestingly, nr-axSpA patients showed increased levels of IL13 compared to controls (2.45±0.968 vs 1.97±0.274, $p = 0.024$) and r-axSpA patients (2.45±0.968 vs 2.07±0.219, $p = 0.033$; Figure 3G). Furthermore, Oncostatin M (OSM) plasma levels were also slight but significantly increased in r-axSpA patients compared to controls (control 7.80±0.828 vs 7.23±0.627, p=0.012; Figure 3H). No

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differences were observed in the plasma levels of Monocyte Chemoattractant Protein-1 (MCP-1), CXC Motif Chemokine Ligand 6 (CXCL6), CXCL5 and CXCL1 among groups (Figures 3I–L respectively).

Regarding the cell surface molecules and receptors involved in inflammatory processes, the plasma levels of the Cluster of Differentiation 6 (CD6) and Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) were similar among all groups (Figures 4A, B

TABLE 1 Clinical and laboratory parameters of all participants.

Variable	Controls (*n* = 20)	nr-axSpA (*n* = 10)	r-axSpA (*n* = 29)	*p*-value
Clinical parameters				
Age, years, mean (SD)	47.35 ± 10.98	38.10 ± 10.06	46.93 ± 13.09	0.104
Female sex, *n* (%)	8 (40)	2 (20)	13 (44.82)	0.379
Smoking				0.055
Never smoked, *n* (%)	9 (45)	4 (40)	9 (31.03)	
Exsmoker, *n* (%)	5 (25)	4 (40)	8 (27.58)	
Active smoker, *n* (%)	0 (0)	2 (20)	12 (41.37)	
Arterial hypertension, *n* (%)	2 (10)	0 (0)	6 (20.68) [a,b]	0.001
Hyperlipidemia, *n* (%)	1 (5)	1 (10)	6 (20.68) [a]	0.002
Exercise				0.553
Never or hardly ever, *n* (%)		3 (30)	14/24 (58.3)	
1–4 times/week, *n* (%)		5 (50)	8/23 (33.3)	
>4 times/week, *n* (%)		2 (20)	2/24 (8.3)	
BASDAI, median (IQR)	–	2.30 (1.45–5.30)	3.80 (2.00–4.65)	0.530
ASDAS, mean ± SD	–	2.09 ± 0.77	2.43 ± 0.96	0.334
BASMI, mean ± SD	–	2.18 ± 1.08	3.22 ± 1.83	0.102
BASFI, median (IQR)	–	1.90 (0.20–4.17)	3.40 (1.50–5.50)	0.281
mSASSS, median (IQR)	–	5.50 (2.75–8.25)	9.00 (3.00–22.00)	0.100
Total hip BMD, g/cm^2, mean ± SD	1.02 ± 0.15	0.88 ± 0.12	1.03 ± 0.11[b]	0.032
Femoral neck BMD, g/cm^2, mean ± SD	1.01 ± 0.13	0.86 ± 0.09	0.98 ± 0.12 a[,b]	0.017
Lumbar BMD, g/cm^2, mean ± SD	1.11 ± 0.14	1.06 ± 0.17	1.11 ± 0.17	0.475
Disease duration, years, median (IQR)	–	5.00 (2.75–17.25)	19.00 (8.00–31.00)	0.024
Laboratory parameters				
HLA-B27, *n* (%)	–	8 (80)	22 (75.86)	0.652
CRP, mg/dL, median (IQR)	0.58 (0.50–3.47)	1.46 (0.12–5.14)	5.81 (2.85–10.19) [a]	0.003
ESR, mm/h, median (IQR)	5.00 (3.00–10.00)	4.00 (4.00–6.00)	4.00 (3.50–6.50)	0.743
Total cholesterol, mg/dL, mean ± SD	197.66 ± 24.37	185.55 ± 25.76	185.73 ± 27.72	0.498
LDL, mg/dL, mean ± SD	119.11 ± 25.81	111.00 ± 32.93	110.00 ± 25.26	0.690
HDL, mg/dL, mean ± SD	54.66 ± 20.06	54.22 ± 19.38	57.30 ± 11.73	0.843
Tryglicerides, mg/dL, median (IQR)	109.00 (64.00–173.00)	91.00 (61.50–146.50)	79.00 (59.00–101.00)	0.409
Glucose, mg/dL, mean ± SD	81.88 ± 6.37	86.66 ± 5.93	82.40 ± 13.06	0.551
Ferritin, ng/mL, mean ± SD	118.57 ± 111.74	130.88 ± 71.75	81.60 ± 69.62	0.264
Corrected calcium for total protein, mg/dL, median (IQR)	9.50 (9.20–9.80)	9.20 (9.10–9.40)	9.30 (9.20–9.40)	0.127
Phosphate, mg/dL, median (IQR)	3.10 (2.70–3.15)	2.70 (2.50–3.25)	3.10 (2.70–3.80)	0.312
25OH vit D, ng/mL, median (IQR)	11.33 (9.43–16.60)	15.91 (13.58–18.58)	15.47 (11.60–20.70)	0.399

(Continued)

TABLE 1 Continued

Variable	Controls (*n* = 20)	nr-axSpA (*n* = 10)	r-axSpA (*n* = 29)	*p*-value
PTH, pg/mL, median (IQR)	40.50 (38.35–57.85)	48.20 (37.85–77.40)	38.20 (22.90–64.80)	0.361
Treatments				
NSAIDs (%)	–	8 (80)	24 (82.75)	0.764
Sulfasalazine (%)	–	0 (0)	2 (6.89)	0.394

ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functionality Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMD, bone mineral density; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; HLA, human leukocyte antigen; IQR, interquartile range; LDL, low-density lipoprotein; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; nr-axSpA, non-radiographic axial spondyloarthritis; NSAIDs, non-steroidal anti-inflammatory drugs; PTH, parathyroid hormone; r-axSpA, radiographic axial spondyloarthritis; SD, standard deviation; 25OH vit D, 25-hydroxyvitamin D^3. Differences between groups; [a]p < 0.05 vs. control; [b]p < 0.05 vs. nr-axSpA.

respectively). However, patients with r-axSpA exhibited a slight but significant increase in the plasma levels of TNFRSF9 compared to controls (6.50±0.420 vs 6.21±0.330, $p = 0.014$; Figure 4C). No differences were observed in the circulating concentration of Cystatin-D (CST5, Figure 4D). Respect to other cytokines and proteins related to inflammation evaluated in this study, we observed that plasma levels of Hepatocyte Growth Factor (HGF) showed a tendency to increase in r-axSpA patients as compared to control group ($p = 0.081$, Figure 4E). Sulfotransferase 1A1 (ST1A1) plasma levels were similar among groups (Figure 4F), while the Extracellular Newly identified Receptor for Advanced Glycation End-products binding protein (EN-RAGE) also showed a tendency to increase in r-axSpA patients compared to the control group ($p = 0.060$, Figure 4G). Plasma levels of Transforming growth factor alpha (TGFα) and Vascular Endothelial Growth Factor A (VEGFA) remained similar in all groups (Figures 4H, I respectively). Of note, r-axSpA patients showed a tendency to increase the plasma levels of tumor necrosis factor β (TNFβ) compared to those with nr-axSpA ($p = 0.076$, Figure 4J). The circulating levels of Tumor Necrosis Factor Superfamily Member 14 (TNFSF14) and TNF were similar in all groups (Figures 4K, L respectively).

