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Vitamin D and mineral ion homeostasis: endocrine dysregulation in chronic diseases

Edited by
Mohammed S. Razzaque and Bara Sarraj

Coordinated by
Rahnuma Ahmad

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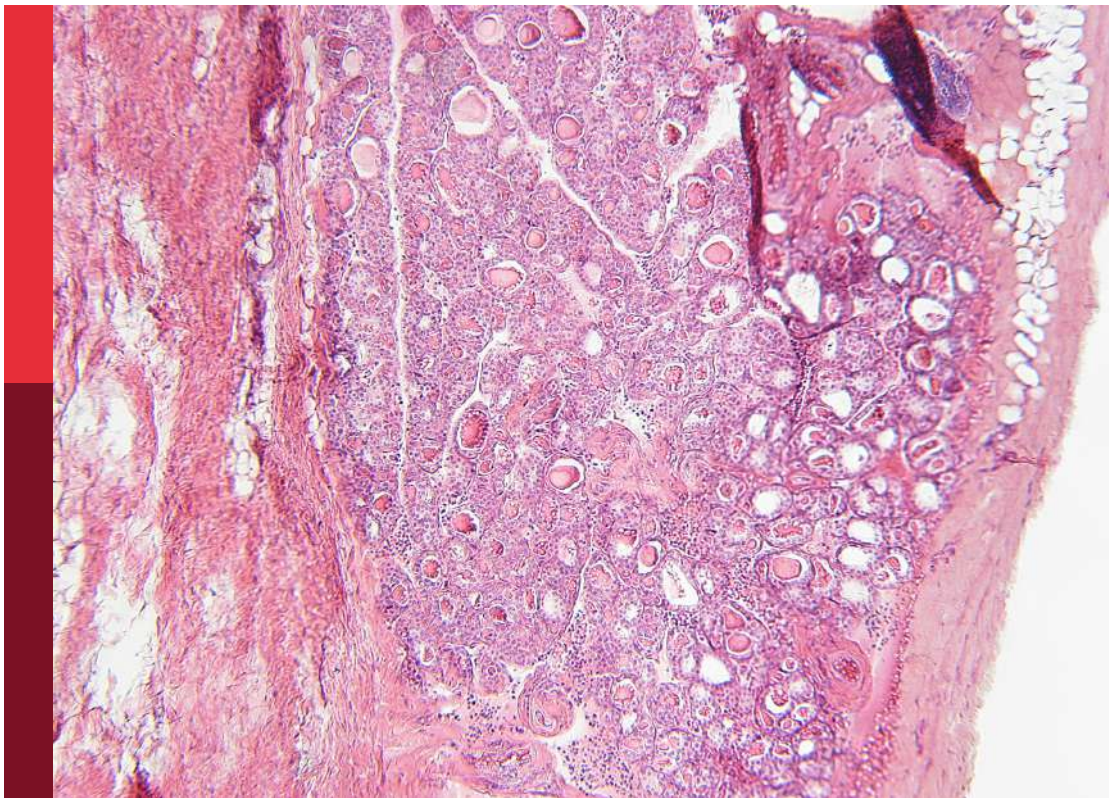

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Vitamin D and mineral ion homeostasis: endocrine dysregulation in chronic diseases

Topic editors

Mohammed S. Razzaque — The University of Texas Rio Grande Valley, United States

Bara Sarraj — Harold Washington College, United States

Topic coordinator

Rahnuma Ahmad — Medical College for Women and Hospital, Bangladesh

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EDITED AND REVIEWED BY
Jeff M. P. Holly,
University of Bristol, United Kingdom

*CORRESPONDENCE
Mohammed S. Razzaque
mohammed.razzaque@utrgv.edu;
msr.nagasaki@gmail.com

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Editorial: Vitamin D and mineral ion homeostasis: endocrine dysregulation in chronic diseases

Rahnuma Ahmad[1], Bara Sarraj[2] and Mohammed S. Razzaque[3]*

[1]Department of Physiology, Medical College for Women and Hospital, Dhaka, Bangladesh, [2]Department of Biological Sciences, Harold Washington College Chicago, Chicago, IL, United States, [3]Department of Medical Education, School of Medicine, University of Texas Rio Grande Valley (UTRGV), Edinburg, TX, United States

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Editorial on the Research Topic

Vitamin D and mineral ion homeostasis: endocrine dysregulation in chronic diseases

This 'Research Topic' is intended to bring together experts to share their experiences in explaining the roles and regulations of vitamin D and mineral ions in various chronic human diseases. A total of 15 articles by 102 authors have been published in this 'Research Topic' to accomplish the intended objectives. Seven of those published articles detail various functional aspects of vitamin D, two articles explain kidney stone-related complications, another two articles discuss parathyroid pathology, and the remaining articles elaborate mineral ion dysregulation in various disease pathologies. Micronutrients, including mineral ions and trace elements, work together to optimize the biological and biochemical functions of the body. Essential components such as calcium, phosphate, zinc, iron, selenium and magnesium, as well as vitamins, play crucial roles in maintaining metabolic balance within the body. Delicate interactions of these nutrients are vital for the physiological functioning of various systems and organs.

Liu et al. studied the relationships of serum 25(OH)D levels with blood pressure and glucose metabolism. They reported that low 25(OH)D levels are correlated with increased diastolic blood pressure, HbA1c, and triglycerides and decreased HDL-C. Vitamin D deficiency may impair glucose tolerance by reducing calcium levels, insulin secretion, and beta-cell function. Vitamin D also suppresses renin synthesis to lower blood pressure. Vitamin D deficiency increases PTH, leading to arterial stiffness and increased lipid factor levels through the expression of receptor for advanced glycation end products (RAGE) and cytokine production (1). Li et al. summarized the vitamin D-diabetes relationship. Studies have shown that serum 25(OH)D levels are negatively correlated with type 2 diabetes risk. Vitamin D supplementation reduces diabetes risk, especially in prediabetic individuals, improves glucose tolerance, and decreases the risk of complications. However, the benefits may be limited to nonobese patients (2). Vitamin D deficiency has been associated with endothelial dysfunction and atherosclerosis. These vascular changes may result in nephropathy and alterations in renal function, particularly in diabetic patients (3).

Hu and Yang studied U.S. vitamin D trends via NHANES data from 2001–2018 and reported that serum 25(OH)D levels increased among adults, with an L-shaped correlation

between vitamin D levels and cardiovascular disease (CVD)/all-cause mortality. Levels below 50 nmol/L were associated with increased CVD and mortality risk. Improved awareness, living standards, dietary inclusion, and supplementation likely contributed to better vitamin D status. The study emphasized the need for continued awareness to prevent vitamin D deficiency. Vitamin D plays an immunomodulatory role, stimulating innate immunity and suppressing acquired immunity. 25(OH)D levels are correlated with autoimmune antibody titers, including those related to thyroid function (4).

Vitamin D has a U-shaped effect on both inflammation and calcium-phosphate metabolism. This was recently demonstrated for 25(OH)D and 1,25$(OH)_2$D in a large cross-sectional study (5). Consequently, it is not surprising that vitamin D supplementation does not benefit everyone. Clear benefits from vitamin D supplementation are observed in patients with vitamin D deficiency (6, 7). However, patients who already have adequate vitamin D levels usually do not benefit from additional supplementation. This highlights the need for the status of vitamin D assessment in chronic kidney disease (CKD) patients to avoid both under- and over-dosing of supplementary vitamin D. The recent study by Li et al. provides useful targets for both 25(OH)D and 1,25$(OH)_2$D levels (5).