Analysis of inflammatory-related proteins and the bone turnover markers as potential biomarkers of disease in axSpA

To further investigate the potential utility of the inflammatory-related proteins and the bone turnover markers in terms of differentiating the two pathological conditions in axSpA, the sensitivity (S) and specificity (E) of the most relevant molecules in this study were evaluated using the ROC curve. A positive significant relation was found for IL6 (AUC 0.78, 95% CI 0.66–0.91, $p = 0.001$; S 72%; E 80%), OSM (AUC 0.72, 95% CI 0.57–0.86, $p = 0.011$; S 69%; E 60%), and TNFRSF9 serum levels (AUC 0.73, 95% CI 0.57–0.86, $p = 0.006$; S 76%; E 65%) in r-axSpA patients compared to controls (Figure 5A). In addition, a negative significant relation was observed for P1NP plasma levels (AUC 0.75, 95% CI 0.60–0.90, $p = 0.003$; S 79%; E 65%) in r-axSpA patients compared to controls (Figure 5B). Finally, there was a positive but not significant association in serum IL13 levels in nr-axSpA patients compared to controls (AUC 0.71, 95% CI 0.47–0.95, $p = 0.065$; S 76%; E 65%; Figure 5C) and in nr-axSpA patients compared to r-

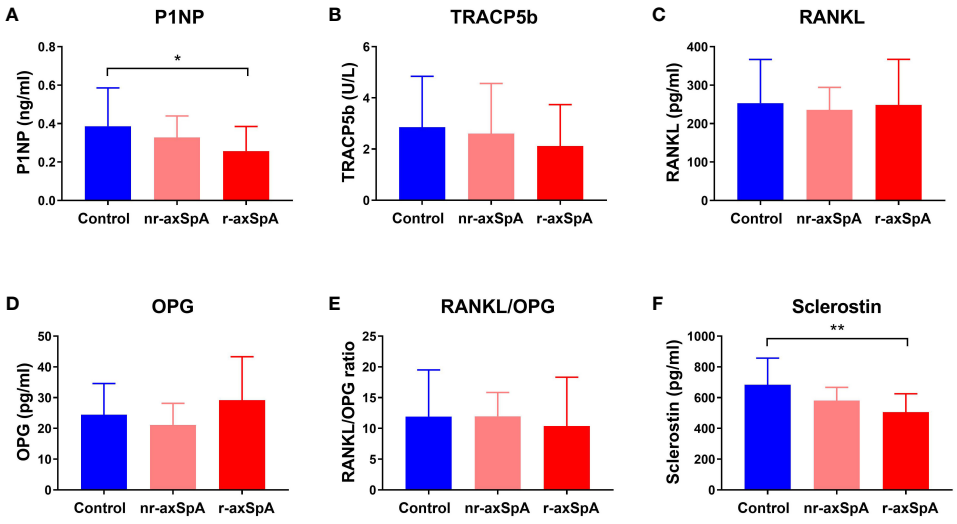

FIGURE 1
Plasma bone markers in radiographic and non-radiographic axial SpA patients, and controls. The circulating levels of **(A)** procollagen type I N-terminal propeptide (P1NP), indicative of osteoblast activity, **(B)** tartrate-resistant acid phosphatase 5b (TRACP5b), and **(C)** receptor activator of nuclear factor kappa-B ligand (RANKL), both markers of osteoclast activity, were measured. Additionally, levels of **(D)** osteoprotegerin (OPG), a RANKL decoy receptor that inhibits osteoclast function, **(E)** RANKL/OPG ratio, and **(F)** Sclerostin, an inhibitor of Wnt signaling that modulates bone formation, were also analyzed. Bars represent mean ± standard deviation. Significant differences: *p < 0.05; **p < 0.01.

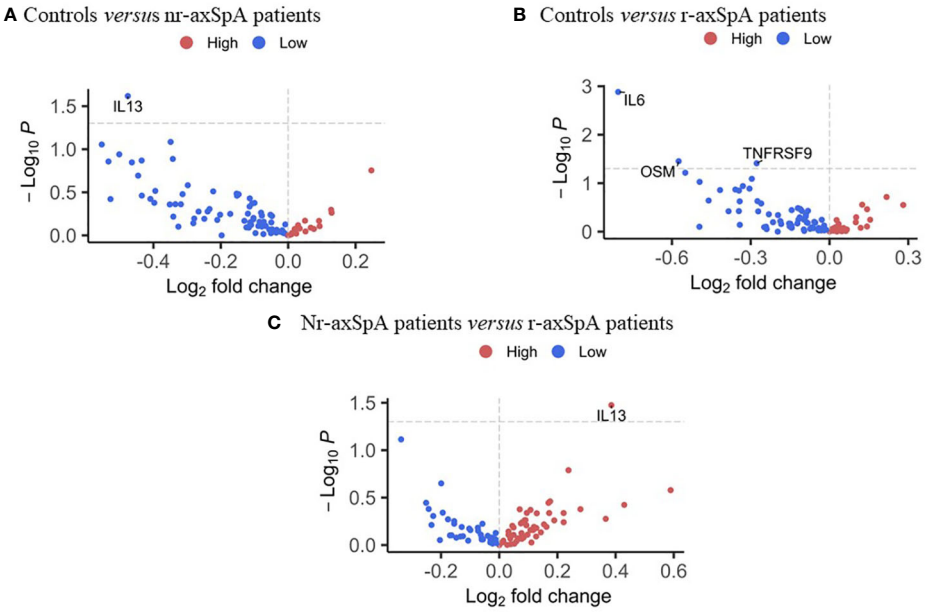

FIGURE 2
Differential protein profile between radiographic and non-radiographic axial SpA patients, and controls. **(A)** Volcano plot of 92 inflammation-related proteins in controls compared to non-radiographic axial SpA patients. **(B)** Volcano plot of 92 inflammation-related proteins in controls compared to radiographic axial SpA patients. **(C)** Volcano plot of 92 inflammation-related proteins in non-radiographic axial SpA patients compared to radiographic axial SpA patients.

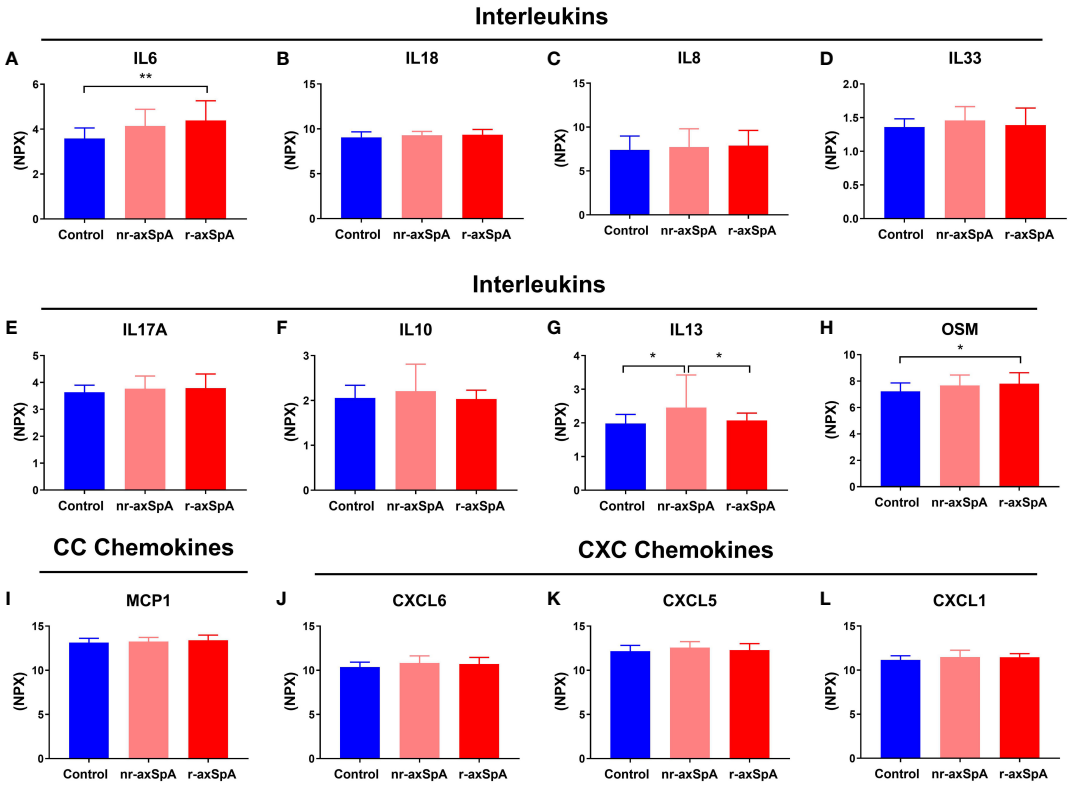

FIGURE 3
Proteins associated with inflammation in patients with radiographic and non-radiographic axial spondyloarthritis (axial SpA) and controls. The proteins were categorized based on their functions into different groups: Interleukins, which includes Interleukin 6 (IL6), IL18, IL8, IL33, IL17A, IL10, IL13 and Oncostatin M (OSM), (**A–H**, respectively); CC chemokines **(I)**, including Monocyte Chemoattractant Protein-1 (MCP1); and CXC chemokines, which includes C-X-C Motif Chemokine Ligand 6 (CXCL6), CXCL5 and CXCL1 (**J–L** respectively). The bars depict the mean ± standard deviation of normalized protein expression units (NPX) values. Significant differences: *$p < 0.05$ and **$p < 0.01$.