Yu et al. investigated the causal link between vitamin D supplementation and autoimmune thyroid disease (AITD) prognosis. They reported that a) higher serum vitamin D levels correlate with reduced AITD risk and that b) vitamin D may inhibit AITD by suppressing T-cell activation, increasing Tregs, inhibiting naïve T-cell differentiation, and reducing HLA II gene expression. While vitamin D is essential for many physiological functions, excess vitamin D can cause severe hypercalcemia, leading to symptoms such as confusion, vomiting, abdominal pain, and polyuria. Although rare, vitamin D toxicity can result from long-term overconsumption, metabolic pathway malfunction, or diseases that cause overproduction of active vitamin D metabolites (8). Xing et al. compared venous and capillary blood collection methods for 25(OH)D detection via a chemiluminescence immunoassay (CLIA): a) venous blood yielded higher 25(OH)D values than capillary blood did, b) capillary blood testing is useful when venous collection is challenging (e.g., obesity, burns, cancer, children), and c) they recommend the use of a truncation value from a linear equation for vitamin D status assessment. Despite global vitamin D deficiency concerns, testing is expensive and not recommended for the general population. The Endocrine Society suggests screening only at-risk individuals. Consequently, a tool for identifying those at risk of vitamin D deficiency is needed to optimize screening efforts. Guo et al. developed a cost-effective tool to predict vitamin D deficiency (<50 nmol/L) via machine learning: a) used easily collectable community data, b) employed the XGBoost method in an online web calculator, and c) allowed clinicians to avoid unnecessary vitamin D testing.

PTH is essential for calcium homeostasis and vitamin D activation. However, thyroidectomy patients are at risk of accidental parathyroid gland removal because of their location behind the thyroid poles (9). Leszczyńska et al. reported a rare case in which a 58-year-old female who underwent parathyroidectomy developed recurrent hypercalcemia 2 years after vitamin D supplementation. Tests revealed suppressed PTH, high serum calcium, and elevated 1,25$(OH)_2$D. A low 24,25(OH)D and high 25(OH)D/24,25(OH)D ratio indicate vitamin D catabolism defects. The patient had a CYP24A1 gene mutation, affecting the 24-hydroxylase enzyme for vitamin D catabolism. Díez et al. compared the development of various comorbidities in patients suffering from long-term hypoparathyroidism resulting from thyroidectomy with comorbidities in subjects without hypoparathyroidism following thyroidectomy. Those authors noted that those with hypoparathyroidism have a greater risk of suffering from chronic kidney disease, cardiovascular disease, and nephrolithiasis. However, these patients have a lower risk of incident fractures. Disease of the kidney may be due to hypercalciuria with the formation of calcium phosphate deposits and their deposition in the renal tubules. Hypocalcemia and PTH deficiency at the vascular and cardiac levels may lead to cardiovascular complications (10). On the other hand, hypercalcemia with hypocalciuria may also result in symptoms such as fatigue, weakness, increased risk of coronary heart disease, chronic kidney disease, chondrocalcinosis, pancreatitis, and femoral fractures (11).

The circadian rhythm affects vitamin D and PTH homeostasis: vitamin D shows diurnal variation, decreases in the morning and plateaus during the day (12). Vitamin D deficiency is linked to inadequate sleep or abnormal light exposure. Night shift workers have lower serum 25(OH)D levels than day workers do because of reduced sunlight exposure (13). He et al. conducted a review in which they highlighted those conditions such as hypertension, metabolic syndrome, microbiome dysbiosis, inflammatory bowel syndrome, vitamin D deficiency, and PTH disorders were related to a disruption in the circadian clock. Each of these diseases also causes the development of kidney stone disease. Oxidative stress, insulin resistance, calcium metabolism disorders, high blood lipid levels, and inflammation may be the underlying pathologies of kidney stone development due to these diseases.

Liu et al. summarized the potential role of magnesium in osteoporosis. Magnesium inadequacy can disrupt the regulation of parathyroid hormone (PTH) and vitamin D, which in turn affects the RANK/RANKL/OPG signaling pathway. This dysregulation results in increased osteoclastic activity, contributing to bone loss and the development of osteoporosis (14). Studies indicate that magnesium supplementation can increase bone density and prevent further bone loss; neuroprotective effects of magnesium in cognitive decline is also reported (15). Therefore, magnesium supplementation represents an easy and cost-effective strategy to delay the progression of osteoporosis, particularly in elderly individuals (16).

Li et al. investigated the potential association between nonalcoholic fatty liver disease (NAFLD) and kidney stone formation. Although they reported no significant link between the two, they proposed that mechanisms such as oxidative stress, insulin resistance, lipotoxicity, and inflammation could contribute to kidney stone formation in individuals with NAFLD. Additionally, elevated blood lipid levels may lead to hyperuricemia. Liu et al. reported that adiposity markers are correlated with hyperuricemia. Lipid parameters strongly predict hyperuricemia, especially in women. Elevated triglycerides and lipid metabolism

disorders may impair renal function, reducing uric acid excretion and causing hyperuricemia. Knowing the levels of serum vitamin D is crucial for individuals with metabolic, cardiovascular, autoimmune, and bone disorders. Both venous and capillary blood can be used for testing (17). Zhang et al. reported a correlation between renal function and vascular damage in the carotid artery among individuals with type 2 diabetes mellitus. Specifically, serum creatinine levels were positively correlated with carotid artery damage, whereas the estimated glomerular filtration rate (eGFR) was negatively correlated with carotid artery atherosclerosis.

Familial hypocalciuric hypercalcemia (FHH) is characterized by increased serum calcium, a normal to high concentration of PTH, and hypocalciuria and is an autosomal disorder. This occurs due to genetic mutation. Lin et al. investigated the genetic cause of FHH. They reported that FHH is caused by a mutation in the CASR gene; the mutation is a *de novo* heterozygous mutation. The specific mutation is c. T1661A,1554 N; this mutation occurs in the cysteine-rich domain of the CASR gene. This finding helps characterize the genetic basis of FHH, providing insight into its development and potential diagnostic markers.

Sun et al. conducted a study examining the relationships among dietary potassium intake, serum potassium levels, and survival in hemodialysis patients, both with and without dietary potassium restrictions. They reported that plant-based foods high in potassium, such as potatoes and melons, also contain significant carbohydrates that can lower plasma potassium levels through insulin release. Animal-based foods are high in potassium but low in carbohydrates, leading to elevated serum potassium levels. In hemodialysis patients, potassium excretion occurs primarily through feces (18). Meat consumption can worsen uremia and cause constipation through the formation of nitride-containing products, whereas a plant-based diet may help reduce uremic toxins through increased fiber intake (19). The authors concluded that there is little to no direct association between dietary potassium and serum potassium levels in these patients. They recommended that dietary considerations should focus not only on potassium content but also on the type of food and its overall nutrient profile.

The articles published in this Research Topic highlighted the significant clinical and biological roles of various minerals and vitamins in maintaining metabolic balance. Minerals and vitamins play crucial roles in maintaining overall health, and proper metabolic balance depends on adequate levels of these nutrients. Additional research into diseases related to mineral ion metabolism is needed to gain a deeper understanding of the conditions associated with vitamin D and mineral ion dysregulation (20–24). Additionally, identifying populations at risk for nutrient deficiencies and encouraging the consumption of diets rich in minerals and vitamins to potentially delay the onset of associated diseases will open new avenues for preventive medicine. Finally, this Research Topic provides valuable insights while also highlighting areas where more research is needed to fully understand the complex relationships between nutrients and health.

Author contributions

RA: Writing – original draft. BS: Writing – review & editing. MR: Conceptualization, Writing – review & editing.

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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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Generative AI statement

The authors declare that Generative AI was used in the creation of this manuscript. OpenAI GPT-3.5 was used to collect information.

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References

1. Wang JM, Ye SD, Li SM, Hu W. Correlations of 25(OH)D level with blood lipid, inflammatory factors and vascular endothelial function in diabetic patients. *Eur Rev Med Pharmacol Sci*. (2018) 22:731–5.