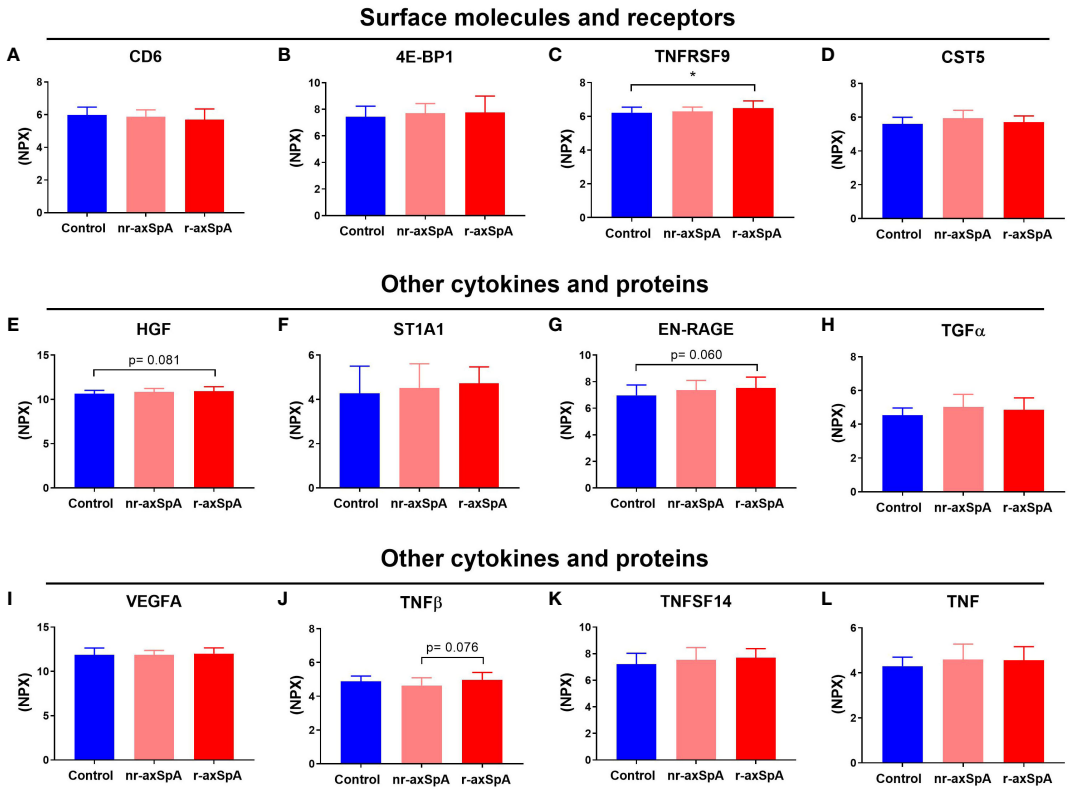

FIGURE 4
Proteins related to inflammation in axial spondyloarthritis (axial SpA) patients, both with and without radiographic changes, and controls. The proteins were categorized into different groups based on their functions: cell surface molecules and receptors, including Cluster of Differentiation 6 (CD6), Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), Tumor Necrosis Factor Receptor Superfamily Member 9 (TNFRSF9), and Cystatin D (CST5), (**A–D** respectively); or other cytokines and proteins, including Hepatocyte growth factor (HGF), Sulfotransferase 1A1 (ST1A1), Extracellular Newly identified Receptor for Advanced Glycation End-products binding protein (EN-RAGE), Transforming Growth Factor Alpha (TGFα), Vascular Endothelial Growth Factor A (VEGFA), Tumor Necrosis Factor β (TNFβ), Tumor Necrosis Factor Superfamily Member 14 (TNFSF14), and Tumor Necrosis Factor TNF, (**E–L**, respectively). The bars indicate the mean ± standard deviation of normalized protein expression units (NPX) values. Significant differences: *$p < 0.05$.

axSpA (AUC 0.63, 95% CI 0.40–0.88, $p = 0.198$; S 70%; E 69%; Figure 5D).

Correlation studies between inflammatory-related proteins, bone turnover markers, and bone mineral density

Results from correlation analysis revealed several significant associations between inflammatory proteins, bone turnover markers, and bone mineral density. Specifically, we found that plasma sclerostin concentration was inversely correlated with interleukins (IL6, IL8, IL7, and OSM), CC chemokines (CCL28 and MCP-2), CXC chemokines (CXCL1, CXCL5, and CXCL6), and other cytokines and proteins (VEGFA, SLAMF1, HGF, TNF, EN-RAGE, and TNFSF14), and a positive correlation with surface proteins and receptors (CD6). On the other hand, P1NP was inversely correlated with interleukins (IL8, OSM, and IL18), CC chemokines (CCL11), and other cytokines and proteins (AXIN1, SIRT2, CASP-8, ST1A1, STAMBP, and TNFSF14) (Figure 6). These findings provide further evidence of the crosstalk between bone metabolism and inflammation in the axSpA pathophysiology. Concerning BMD test, lumbar BMD was inversely associated with interleukins (IL1α, IL2, IL20, IL33, and IL5). Femoral neck BMD was inversely correlated with interleukins (IL12B), CXC chemokines (CXCL9 and CX3CL1), surface molecules and receptors (IL22RA1), and other cytokines and proteins [colony-stimulating factor 1 (CSF1), caspase 8 (CASP8), artemin (ARTN), and fibroblast growth factor 5 (FGF5)]. Finally, total hip BMD was also inversely correlated with surface molecules and receptors (IL10RA, IL15RA, IL10RB, and IL22RA1), CXC chemokines (CX3CL1), and other cytokines and proteins [thymic stromal lymphopoietin (TSLP), stem cell factor (SCF), FGF5, beta nerve growth factor (BETA-NGF), ARTN, FMS-like tyrosine kinase 3 ligand (Flt3l), and CASP8] (Supplementary Table 1).

Discussion

Our data showed differential inflammatory status and bone remodeling in axSpA patients, as reflected by an augmentation of serum pro-inflammatory cytokines and a decrease in bone formation in r-axSpA patients. Consequently, the inflammatory status of r-axSpA patients may result in altered bone turnover,

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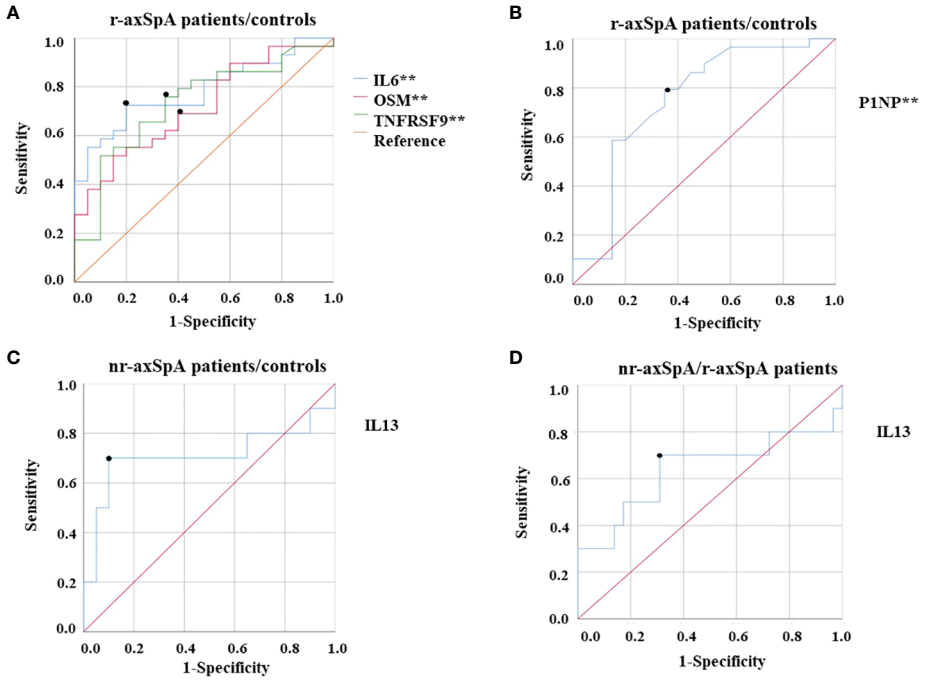

FIGURE 5
ROC curve analyses of circulating levels of inflammatory-related proteins and bone turnover markers as potential biomarkers of disease in axSpA. **(A)** Positive relation of inflammatory-related proteins in r-axSpA patients compared with controls [IL6 (AUC 0.78; p = 0.001), OSM (AUC 0.72; p = 0.011), and TNFRSF9 (AUC 0.73; p = 0.006)]. **(B)** Negative relation in P1NP in r-axSpA patients compared to controls (AUC 0.75; p = 0.003). **(C)** Positive relation in IL13 in nr-axSpA patients compared to controls (AUC 0.71; p = 0.065). **(D)** Positive relation in IL13 in nr-axSpA patients compared to r-axSpA patients (AUC 0.63; p = 0.198). Significant differences $^{**}p < 0.01$.

resulting in decreased bone quality and an increased risk of fractures and deformities (22). These findings found in r-axSpA patients were not observed in nr-axSpA patients, who also did not show any differences in serum bone markers. Of note, nr-axSpA patients had higher levels of serum IL13, which could exert a preventive effect on inflammation (23). These findings may suggest that the differential inflammatory profile could be a primary cause of bone alterations.

We observed that the circulating levels of the P1NP, a bone formation marker (24), were decreased in r-axSpA as compared with the control group, indicating decreased osteoblast activity, whereas nr-axSpA remained similar. These results indicate that, at the systemic level, bone formation is reduced in r-axSpA despite the aberrant new bone formation, which may explain, at least in part, the osteopenia and osteoporosis conditions in these patients (25). No differences among groups were observed in the markers of osteoclast activity, TRACP5b (26) and RANKL (21), as well as in OPG plasma concentration, a decoy receptor for RANKL and inhibiting RANKL-RANK binding through it (27). Consistently, the RANKL/OPG ratio remained similar among the three groups, indicating similar osteoclast activity. As compared with the control group, we observed a significant reduction in plasma sclerostin concentration in r-axSpA patients. It is interesting to note that sclerostin is a well-known inhibitor of osteogenesis, blocking the activation of the canonical Wingless-related integration site (Wnt) pathway, which plays a key role in bone formation (28). Therefore, the lower levels of sclerostin in r-axSpA patients are not in line with

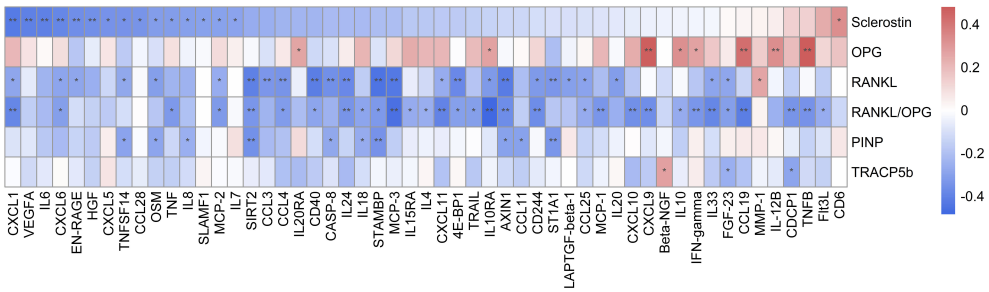

FIGURE 6
Heatmap representing correlations between bone remodeling markers and inflammatory-related proteins. Significant differences: $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$.

the expected decrease in plasma P1NP concentration. However, previous studies have also observed lower levels of sclerostin in patients with r-axSpA, which has been linked to increased structural damage and disease progression in these patients (12, 29). In the same way, Luchetti MM et al. also observed low serum levels of sclerostin in patients with axSpA/IBD. These authors suggested that this reduction in sclerostin levels might contribute to the development of axial joint inflammation (30). Moreover, an association of serum Dickkopf-1(DKK1) levels has been reported, which is also an inhibitor of the Wnt pathway, with BMD values and the prevalence of vertebral fractures, suggesting that DKK1 could also contribute to bone abnormalities in individuals with axSpA (31). Additionally, sclerostin is also associated with other functions such as pro-inflammatory processes (32).

The underlying pathophysiology of axSpA is thought to be driven by inflammation, which results in systemic and local bone loss and local new bone formation (33). Inflammation in this pathology is mediated by pro-inflammatory cytokines, such as IL6, TNF-α, and IL17 (34, 35).

Our data demonstrated an inflammatory profile in r-axSpA, displayed by significantly high concentrations of pro-inflammatory markers in serum such as IL6, OSM, and TNFRSF9 (HGF, EN-RAGE, and TNFβ showed a tendency) as compared to nr-axSpA controls. Some factors could influence the inflammatory profile, such as smoking or exercise, but non-significant differences were observed in our study (36, 37).

Regarding IL6, which is a pleiotropic cytokine produced by various cell types in the context of infection, inflammation, and malignancy, our findings were in line with other works that found elevated IL6 levels in the serum of SpA patients (38, 39). Interestingly, other authors observed that Infliximab treatment decreases IL6 serum levels in patients with ankylosing spondylitis, and this depletion correlated with improvement in disease activity and bone mineral density (40).

In addition, elevated levels of OSM were observed in r-axSpA patients in this study. OSM is a cytokine that belongs to the IL6 family and plays a role in inflammation and bone remodeling. Tsui FWL et al. proposed that both Lipocalin 2 and OSM-associated pathways were involved in axSpA pathogenesis (41). Of note, it has been demonstrated that OSM inhibits sclerostin production in primary murine osteoblasts, also indicating a principal effect of inflammation in the regulation of bone markers (42), as may also occur in r-axSpA patients.

Another inflammatory-related marker increased in r-axSpA patients was TNFRSF9, which is involved in the modulation of inflammation and its dysregulation has been linked to various pathological conditions, including cancer, chronic viral infections, and autoimmune disorders. According to our results, a recent study has observed a high expression of *TNFRSF9* in the Treg cells of synovial fluid of SpA patients (43). Whether TNFRSF9 levels in plasma are related to higher *TNFRSF9* expression in the Treg cells must be clarified.

ROC curve analysis revealed that serum levels of IL6, OSM, and TNFRSF9 could be used as predictive biomarkers for radiological axSpA progression.

In addition, it has been reported that axSpA patients show high levels of IL17, which might increase bone resorption and compromise bone homeostasis (44). In fact, IL17 inhibitors have been suggested as potential treatments to prevent bone loss (45, 46). Nevertheless, other studies have shown that IL17 can promote increased osteoblastic differentiation, which could potentially contribute to excessive osteogenesis and bone formation (47, 48). This phenomenon may be, at least in part, attributed to the activation of the Janus Kinase (JAK) 2/STAT3 pathway (49). In our study, we did not observe any change regarding serum levels of IL17A and plasma levels of osteoclast activity markers such as RANKL or TRACP5b.

On the other hand, our nr-axSpA patients displayed increased levels of IL13 compared to controls and r-axSpA patients. This cytokine has presented contradictory results in rheumatoid arthritis (RA) patients. Some studies have found upregulated levels of IL13 in serum (50) and other studies did not differ in IL13 levels from the control group (51). IL13 is a component of Th-2-mediated immunity, and it has been proposed that IL13 can downregulate IL23 from antigen-presenting cells or IL17 from T cells, thus blocking IL17-driven inflammation (52). This finding could suggest that cytokines such as IL13, with anti-inflammatory properties, may have the potential to prevent bone alterations. However, further in-depth studies are necessary to clarify these findings. For example, fetuin-A, a mineral metabolism protein, has been proposed as a promising marker in axSpA severity. In this respect, Favero et al. (53) have suggested that fetuin-A levels could be a useful biomarker for identifying axSpA patients with a high risk of developing severe disease and early structural damage.