2. Pittas A, Dawson-Hughes B, Staten M. Vitamin D supplementation and prevention of type 2 diabetes. *Reply. N Engl J Med*. (2019) 381:1785–6. doi: 10.1056/NEJMoa1900906

3. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. *Nutrients*. (2020) 12. doi: 10.3390/nu12020575

4. Zhang H, Liang L, Xie Z. Low vitamin D status is associated with increased thyrotropin-Receptor antibody titer in graves disease. *Endocr Pract*. (2015) 21:258–63. doi: 10.4158/EP14191.OR

5. Li X, Liu Y, Chen X, Reichetzeder C, Elitok S, Krämer BK, et al. Target values for 25-hydroxy and 1,25-dihydroxy vitamin D based on their associations with inflammation and calcium-phosphate metabolism. *Nutrients*. (2024) 16. doi: 10.3390/nu16162679

6. Serra MO, de Macedo LR, Silva M, Lautner RQ. Effect of Vitamin D supplementation on blood pressure in hypertensive individuals with hypovitaminosis D: a systematic review and meta-analysis. *J Hypertens*. (2024) 42:594–604. doi: 10.1097/HJH.0000000000003646

7. Arabi SM, Shahraki-Jazinaki M, Chambari M, Bahrami LS, Sabeti S, Gubari MIM, et al. The effect of oral supplementation of Paricalcitol on C-reactive protein levels in chronic kidney disease patients: GRADE-assessed systematic review and dose-response meta-analysis of data from randomized controlled trials. *BMC Pharmacol Toxicol*. (2024) 25:19. doi: 10.1186/s40360-024-00740-y

8. Marcinowska-Suchowierska E, Kupisz-Urbanska M, Lukaszkiewicz J, Pludowski P, Jones G. Vitamin D toxicity-A clinical perspective. *Front Endocrinol (Lausanne)*. (2018) 9:550. doi: 10.3389/fendo.2018.00550

9. Arslan HE, Zeren S, Yildirim AC, Ekici MF, Arik O, Algin MC. Factors affecting the rates of incidental parathyroidectomy during thyroidectomy. *Ann R Coll Surg Engl*. (2024) 106:454–60. doi: 10.1308/rcsann.2024.0019

10. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Cardiovascular and renal complications to postsurgical hypoparathyroidism: a Danish nationwide controlled historic follow-up study. *J Bone Miner Res*. (2013) 28:2277–85. doi: 10.1002/jbmr.1979

11. Josephs TM, Zhang F, Dinh LV, Keller AN, Conigrave AD, Capuano B, et al. Personalised medicines for familial hypercalcemia and hyperparathyroidism. *J Mol Endocrinol*. (2022) 69:243–57. doi: 10.1530/JME-21-0263

12. Masood T, Kushwaha RS, Singh R, Sailwal S, Pandey H, Varma A, et al. Circadian rhythm of serum 25 (OH) vitamin D, calcium and phosphorus levels in the treatment and management of type-2 diabetic patients. *Drug Discovery Ther*. (2015) 9:70–4. doi: 10.5582/ddt.2015.01002

13. Lehnert M, Beine A, Burek K, Putzke S, Schlosser S, Pallapies D, et al. Vitamin D supply in shift working nurses. *Chronobiol Int*. (2018) 35:724–9. doi: 10.1080/07420528.2018.1424719

14. Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino AC, et al. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D3. *Bone*. (2007) 40:1517–28. doi: 10.1016/j.bone.2007.02.024

15. Patel V, Akimbekov NS, Grant WB, Dean C, Fang X, Razzaque MS. Neuroprotective effects of magnesium: Implications for neuroinflammation and cognitive decline. *Front Nutr (Lausanne)*. (2024) 15:1406455. doi: 10.3389/fendo.2024.1406455

16. Fouhy LE, Mangano KM, Zhang X, Hughes BD, Tucker KL, Noel SE. Association between a calcium-to-Magnesium ratio and osteoporosis among puerto rican adults. *J Nutr*. (2023) 153:2642–50. doi: 10.1016/j.tjnut.2023.05.009

17. Li LL, Li XN, Jia FY, Chi MZ, Wen ZH, Yang F, et al. Standardization of clinical application of mass spectrometry method for measurement of vitamin D in capillary blood of children: a multicenter study. *Zhonghua Er Ke Za Zhi*. (2022) 60:1282–7. doi: 10.3760/cma.j.cn112140-20220731-00689

18. St-Jules DE, Goldfarb DS, Sevick MA. Nutrient non-equivalence: does restricting high-Potassium plant foods help to prevent hyperkalemia in hemodialysis patients. *J Ren Nutr*. (2016) 26:282–7. doi: 10.1053/j.jrn.2016.02.005

19. Sakaguchi Y, Kaimori JY, Isaka Y. Plant-dominant low protein diet: A potential alternative dietary practice for patients with chronic kidney disease. *Nutrients*. (2023) 15. doi: 10.3390/nu15041002

20. Chittilla M, Uzoma C, Brewer D, Razzaque MS. Potential association between arsenic and vitamin D. *Front Endocrinol (Lausanne)*. (2024) 15:1430980. doi: 10.3389/fendo.2024.1430980

21. Akimbekov NS, Coban SO, Atfi A, Razzaque MS. The role of magnesium in pancreatic beta-cell function and homeostasis. *Front Nutr*. (2024) 11:1458700. doi: 10.3389/fnut.2024.1458700

22. Ahmad R, Shaju R, Atfi A, Razzaque MS. Zinc and diabetes: A connection between micronutrient and metabolism. *Cells*. (2024) 13(16):1359. doi: 10.3390/cells131613592024;13(16):1359.

23. Razzaque MS. Magnesium: are we consuming enough. *Nutrients*. (2018) 10. doi: 10.3390/nu10121863

24. Erem S, Razzaque MS. Dietary phosphate toxicity: an emerging global health concern. *Histochem Cell Biol*. (2018) 150:711–9. doi: 10.1007/s00418-018-1711-8

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Yu Pan,
Shanghai Jiao Tong University, China
Ricardo Adrian Nugraha,
Airlangga University, Indonesia
Jens Djurhuus,
Aarhus University, Denmark

*CORRESPONDENCE
Yun-Jin Bai
baiyunjin@wchscu.cn

†These authors have contributed equally to this work

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Sleep and circadian rhythm disturbance in kidney stone disease: a narrative review

Si-Ke He[1†], Jia-Hao Wang[1†], Tao Li[2†], Shan Yin[3], Jian-Wei Cui[1], Yun-Fei Xiao[1], Yin Tang[1], Jia Wang[1] and Yun-Jin Bai[1*]

[1]Department of Urology, Institute of Urology, West China Hospital, Sichuan University, Chengdu, China, [2]Department of Urology, Affiliated Hospital of Guizhou Medical University, Guiyang, China, [3]Department of Urology, Affiliated Hospital of North Sichuan Medical College, Nanchong, China

The circadian rhythm generated by circadian clock genes functions as an internal timing system. Since the circadian rhythm controls abundant physiological processes, the circadian rhythm evolved in organisms is salient for adaptation to environmental change. A disturbed circadian rhythm is a trigger for numerous pathological events. Recently, accumulated data have indicated that kidney stone disease (KSD) is related to circadian rhythm disturbance. However, the mechanism between them has not been fully elucidated. In this narrative review, we summarized existing evidence to illustrate the possible association between circadian rhythm disturbance and KSD based on the epidemiological studies and risk factors that are linked to circadian rhythm disturbance and discuss some chronotherapies for KSD. In summary, KSD is associated with systemic disorders. Metabolic syndrome, inflammatory bowel disease, and microbiome dysbiosis are the major risk factors supported by sufficient data to cause KSD in patients with circadian rhythm disturbance, while others including hypertension, vitamin D deficiency, parathyroid gland dysfunction, and renal tubular damage/dysfunction need further investigation. Then, some chronotherapies for KSD were confirmed to be effective, but the molecular mechanism is still unclear.