To additionally explore the relationship between inflammatory markers and bone remodeling markers, we next performed correlation studies and observed an inverse correlation between P1NP and sclerostin with pro-inflammatory markers. Specifically, we found that plasma levels of sclerostin were significantly inversely correlated with serum cytokine levels (IL6 and OSM). A study by Wehmeyer et al. (54) observed that the lack of sclerostin or its inhibition with neutralizing antibodies led to increased activation of pathways involved in inflammatory states in a mouse model of RA, indicating that sclerostin could have a protective effect against TNF-dependent inflammation. Conversely, another study reported that RA patients treated with anti-TNFα showed increased levels of sclerostin (55). A wide range of effects on bone turnover is likely to occur as a result of sclerostin modulation in inflammatory and non-inflammatory conditions. As an example, anti-TNFα agents were shown to increase serum levels of bone formation markers, such as P1NP, and suppress bone resorption markers in RA and SpA (56). These findings suggest that changes in bone biomarkers due to anti-inflammatory therapies are associated with improvements in the disease activity of RA and SpA. Therefore, it would be interesting to comprehensively study other therapies that act on different pathways of inflammation such as IL6, and observe if bone remodeling markers undergo changes that translate into an improvement in patients with r-axSpA.

Even though the number of participants collected in each group is one of the limitations of our study, these individuals were well-

phenotyped and many variables have been collected. Additionally, another limitation of our study was the unavailability of trabecular bone score measurements. However, although further studies are required, our results reinforce what has been described in the literature about the relationship between inflammatory markers and bone turnover markers, which could lead to new insights into the pathogenesis of the disease and potential therapeutic targets.

Conclusion

In summary, r-axSpA patients showed decreased bone formation as assessed by lower plasma levels of P1NP. In addition, these patients showed decreased sclerostin levels compared to controls, which were related to the plasma levels of inflammatory cytokines rather than bone formation markers in r-axSpA. Conversely, nr-axSpA patients demonstrated elevated levels of the anti-inflammatory cytokine IL13, suggesting potential protective effects. Further studies are required to elucidate the role of sclerostin in the inflammatory processes in r-axSpA patients and the potential protective actions of IL13 in those lacking radiographic features. While further investigation is required, these findings offer insights that may contribute to identifying novel targets and therapies for patients with axSpA in relation to bone alterations and inflammation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of the Reina Sofía University Hospital, Córdoba, Spain. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

IG-G, MLL-P, JMD-T, and CL-M wrote the first draft of the manuscript. IG-G, MLL-P, CL-M, MCA-A, DR-V, AE-C carried out patient recruitment and data collection. JMD-T and PR-L were major contributors to laboratory determinations and contributors to the interpretation of laboratory data. GP-L, AG-J, and JAGR performed statistical analyses. FJT, IM-I, reviewed and edited. PR-L, EC-E were contributors to the design of the study and interpretation of patient data, and major contributors in writing the manuscript. All authors had final approval of the submitted and published versions. All authors have read and agreed to the published version of the manuscript.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded by Instituto Salud Carlos III (ISCIII; PI19/00701), co-funded by the European Regional Development Fund (ERDF), and Junta de Andalucía (grant number PI0151-2018). PR-L was supported by a "Miguel Servet" postdoctoral contract (CP22/00096) by the ISCIII-Madrid (Spain) and co-funded by the European Union. JMD-T was supported by a "Sara Borrell" postdoctoral contract (CD19/00055) by the ISCIII-Madrid (Spain), co-funded by the ERDF. GP-L was supported by the Institute of Health Carlos III (project IMPaCT-Data, exp. IMP/00019), co-funded by the European Union, European Regional Development Fund (ERDF, "A way to make Europe"). IM-I was supported by the "Miguel Servet Type II" program (CPII21/00013) of the ISCIII-Madrid (Spain) and co-funded by the European Union.

Acknowledgments

We would like to thank all the patients and control participants for their kind participation in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1227196/full#supplementary-material

References

1. Dougados M, Baeten D. Spondyloarthritis. *Lancet* (2011) 377(9783):2127–37. doi: 10.1016/S0140-6736(11)60071-8

2. Michelena X, López-Medina C, Marzo-Ortega H. Non-radiographic versus radiographic axSpA: what's in a name? *Rheumatol (Oxford)* (2020) 59(Suppl4):IV18–24. doi: 10.1093/rheumatology/keaa422

3. Arends S, Spoorenberg A, Efde M, Bos R, Leijsma MK, Bootsma H, et al. Higher bone turnover is related to spinal radiographic damage and low bone mineral density in ankylosing spondylitis patients with active disease: a cross-sectional analysis. *PloS One* (2014) 9(6):e99685. doi: 10.1371/journal.pone.0099685

4. Baraliakos X, Listing J, Rudwaleit M, Haibel H, Brandt J, Sieper J, et al. Progression of radiographic damage in patients with ankylosing spondylitis: defining the central role of syndesmophytes. *Ann Rheum Dis* (2007) 66(7):910–5. doi: 10.1136/ard.2006.066415

5. Davey-Ranasinghe N, Deodhar A. Osteoporosis and vertebral fractures in ankylosing spondylitis. *Curr Opin Rheumatol* (2013) 25(4):509–16. doi: 10.1097/BOR.0b013e3283620777

6. El Maghraoui A. Osteoporosis and ankylosing spondylitis. *Joint Bone Spine.* (2004) 71(4):291–5. doi: 10.1016/j.jbspin.2003.06.002

7. Poddubnyy D, Haibel H, Listing J, Märker-Hermann E, Zeidler H, Braun J, et al. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. *Arthritis Rheumatol* (2012) 64(5):1388–98. doi: 10.1002/art.33465

8. Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *Proc Natl Acad Sci* (1993) 90(24):11924–8. doi: 10.1073/pnas.90.24.11924

9. Roodman GD, Kurihara N, Ohsaki Y, Kukita A, Hosking D, Demulder A, et al. Interleukin 6. A potential autocrine/paracrine factor in Paget's disease of bone. *J Clin Invest.* (1992) 89(1):46–52. doi: 10.1172/JCI115584

10. Gamez-Nava J, de la Cerda-Trujillo L, Vazquez-Villegas M, Cons-Molina F, Alcaraz-Lopez M, Zavaleta-Muñiz S, et al. Association between bone turnover markers, clinical variables, spinal syndesmophytes and bone mineral density in Mexican patients with ankylosing spondylitis. *Scand J Rheumatol* (2016) 45(6):480–90. doi: 10.3109/03009742.2016.1152399

11. Heiland GR, Appel H, Poddubnyy D, Zwerina J, Hueber A, Haibel H, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. *Ann Rheum Dis* (2012) 71(4):572–4. doi: 10.1136/annrheumdis-2011-200216

12. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheumatol* (2009) 60(11):3257–62. doi: 10.1002/art.24888

13. Rudwaleit M, van der Heijde D, Landewé R, Listing J, Akkoc N, Brandt J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* (2009) 68(6):777–83. doi: 10.1136/ard.2009.108233

14. Van Der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheumatol* (1984) 27(4):361–8. doi: 10.1002/art.1780270401

15. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* (1994) 21(12):2286–91.

16. Van Der Heijde D, Lie E, Kvien TK, Sieper J, Van Den Bosch F, Listing J, et al. ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. *Ann Rheum Dis* (2009) 68(12):1811–8. doi: 10.1136/ard.2008.100826

17. Jenkinson TR, Mallorie PA, Whitelock HC, Kennedy LG, Garrett SL, Calin A. Defining spinal mobility in ankylosing spondylitis (AS). *Bath AS Metrology Index J Rheumatol* (1994) 21(9):1694–8.

18. Creemers MCW, Franssen MJAM, Van't Hof MA, Gribnau FWJ, Van De Putte LBA, Van Riel PLCM. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. *Ann Rheum Dis* (2005) 64(1):127–9. doi: 10.1136/ard.2004.020503

19. Ruiz-Limon P, Ladehesa-Pineda ML, Castro-Villegas MDC, Abalos-Aguilera MDC, Lopez-Medina C, Lopez-Pedrera C, et al. Enhanced NETosis generation in radiographic axial spondyloarthritis: Utility as biomarker for disease activity and anti-TNF-α therapy effectiveness. *J BioMed Sci* (2020) 27(1):1–16. doi: 10.1186/s12929-020-00634-1

20. Marini F, Giusti F, Palmini G, Brandi ML. Role of Wnt signaling and sclerostin in bone and as therapeutic targets in skeletal disorders. *Osteoporos Int* (2023) 34(2):213–38. doi: 10.1007/s00198-022-06523-7

21. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* (2008) 473(2):139–46. doi: 10.1016/j.abb.2008.03.018

22. Briot K, Roux C. Inflammation, bone loss and fracture risk in spondyloarthritis. *RMD Open* (2015) 1(1):e000052. doi: 10.1136/rmdopen-2015-000052

23. Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat Rev Rheumatol* (2018) 15(1):9–17. doi: 10.1038/s41584-018-0109-2

24. Lee J, Vasikaran S. Current recommendations for laboratory testing and use of bone turnover markers in management of osteoporosis. *Ann Lab Med* (2012) 32(2):105–12. doi: 10.3343/alm.2012.32.2.105

25. Ramírez J, Nieto-González JC, Curbelo Rodríguez R, Castañeda S, Carmona L. Prevalence and risk factors for osteoporosis and fractures in axial spondyloarthritis: A systematic review and meta-analysis. *Semin Arthritis Rheumatol* (2018) 48(1):44–52. doi: 10.1016/j.semarthrit.2017.12.001

26. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Väänänen HK. Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. *J Bone Mineral Res* (2000) 15(7):1337–45. doi: 10.1359/jbmr.2000.15.7.1337

27. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* (2003) 423(6937):337–42. doi: 10.1038/nature01658

28. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner Res* (2008) 23(6):860–9. doi: 10.1359/jbmr.080216

29. Papagoras C, Tsiami S, Chrysanthopoulou A, Mitroulis I, Baraliakos X. Serum granulocyte-macrophage colony-stimulating factor (GM-CSF) is increased in patients with active radiographic axial spondyloarthritis and persists despite anti-TNF treatment. *Arthritis Res Ther* (2022) 24(1):195. doi: 10.1186/s13075-022-02888-6

30. Luchetti MM, Ciccia F, Avellini C, Benfaremo D, Guggino G, Farinelli A, et al. Sclerostin and antisclerostin antibody serum levels predict the presence of axial spondyloarthritis in patients with inflammatory bowel disease. *J Rheumatol* (2018) 45(5):630–7. doi: 10.3899/jrheum.170833

31. Rossini M, Viapiana O, Idolazzi L, Ghellere F, Fracassi E, Troplini S, et al. Higher level of dickkopf-1 is associated with low bone mineral density and higher prevalence of vertebral fractures in patients with ankylosing spondylitis. *Calcif Tissue Int* (2016) 98(5):438–45. doi: 10.1007/s00223-015-0093-3

32. Liao C, Liang S, Wang Y, Zhong T, Liu X. Sclerostin is a promising therapeutic target for oral inflammation and regenerative dentistry. *J Transl Med* (2022) 20(1):221. doi: 10.1186/s12967-022-03417-4

33. Roux C. Osteoporosis in inflammatory joint diseases. *Osteoporos Int* (2011) 22(2):421–33. doi: 10.1007/s00198-010-1319-x

34. Ruiz-Limon P, Ladehesa-Pineda ML, Lopez-Medina C, Lopez-Pedrera C, Abalos-Aguilera MC, Barbarroja N, et al. Potential role and impact of peripheral blood mononuclear cells in radiographic axial spondyloarthritis-associated endothelial dysfunction. *Diagnostics (Basel)* (2021) 11(6):1037. doi: 10.3390/diagnostics11061037

35. How Shing Koy E, Labauge P, Baillet A, Prati C, Marotte H, Pers YM. Immunomodulation with IL-17 and TNF-α in spondyloarthritis: focus on the eye and the central nervous system. *Ther Adv Musculoskelet Dis* (2021) 13:1759720X211025894. doi: 10.1177/1759720X211025894

36. Villaverde-García V, Cobo-Ibáñez T, Candelas-Rodríguez G, Seoane-Mato D, del Campo-Fontecha PD, Guerra M, et al. The effect of smoking on clinical and structural damage in patients with axial spondyloarthritis: A systematic literature review. *Semin Arthritis Rheumatol* (2017) 46(5):569–83. doi: 10.1016/j.semarthrit.2016.11.004

37. Millner JR, Barron JS, Beinke KM, Butterworth RH, Chasle BE, Dutton LJ, et al. Exercise for ankylosing spondylitis: An evidence-based consensus statement. *Semin Arthritis Rheumatol* (2016) 45(4):411–27. doi: 10.1016/j.semarthrit.2015.08.003

38. Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet* (2013) 45(7):730–8. doi: 10.1038/ng.2667