KEYWORDS

circadian rhythm disturbance, circadian clock, sleep disorder, circadian clock gene, kidney stone disease

1 Introduction

With the Earth's planetary rotation, there is a 24-h oscillating light-dark cycle (1). To adapt to this environmental cycle, all animals and plants have evolved universal internal circadian rhythms (2). Such rhythms are observed in cellular, physiological, and biological behavioral processes within a 24-hour cycle. For instance, heart rate and body temperature increase in the morning and decrease in the evening (1, 3). It is also found in the diet, sleep-wake cycle, endocrine, absorption, and reproduction (4–7). Hence, circadian rhythm is of great significance for maintaining homeostasis and normal physiological activities.

Before artificial light was created, humans adjusted their lives to a natural day-night alteration cycle (8). With the great development of technology and society, life patterns have changed greatly, and the phenomenon of circadian rhythm disorder is now common due to social jet lag (SJL), shift work, and sleep disruption, which all contribute to abnormal daily rest/wake cycles and chronically disrupt endogenous circadian rhythms. The asynchrony between endogenous circadian rhythm and the sleep-wake cycle is defined as circadian rhythm disruption (9, 10). Growing epidemiological and genetic evidence shows that circadian disruption leads to various diseases, such as insomnia, hypertension, type 2 diabetes (T2D), chronic kidney disease (CKD), and even cancer (10–12). All of these factors finally cause immense loss of public health.

Kidney stone disease (KSD) is a common health concern with increasing incidence during the past few decades and occurs in a wide range of ages, including children, adolescents, and adults (13). The prevalence is approximately 10% globally, with a high recurrence rate of 50% within 5-10 years and 75% within 20 years (14–16). It causes such a large burden on public health since it is not only a transient acute symptom but is also linked to cardiovascular disease (CVD), CKD, end-stage renal disease (ESRD), and renal cancer (17, 18). An updated meta-analysis signifies that KSD is associated with an approximately 20%-40% higher risk for coronary artery disease, transient ischemia/stroke, and arterial disease (19). Then, the risks of ESRD, renal cell carcinoma, and transient cell carcinoma are all increased in patients with prior KSD history (18, 20). Although great progress has been achieved in traditional surgical management to provide better prognosis, the incidence and recurrence rates are still very high with little breakthrough in prevention methods (especially drug interventions) (21). Therefore, it is pivotal to explore the potential pathophysiological mechanisms of KSD to provide insights for prevention and therapy.

There is plenty of evidence to suggest that a circadian rhythm disturbance can promote KSD (22, 23). Herein, this review summarizes the relationship between circadian rhythm disturbance and KSD and discusses the potential mechanisms by which circadian rhythm affects KSD.

2 Biological characteristics of the circadian clock

The central circadian rhythm clock in the suprachiasmatic nucleus (SCN) is in the anterior part of the hypothalamus. It synchronizes with Earth's time and feeds back to the downstream brain and peripheral regions by sympathetic nervous system transduction and hormone secretion after light signals from the light-dark cycle are received by the retina and transmitted to the SCN as electrical signals (10, 24). Some synchronization factors also called "time givers" or *zeitgebers* vary with temperature, diet, pharmacological manipulation, and social interactions (25). Additionally, peripheral organs, including the heart, liver, and kidney, participate in the "peripheral clock" and regulate cyclic physiological functions by manipulating the transcription of circadian genes, protein synthesis, energy metabolism, and so on. Both the central and peripheral clocks essentially share the same molecular structure, but the relationship between them remains unclear (10, 26).

At the molecular level, approximately 10% of genes are clock-controlled genes (CCGs) with circadian oscillations, also called circadian clock genes. The maintenance of circadian rhythm depends on a transcription-translation negative feedback loop formed by a series of interacting clock genes (25, 27, 28) (see Figure 1). The cycle of potential molecular mechanisms generating circadian rhythm is approximately 24 hours without synchronizing input; the central-peripheral network can adapt to a limited range of day lengths (29, 30). Some genes and regulators that are vital for initiating and maintaining circadian rhythm have been well investigated, such as the circadian locomotor output cycles kaput (*CLOCK*), a gene encoding the protein related to the length and persistence of a circadian circle (2); brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 coding gene (*BMAL1*), encoding the basic-helix–loop-helix transcription factor BMAL1 protein (2); the period family (*PER1/2/3*), key regulators in the cell cycle (31); cryptochrome 1&2 (*CRY1/2*), the main part of the negative feedback loop of the circadian clock and *REV-ERBα/β*, members of the orphan nuclear receptor family, which play a key role in regulating the expression of *CLOCK* and *BMAL1* (32, 33). These circadian clock genes regulate the day-night cycles by both positive and negative feedback cycles in the SCN and peripheral tissues and organs (10).

3 Mechanism of KSD

KSD is a common urological disease with a complicated pathological process. The major types include calcium stones (calcium oxalate [CaOx] & calcium phosphate [CaP]), uric acid (UA) stones (UAS), struvite stones (infection stones), and cystine stones. The biochemical process of stone formation is successive and complex and involves various physicochemical changes. Generally, four steps participate in pathogenesis: urinary supersaturation, crystal nucleation, growth, and aggregation. Crystal formation from supersaturated urine retained in the kidney is the driving force (16, 34). Then, the crystals gather together to grow to a size as further aggregation, which can interact with intrarenal structures (also called crystal-cell interaction) to cause renal tubular epithelial cell (RTEC) injury (34, 35). Crystal-cell interaction leads to the movement of crystals from the basolateral side of cells to the basement membrane and results in the retention of crystals in the kidney or collecting duct to eventually form the clinical stone (34, 36). Furthermore, a plaque of calcium deposited in the interstitial tissue of the renal papilla observed by electron microscopy, called Randall's plaque (RP), appears to be the origin of urinary stones since it contributes greatly to crystal retention (37, 38). It should be noted that some factors are reported to be critical modulators for stone formation, including promoters and inhibitors. Promoters are receptors or receptor-like features that play vital roles in crystal–cell interactions for crystal retention in the kidney, while inhibitors can decrease crystallization and inhibit crystal aggregation and/or adhesion to RTECs. For example, serum calcium and vitamin D (vit D) act as promoters in KSD, while some metallic cations, such as magnesium,

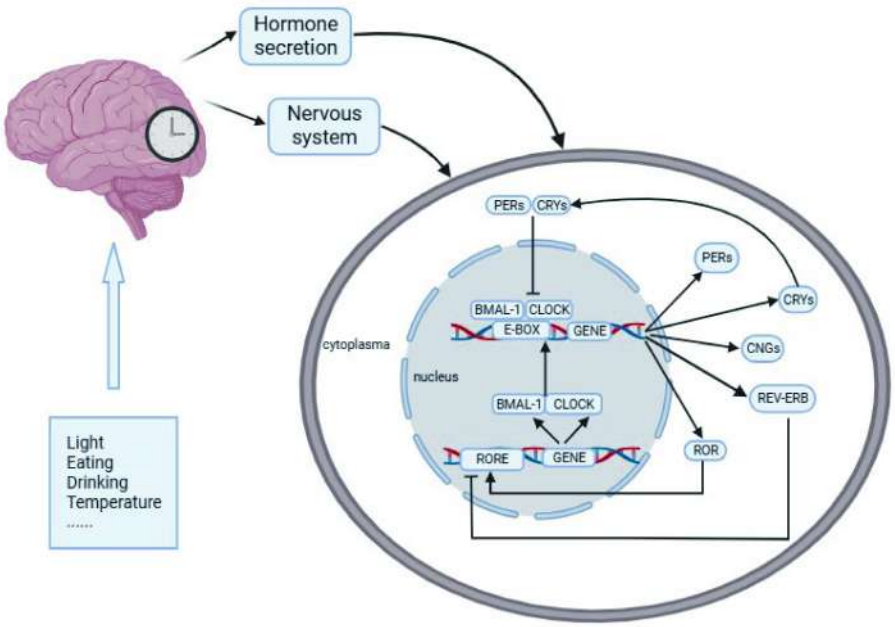

FIGURE 1
Molecular mechanism of the circadian rhythm. After cues from *Zeitgebers* of light, temperature, and feeding are perceived and transmitted to the SCN as electrical signals, the central circadian clock system will synchronize with geophysical time and feedback to the downstream brain regions and peripheral organs through the nervous system and hormone release. CLOCK and BMAL1 act as the center transcription factors of a heterodimer complex and activate the transcription of PER, CRY, REV-ERβ and RORα by cis-acting E-box and ROR elements. A multimeric complex formed by the PER/CRY proteins subsequently enters the nucleus to inhibit CLOCK/BMAL1 activity. Then, REV-ERBα and ROR proteins compete for response RORα/CLOCK/BMAL1 transcription. REV-ERBα reduces CLOCK/BMAL1 transcription, while RORα induces it. The main circadian genes are reactivated by the last with a new cycle beginning, and this feedback loop occurs at approximately 24 h.

can inhibit crystal growth and aggregation (16, 39, 40). It is now widely accepted that stone formation usually depends on the imbalance between promoters and inhibitors.