39. Visvanathan S, Wagner C, Marini JC, Baker D, Gathany T, Han J, et al. Inflammatory biomarkers, disease activity and spinal disease measures in patients with ankylosing spondylitis after treatment with infliximab. *Ann Rheum Dis* (2008) 67(4):511–7. doi: 10.1136/ard.2007.071605

40. Rodriguez VR, Poddubnyy D. Old and new treatment targets in axial spondyloarthritis. *RMD Open* (2015) 1(Suppl 1):e000054. doi: 10.1136/rmdopen-2015-000054

41. Tsui FWL, Lin A, Sari I, Zhang Z, Tsui HW, Inman RD. Serial Lipocalin 2 and Oncostatin M levels reflect inflammation status and treatment response in axial spondyloarthritis. *Arthritis Res Ther* (2021) 23(1):141. doi: 10.1186/s13075-021-02521-y

42. Walker EC, McGregor NE, Poulton IJ, Solano M, Pompolo S, Fernandes TJ, et al. Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. *J Clin Invest* (2010) 120(2):582–92. doi: 10.1172/JCI40568

43. Simone D, Penkava F, Ridley A, Sansom S, Al-Mossawi MH, Bowness P. Single cell analysis of spondyloarthritis regulatory T cells identifies distinct synovial gene expression patterns and clonal fates. *Commun Biol* (2021) 4(1):1395. doi: 10.1038/s42003-021-02931-3

44. Groen SS, Sinkeviciute D, Bay-Jensen AC, Thudium CS, Karsdal MA, Thomsen SF, et al. Exploring IL-17 in spondyloarthritis for development of novel treatments and biomarkers. *Autoimmun Rev* (2021) 20(3):102760. doi: 10.1016/j.autrev.2021.102760

45. Fassio A, Gatti D, Rossini M, Idolazzi L, Giollo A, Adami G, et al. Secukinumab produces a quick increase in WNT signalling antagonists in patients with psoriatic arthritis. *Clin Exp Rheumatol* (2019) 37(1):133–6.

46. van Tok MN, van Duivenvoorde LM, Kramer I, Ingold P, Pfister S, Roth L, et al. Interleukin-17A inhibition diminishes inflammation and new bone formation in experimental spondyloarthritis. *Arthritis Rheumatol* (2019) 71(4):612–25. doi: 10.1002/art.40770

47. Bertoldo E, Adami G, Rossini M, Giollo A, Orsolini G, Viapiana O, et al. The emerging roles of endocrine hormones in different arthritic disorders. *Front Endocrinol (Lausanne)* (2021) 12. doi: 10.3389/fendo.2021.620920

48. Rossini M, Viapiana O, Adami S, Idolazzi L, Fracassi E, Gatti D. Focal bone involvement in inflammatory arthritis: the role of IL17. *Rheumatol Int* (2016) 36 (4):469-82. doi: 10.1007/s00296-015-3387-x

49. Gravallese EM, Schett G. Effects of the IL-23–IL-17 pathway on bone in spondyloarthritis. *Nat Rev Rheumatol* (2018) 14(11):631–40. doi: 10.1038/s41584-018-0091-8

50. Iwaszko M, Biały S, Bogunia-Kubik K. Significance of interleukin (IL)-4 and IL-13 in inflammatory arthritis. *Cells* (2021) 10(11):3000. doi: 10.3390/cells10113000

51. Azizieh FY, Al Jarallah K, Shehab D, Gupta R, Dingle K, Raghupathy R. Patterns of circulatory and peripheral blood mononuclear cytokines in rheumatoid arthritis. *Rheumatol Int* (2017) 37(10):1727–34. doi: 10.1007/s00296-017-3774-6

52. Bridgewood C, Sharif K, Freeston J, Saleem B, Russell T, Watad A, et al. Regulation of entheseal IL-23 expression by IL-4 and IL-13 as an explanation for arthropathy development under dupilumab therapy. *Rheumatol (Oxford).* (2021) 60 (5):2461–6. doi: 10.1093/rheumatology/keaa568

53. Favero M, Ometto F, Belluzzi E, Cozzi G, Scagnellato L, Oliviero F, et al. Fetuin-A: A novel biomarker of bone damage in early axial spondyloarthritis. Results of an interim analysis of the SPACE study. *Int J Mol Sci* (2023) 24(4):3203. doi: 10.3390/ijms24043203

54. Wehmeyer C, Frank S, Beckmann D, Böttcher M, Cromme C, König U, et al. Sclerostin inhibition promotes TNF-dependent inflammatory joint destruction. *Sci Transl Med* (2016) 8(330):330ra35. doi: 10.1126/scitranslmed.aac4351

55. Lim MJ, Kwon SR, Joo K, Son MJ, Park SG, Park W. Early effects of tumor necrosis factor inhibition on bone homeostasis after soluble tumor necrosis factor receptor use. *Korean J Intern Med* (2014) 29(6):807–13. doi: 10.3904/kjim.2014.29.6.807

56. Szentpétery Á, Horváth Á, Gulyás K, Pethö Z, Bhattoa HP, Szántó S, et al. Effects of targeted therapies on the bone in arthritides. *Autoimmun Rev* (2017) 16(3):313–20. doi: 10.1016/j.autrev.2017.01.014

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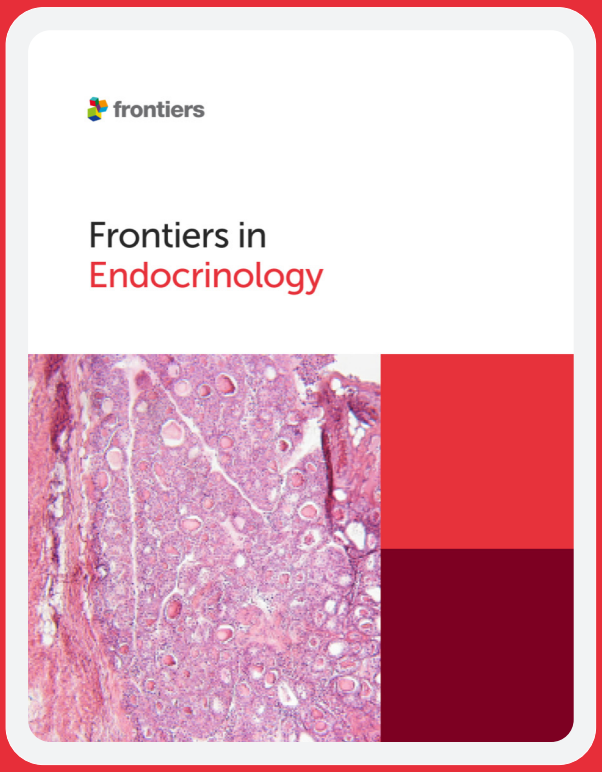

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