4 Epidemiological evidence of circadian rhythm disruption in KSD

Light is the main *zeitgeber* that regulates the 24-h circadian cycle. Sleep and wakefulness can coregulate the circadian rhythm and maintain sleep homeostasis with light exposure. However, the day-night circadian rhythm has been altered greatly with the invention of incandescent lighting (2, 41). Circadian sleep disorder is defined medically as an inability to sleep at the desired time rather than an inability to sleep, for instance, staying up late working (night shift work) and rapidly travelling to new time zones (social jet lag [SJL]). Both shift work and SJL are popular and involve a large population and diverse professions (42, 43). Altered sleep-wake cycles disturb the internal circadian clock. Growing evidence shows that disordered circadian sleep and sleep insufficiency are closely related to multiple disorders, including KSD. Yin et al. (44) investigated the relationship between sleep status and KSD risk in a cross-sectional study and found that short sleep duration (< 7 h) was associated with a higher KSD prevalence than normal sleep duration (7-9 h) (odds ratio [OR] = 1.21, 95% confidence interval [CI]: 1.08 to 1.35). Another large population-based study obtained similar results, and it estimated the sleep score to assess sleep quality according to a previous study and indicated that a reduced sleep score led to increased KSD risk (hazard ratio [HR] = 1.07, 95% CI: 1.05 to 1.10) (45, 46). A prospective study in China measured sleep quality using the Pittsburgh Sleep Quality Index (PSQI) and KSD prevalence, and the PSQI was positively correlated with KSD prevalence (OR=1.18, 95% CI: 1.08 to 1.28) (47). Another sleep disturbance, obstructive sleep apnea (OSA), is common, with an incidence of 4.0% to 32.8% for middle age and 22.4% for older than 60 years (42), and has been confirmed to be a risk factor for KSD (HR=1.35, 95% CI: 1.23 to 1.48) (48). A retrospective study including 127 patients performed a univariable comparison of 24-h urine components and found that OSA is related to changes in urinary analytes that promote KSD (49).

Although several studies have elucidated that disturbed circadian rhythm can induce KSD, the endogenous mechanism is poorly understood. KSD is a multifactorial systemic disease with diverse triggers. Based on epidemiological studies, metabolic syndrome, hypertension, inflammatory bowel disease, microbiome dysbiosis, parathyroid hormone disorder, and vitamin D deficiency can induce KSD, and they are related to a disrupted circadian clock (50–52) (Figure 2).

5 Association between circadian rhythm disturbance and KSD

Sleep and circadian rhythm disturbance is associated with alterations in circadian gene expression, and mammals with

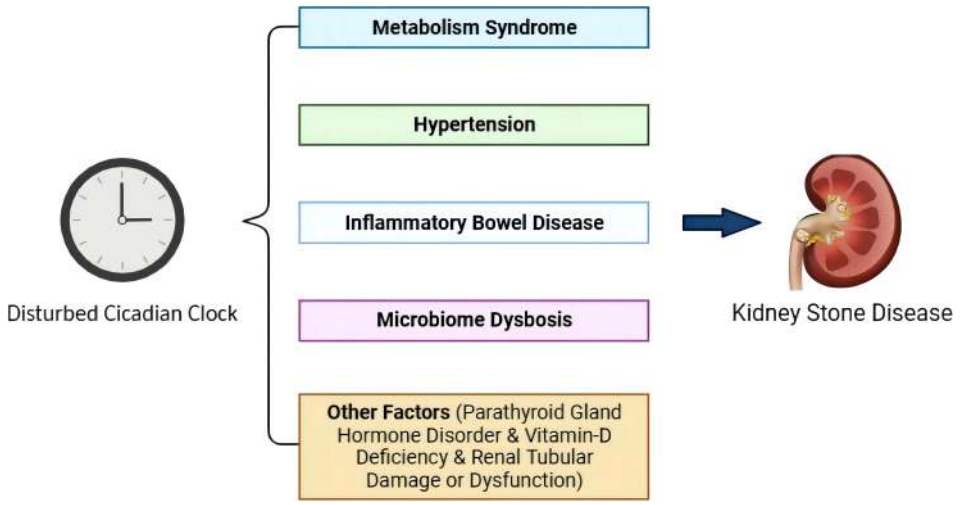

FIGURE 2
Involvement of circadian rhythm disturbance in KSD.

specific circadian gene deficiencies or mutations exhibit abnormal sleep/awake rhythms (1, 53–55). Here, we comprehensively summarize some potential links between circadian rhythm disturbance and KSD. The factors related to KSD that are affected by sleep and circadian rhythm disturbance and the mechanisms are described as follows.

5.1 Glycolipid metabolism disorder (metabolic syndrome)

Metabolic syndrome (MetS) is a cluster of conditions that usually occur together to increase the risk of T2D and CVD, including hypertension, chronic hyperglycemia (T2D and impaired glucose tolerance [IGT]), and dyslipidemia (obesity, decreased high-density lipoprotein [HDL] level, increased low-density lipoprotein [LDL] or total cholesterol [TC] level), and its prevalence is approximately 25% of adults with an increasing trend at advanced ages (56, 57). MetS is confirmed to be an independent risk factor for KSD (OR=1.49, 95% CI: 1.26 to 1.76) (58, 59). The pathological mechanism of MetS in KSD mainly consists of insulin resistance (IR), hyperglycemia, and vascular dysfunction (59, 60).

MetS is significantly associated with circadian rhythm disturbance. A new concept, circadian syndrome (CirS), was suggested to indicate MetS related to disrupted circadian rhythm (61, 62). Xiao et al. first reported the relationship between CirS and KSD prevalence in a cross-sectional study and obtained similar results (OR=1.42, 95% CI: 1.06 to 1.91) (23). A longitudinal analysis from Canada investigated 393 participants for a mean 6-year follow-up and found that short sleepers were at significantly higher risk of MetS (relative risk [RR]=1.74; 95% CI: 1.05-2.72) (63). The large population-based study by Bayon et al. revealed that shift work was correlated with higher MetS risk (OR=4.45, 95% CI: 1.36 to 14.56) (64). The cross-sectional data from the New Hoorn Study cohort indicated that SJL was correlated with MetS with a prevalence ratio of 1.62 (65). Additionally, OSA can cause MetS and increased waist circumference (WC) and triglyceride (TG) levels in MetS can induce OSA (66, 67). Here, we reviewed the effects of MetS on KSD from two aspects.

5.1.1 Type 2 Diabetes (T2D)

The KSD prevalence was higher in T2D patients than in healthy controls (21% *vs.* 8%, $p < 0.05$), and the recurrence rate was twice as high (2.1 *vs.* 1.3, $p < 0.05$) (68, 69). A cohort study showed that the population with T2D had a higher KSD risk (OR=2.44, 95% CI: 1.84 to 3.25) (70). IR can hamper the ability of ammonia genesis to respond to acid load in the kidney, which results in hyperacid urine and elevated urinary calcium excretion by compensatory hyperinsulinemia (60, 68). Oxidative stress (OS) is another link between T2D and KSD; increased reactive oxygen species (ROS) generated by disordered glycometabolism, oxidative damage of β-cells in the pancreas, and endothelial dysfunction cause OS and inflammation in RTECs, resulting in increased levels of superoxide dismutase and malondialdehyde and decreased levels of antioxidants (71). These factors eventually lead to KSD.

Glycometabolism is a complicated physiological process and presents a significant diurnal oscillation. Variations in both the secretion and sensitivity of insulin display obvious circadian rhythms, and glucose tolerance (GT) is higher in the morning than in the evening in healthy people, but this oscillation disappears in T2D patients and IGT appears when the circadian oscillation is damaged (72–74). Many studies have revealed that the risk of IR and T2D is higher in populations with sleep disorders, SJL, and shift work, and it may be reduced by extended sleep duration and circadian readjustment strategies (11, 75). Cui et al. investigated the association between sleep duration and the risk of T2D in a case-control study and found a U-shaped association (≤ 6 h, OR = 1.74, 95% CI: 1.01 to 3.01; 8-9 h, OR = 1.46, 95% CI: 1.04 to 2.06) compared to sleep duration of 6-8 h, in which abnormal sleep duration increased the T2D risk (76). A prospective study including night workers and day workers with 4 years of follow-up showed that the risk of obesity and T2D was 5 times higher in night shift

workers than in daytime workers (77). Then, a meta-analysis assessed the association between shift work and T2D and revealed that shift work increased the risk of T2D (RR=1.10, 95% CI: 1.05 to 1.14), and the trend was similar in the population with SJL (78, 79).

The possible relationship between disrupted circadian rhythm and T2D has been clarified by numerous studies. Rodents with SCN dysfunction display global or partial circadian gene disruption and easily developed IGT, hyperglycemia, hyperinsulinemia, decreased insulin secretion and sensitivity, and β-cell defects in the pancreas (80–82). *Clock* mutant mice show hyperglycemia, decreased levels of expression, and phase shifts of RNA oscillation of genes that participated in glucose sensing, insulin signaling, islet growth, and development (83, 84). It also disrupts hepatic glycogen oscillation and changes the circadian mRNA and protein expression of glycogen synthase 2 (limiting enzyme in glycogenesis) in mice (85). Whole-body *Bmal1* knockout (KO) obliterates the systemic insulin sensitivity rhythm to develop increased fasting blood glucose, glucose intolerance, and hypoinsulinemia (83, 86). Severe IGT, increased glycemia levels, and decreased insulin secretion are found in pancreas- or β-cell-specific *Bmal1* KO mice (87). The whole-body *Cry1/2* KO severely damages glucose homeostasis with IGT (88). Another study by Zhang et al. demonstrated that the hepatic overexpression of *Cry1* results in decreased gluconeogenesis and lower glycemia in diabetic mice (89). Qian et al. investigated *Per1*:LUC transgenic rats exposed to light during the night for 10 weeks and showed disruption of islet circadian clock through impairment in the amplitude, phase, islet synchrony of clock transcriptional oscillations, and diminished glucose-stimulated insulin secretion (90). Furthermore, *Per2* inhibition decreases glycemia levels and gluconeogenesis and stimulates insulin secretion (91). Then, global *Rer-erbα/β* dual KO mice exhibit hyperglycemia by disturbing the insulin signaling pathway (92). Meanwhile, *Rev-erbα* mutant mice fed a chow diet have slight hyperglycemia without IR, which can be explained by *REV-ERBα* affecting glycemia by regulating glucose-6-phosphatase and phosphoenolpyruvate carboxylase (93).

In summary, primary findings indicated that a disturbed circadian rhythm leads to T2D mainly by regulating enzymes and insulin in glycometabolism. However, direct evidence about how circadian disorders affect T2D and KSD remains unclear.

5.2 Lipid abnormalities and obesity

Lipid metabolism disorders are common in KSD patients. A retrospective study enrolled 2242 patients with KSD and found that high TC levels were significantly associated with higher UAS risk (p=0.006), and the 24-h urine analysis presented a significant positive correlation between low HDL levels and lower urine pH and higher urinary oxalate and uric acid levels (94, 95). A demonstrably increased KSD risk in the obese population was observed in a cohort study, and in particular, an increased level of visceral adiposity was a risk factor for hypercalciuria and UAS (OR=3.64, 95% CI: 1.22 to 10.85) (96, 97). Obesity promotes systemic inflammation and OS, leading to tissue immune cell infiltration and contributing to stone formation. It facilitates the expression of adipokines and some inflammatory molecules, such as tumor necrosis factor-α and interleukin-6, which were detected in the renal tissue and urine of KSD patients (98). Furthermore, calcifications by lipid deposition within the hyperosmotic turbulent vasa recta erode into the nearby collecting system and interstitium to promote RP formation, which is further confirmed by the presence of cholesterol identified in stones and renal papillary (95).

Lipid metabolism is precisely regulated by the circadian clock. Plasma lipids present a day-night variation within a narrow range independent of food intake, with the peak level of HDL in the early rest phase and a decrease in the active phase (99, 100). Circadian rhythm disturbance causes lipid abnormalities and obesity. A study investigated the association between sleep duration and obesity in children and adolescents and revealed that short sleep duration increases the risk of obesity (OR=1.69, 95% CI: 1.25 to 2.29) and elevated WC (OR=1.49, 95% CI: 1.13 to 1.97), which is similar to the study by Brocato et al. (101, 102). Another longitudinal investigation including 815 workers showed that workers with greater SJL are more likely to be obese (OR=1.20, 95% CI: 1.00 to 1.50) (103). In addition, circadian rhythm disturbance alters the plasma lipid profile by increasing the levels of cholesterol, TGs, and LDL and decreasing the level of HDL (104, 105).

Clock gene expression alteration has been comprehensively verified in both human and animal models. Vieira et al. analyzed the 24-h pattern of clock gene expression in an obese population and showed that the expression of *CRY2* and *REV-ERBα* was upregulated in obese participants. A positive correlation was observed between *REV-ERBα* expression and body mass index and WC in the obese population. The expression of *CLOCK* was positively correlated with LDL and *RORα* with HDL levels. Obese people with MetS presented a positive correlation between *PER2* expression and LDL, while *REV-ERBα* was correlated with WC. *CRY2* and *REV-ERBα* are considered clock genes upregulated in obesity (106). Hepatic *PER2*, *PER3*, and *CRY2* showed lower expression in the obese groups than in the normal control group (107). In animal experiments, the diurnal feeding rhythm is significantly impaired in homozygous *Clock* mutant mice, and they are more likely to develop hyperphagia, obesity, hyperleptinemia, and hepatic steatosis (80, 83). Microsomal TG transfer protein levels in the enterocytes of $Clock^{\Delta19/\Delta19}Apoe^{-/-}$ mice are higher, and enterocytes secret more chylomicrons. $Clock^{\Delta19/\Delta19}$ protein enhances intestinal cholesterol absorption, as well as the secretion of chylomicrons and cholesterol (108). The *Bmal1* KO mice display ectopic body and liver fat formation, hyperlipidemia, increased circulating leptin levels, and the absence of glucose fluctuation, also presenting earlier signs of obesity under a high-fat diet (HFD) (86, 109). In liver-specific and global $Bmal1^{-/-}$ mice, an elevation of circulating free fatty acids and higher TG formation is detected and can be reversed by *Bmal1* overexpression (110). Adipose-specific $Bmal1^{-/-}$ mice show increased weight gain and fat formation with increased calorie intake during the daytime (84). *Per1/2/3* triple-deficient mice are more likely to be obese, suggesting a potential role in body weight regulation (111). Moreover, *PER1* is identified to bind with major hepatic enzymes in bile acid synthesis, and *Per1* expression can be

enhanced to increase fat absorption and accumulation in mice (112). *Per2* KO mice gain an altered lipid profile and downregulated triacylglycerol levels, while *Per2* deficiency in fibroblasts can promote adipocyte differentiation via the direct interaction with PPARα/γ and their target genes (113, 114). *Per3* KO promotes adipogenesis *in vivo* by a clock output pathway in which *PER3* and *BMAL1* directly affect transcription factor Klf15 expression in adipocyte precursor cells (115). In addition, *Cry1/2* double null mice have abnormal serum and hepatic TG levels (88). Furthermore, *Rev-erbα* deficiency increases plasma lipid levels and decreases hepatic cholesterol and TG levels (116).

Although circadian genes in lipid abnormalities have been extensively studied, the pathological process in KSD deserves further research.

5.3 Hypertension

The kidney is the central organ that regulates blood pressure (BP). Poorly controlled BP results in kidney diseases and influences BP regulation in positive feedback (117). According to historical clinical investigations, hypertension (HTN) may correlate with KSD. Cappuccio et al. recruited 688 male workers in a cross-sectional study and found the relative risk of hypertensive participants having a history of KSD was twice that of the normal group (OR=2.11, 95% CI: 1.17 to 3.81), which is similar to the larger cohort study based on U.S. population by Hill et al. (RR=1.79, 95% CI: 1.19 to 2.71) (118). Data from a prospective cohort proved this finding (119, 120). A study confirmed that HTN can be an independent predictive determinant for recurrent KSD, especially in non-obese SFs (121). Inconsistently, the studies published by Madore et al. showed that the KSD incidence was comparable in both the hypertensive population and normal population (OR= 0.99, 95% CI: 0.82 to 1.21), and similar results were obtained when limited to middle-aged women (122, 123). The inconsistency of diagnostic criteria for HTN can lead to opposite results, and it is essential to remeasure the association in new criteria. Considering the widespread popularity of HTN and the link between HTN and metabolism, it cannot be ignored. Currently, the etiology of HTN in KSD consists of an alteration of urine components, IR, inflammation, and OS (124). Increased urine calcium excretion is caused by central volume expansion (the 'central blood volume' theory), and higher excretion levels of oxalate and uric acid were detected in hypertensive patients (119, 124, 125). Furthermore, ROS overgeneration by the activated renin-angiotensin-aldosterone system (RAAS) promotes RTEC injury and crystal formation (124, 126).

BP in healthy individuals exhibits precise daily variation, which is characterized by an increase after awakening followed by a decrease at night during sleep (1, 127). Disturbed circadian rhythm eliminates the diurnal rhythm of BP, resulting in elevated BP that transfers to HTN (127–129). Grandner et al. analyzed more than 700,000 adults from two large cohorts to illustrate that the HTN risk was higher in sleep deficiency compared to 7 h (≤ 4 h: OR=1.86, ≤ 5 h: OR=1.56, ≤ 6 h: OR=1.27, $p < 0.0005$ for all) (130). A dose-response meta-analysis found a higher HTN risk for shorter sleep duration, and previous meta-analyses reported similar results to strengthen this association (47, 131). For OSA patients, the HTN risk was higher than that in healthy controls (OR=2.84, 95% CI: 1.70 to 3.98) and was positively correlated with the OSA grade (132). Additionally, night shift workers have a higher HTN risk than normal controls, which increases with an increasing frequency of night shift work (133). Although there is no significant association between SJL and HTN in historical studies, it is worth noting that a recent study presented a morning BP surge caused by acute SJL (134, 135).

The underlying mechanism of disturbed circadian rhythm in HTN is complicated. First, in OSA patients, sympathetic nervous system overactivity, disruption by OS, and inflammation in vascular structure and functions contribute to the abnormal diurnal pattern of BP (132, 136). Intermittent hypoxia (IH) and negative pressure against obstruction activate adrenal, renal, and peripheral chemoreceptors to increase the circulating levels of hormones such as renin and catecholamine and decrease nitric oxide (NO) synthesis, leading to upregulated sympathetic system activity (136, 137). The interactions between the sympathetic system and the kidney secondarily activate RAAS to increase BP (138). Additionally, IH disrupts endothelial NO expression by promoting ROS generation. Endothelial dysfunction is mainly caused by inhibited NO bioactivity and bioavailability impairs vascular vasodilation and enhances vasoconstriction (25, 136).

Circadian gene abnormalities also trigger HTN. *Clock* mutation represses the expression level of *Atp1b1*, which encodes the β1 subunit of the Na^+/K^+-ATPase to elevate BP (139). In hypertensive rodents, myeloid-specific deficiency of *Bmal1* exacerbates vascular remodeling and accelerates HTN formation by influencing the profibrotic macrophage phenotype (140). A human study showed a higher level of *Per1* mRNA in the renal medulla in the hypertensive group than in the normal control group, suggesting a role for *Per1* in the regulation of BP by renin (141). Doi et al. examined BP regulation in global *Cry1/2* double null mice and revealed that *Cry1/2* KO mice developed salt-sensitive HTN compared to wild-type (WT) mice (142). Surprisingly, KO or mutation of circadian genes also causes the absence of diurnal rhythm in BP and significantly decreased BP (127, 128, 143). To explain this, glycolipid metabolism disorders should be considered due to the close link between HTN and MetS (143, 144). Moreover, the relationship between circadian genes and the proteins expression in the local kidney that play roles in water and electrolyte balance is inspiring. The sodium chloride (NaCl) co-transporter (NCC) is involved in sodium reabsorption and BP maintenance and Richards et al. proved that *Per1* inhibition reduces NCC expression and results in lower BP in mice (145). Zuber et al. also investigated the intrinsic circadian rhythm system and found that *Clock* mutant mice exhibit significant alterations in the renal expression of several key regulators of water or sodium balance (vasopressin V2 receptor, aquaporin-2, aquaporin-4, epithelial sodium channel), which functionally leads to dysregulation of sodium excretion rhythms and a significant decrease in BP (146). In addition to circadian genes, serum- and glucocorticoid-induced kinase 1 (*SGK1*) in renal tubules, a clock-controlled and glucocorticoid receptor- and mineralocorticoid

receptor-induced gene was recently shown to participate in BP circadian regulation. Staub et al. generated a tubular-specific *Sgk1* KO model and found that *Sgk1* deletion elevates pulse pressure by increasing the circulating aldosterone level and disrupts the BP rhythm (147).

Together, these studies indicated that circadian rhythm disturbance can disrupt BP homeostasis bidirectionally. However, since epidemiological studies focused on KSD and HTN are full of controversies, this hypothesis needs further verification.

5.4 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is an autoimmune disease characterized by chronic intestinal granulomatous inflammation and includes two main types: Crohn's disease (CD) and ulcerative colitis (UC) (148, 149). KSD is one of the most frequent extraintestinal manifestations of IBD, with a higher prevalence in IBD patients than in normal controls. In the cohort from Mississippi, 6% and 6.7% of CD and UC patients developed KSD, respectively, which is similar to the data in Switzerland (150–152). An observational study revealed that UC was a risk factor for KSD (OR = 4.2, 95% CI: 1.1-15), and the KSD prevalence in UC and CD was comparable (153). The pathological process of IBD in KSD was exhaustively reviewed by Corica et al. (152). Briefly, UA supersaturation by low urine volume and pH, hypercalciuria by bile salt malabsorption, increased colonic epithelium permeability to oxalate, and decolonization of *Oxalobacter formigenes* (*O. formigenes*) are radical.

IBD is strongly affected by circadian rhythm disturbance. The IBD incidence was significantly higher in patients with shorter sleep durations than in normal controls (HR = 1.51, 95% CI: 1.10 to 2.09) in a 10-year follow-up analysis (154). A retrospective study enrolled 115 IBD subjects and 76 healthy controls to measure chronotype, SJL and sleep debt (SD), which showed that later chronotype was negatively correlated with severe IBD ($r = -0.209$, $p < 0.05$) and that SJL was higher in the IBD group than in the controls (1.32 h ± 1.03 *vs.* 1.05 h ± 0.97, $p < 0.05$), while SD was also elevated in the IBD group compared to the controls (21.90 min ± 25.37 *vs.* 11. 49 min ± 13.58, $p < 0.05$) (155).

In IBD patients and animals, abnormal expression and status of clock genes are considered initial manifestations. Weintraub et al. analyzed clock genes in peripheral blood and intestinal mucosa and found that the expression levels of clock genes (*CLOCK*, *BMAL1*, *CRY1*, *CRY2*, *PER1*, and *PER2*) were significantly lower in both inflamed intestinal mucosa and leukocytes than in healthy controls, which was also reported in different tissues (peripheral blood monocytes, colon) (156–158). Rodents with artificially induced colitis displayed decreased *Per2*, *Cry1*, *Rev-erbα*, and *Npas2* levels and increased *Rorα* in colon tissue (159). Kyoko et al. measured the tight junction proteins occludin and claudin-1 in *Clock* mutation ($(Clock^{\triangle 19/\triangle 19})$) mice to show that mice lacking *Clock* have persistently low levels of these two proteins and were more susceptible to intestinal injury (160). Similarly, *Bmal1* KO mice show worse UC and the absence of time-dependent variation in disease activity compared to $Bmal1^{+/+}$ controls, and epithelial proliferation in the colon presents a daily rhythm in $Bmal1^{+/+}$ controls but is absent in the *Bma1 KO* group, resulting in poor regeneration (161). Mice lacking *Rorα* or *Bmal1*-driven Lnc-UC (a long noncoding RNA that is associated with colitis, particularly by reducing *Rev-erbα* expression) are more likely to have colitis than the control group. Lnc-UC deactivates the activity of NLR family pyrin domain (NLRP) 3, which is essential in the induction of proinflammatory cytokines (159, 162, 163). *Bmal1* KO also leads to a lower level of regulatory B cells in the intraepithelial region, which expresses highly programmed death ligand 1 to alleviate colitis severity (164). Then, colitis is more severe in *Per1/2* KO mice than in WT mice, with decreased Paneth cells, goblet cells, lysozyme transcripts, and lysozyme proteins (165). Oh et al. presented that intestinal epithelial-specific *Rorα* KO leads to severe inflammation by reducing the level of Ki67, a cell proliferation marker, and p-DRP1, a molecule active in ATP production (162). All the evidence indicates that IBD is related to abnormal expression of circadian genes.

All these studies demonstrated that circadian rhythm disturbance can promote KSD via IBD. However, further research is necessary to better clarify the internal molecular pathways in the process of KSD.

5.5 Microbiome dysbiosis

Microbiomes coexisting with humans are important in maintaining health and causing diseases. The gut microbiome (GMB) is a large set of microorganisms that colonize our digestive tract, and the diverse mixture of bacteria within the genitourinary tract (often at low levels) is defined as the urinary microbiome (UMB) (166–168). Dysbiosis of GMB and UMB contributes greatly to KSD (168). The colonization rate of *O. formigenes* in CaOx stones has attracted great attention. Several studies have proven that the colonization rate of *O. formigenes* is lower in SFs than in controls (169–171). Stern et al. studied the distinct GMB in SFs and non-SFs and revealed that *Bacteroides* was 3.4 times more abundant in SFs than in non-SFs (34.9% *vs.* 10.2%, $p = 0.001$), and *Prevotella* was 2.8 times more abundant in non-SFs than in SFs (34.7% *vs.* 12.3%, $p = 0.005$). In urinary analysis, *Eubacterium* was negatively correlated with oxalate levels, and *Escherichia* tended to have an inverse correlation with citrate levels (172). Compared to GMB, UMB in KSD is underexplored due to dramatic variation (168, 173). A case-control investigation profiling the UMB in male patients with calcium-based stones found that the UMB diversity was markedly lower than that in healthy controls, and the components were also different in the two groups ($p < 0.001$). The taxa at the genus level that significantly differentiated the two groups were *Prevotella* in the normal group and *Acinetobacter* in the KSD group (174). A meta-analysis including 8 studies indicated that the abundance of *Bacteroides*, *Lactobacillus*, and *Prevotella* showed the most significant difference in GMB between KSD patients and healthy controls (175). In UMB, *Escherichia coli* (*E. coli*), *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and *Klebsiella* are considered vital in KSD based on evidence *in vivo* and *in vitro* (166, 168, 176–178). The pathogenic

mechanism of GMB is poorly understood, oxalate accumulation by oxalate degradation dysfunction and related metabolic disorders is widely recognized (166, 179). Meanwhile, urease enzymes and inflammation are also important (16, 180).

Microbiomes have been found to follow a strict circadian rhythm (167, 181). Up to 60% of the total microbial composition oscillates rhythmically, which translates to diurnal fluctuations in 20% of commensal species of the GMB in mice and 10% in humans. The GMB abundance of ad libitum-fed mice under a strict light-dark cycle was measured at the changing point, and significant diurnal fluctuations were identified in the abundance of more than 15% of the GMB (182). Circadian misalignment disrupts GMB homeostasis and causes diseases. Smith et al. explored the correlation between GBM diversity and sleep physiology to show that diversity was positively correlated with sleep efficiency and duration. Sleep duration reduction can significantly decrease GBM diversity (183, 184). Additionally, exposure to SJL exacerbated GBM and metabolite homeostasis in the jejunum and colon of mice and was also detected in humans (181, 185).

Studies support that clock gene expression alterations contribute to GMB dysbiosis. Compared to WT mice, *Clock* mutant (*Clock*$^{\Delta19/\Delta19}$) mice have a decreased *Firmicutes*/*Bacteroidetes* ratio, especially when paired with alcohol consumption or HFD (186). Liang et al. constructed *Bmal1* KO mice and found that *Bmal1* KO abolishes the oscillation and composition of GMB with a decreased abundance of *Prevotella* and an increased abundance of *Bacteroides* (187). In *Per1/2*-deficient mice, *Bacteroides* and *Lactobacillus* lost oscillations in relative abundance (182). Furthermore, in the interaction between GBM and MetS, dysbiosis significantly promotes the development of MetS. A high-sugar diet and HFD can aggravate the impact of circadian disorganization on GBM and further disturb glycolipid metabolism (188–190). In UMB, studies are limited. The diurnal oscillation in *Streptococcus pneumoniae (S. pneumoniae*) is driven by external clues, such as temperature (191). *Per1* mutant flies are more sensitive to *S. pneumoniae*, and the elevated infection sensitivity can be a consequence of the circadian regulation disturbance of phagocytosis in these fly mutants (192, 193). In *E. coli*, circadian rhythms are driven by special genes, including *radA* and *KaiC* (194). Furthermore, a red and blue photoreceptor was contained in *E. coli* to adapt to the day-night cycle, which may provide evidence for dysbiosis in abnormal light exposure by circadian disturbance (195).

These studies provide a preliminary indication of the correlation between MB and KSD. However, data from mammals are still necessary, and since there is an interaction between GMB and UMB, further research is needed.

5.6 Other factors

5.6.1 Parathyroid gland hormone disorder

Disordered calcium metabolism contributes greatly to KSD. Goodman et al. concluded that the risk of hypercalcemia was 9 times higher in KSD patients than in normal controls (196). Parathyroid hormone (PTH) is essential to enhance calcium reabsorption and inhibit phosphorus reabsorption in the kidney (197). Elevated PTH with hypercalcemia in primary hyperparathyroidism (PTHP) is a well-recognized reason for KSD (198). An observational study revealed that parathyroidectomy was effective in the therapy of KSD recurrence by idiopathic hypercalciuria and that stone activity significantly decreased after surgery (0.05-0.15 *vs* 0.50-0.75, $p < 0.001$) (199, 200).

The PTH level exhibits a bimodal pattern over 24 hours, with a maximum peak in the afternoon and a small peak at night (201). Circadian impairment leads to abnormal PTH levels. Higher serum PTH level was observed in patients with moderate OSA and severe OSA than in healthy controls (63.11 ± 36.11 and 53.16 ± 25.29 *vs* 43.71 ± 24.45, $p < 0.05$) (202). Then, Sleep disturbance is common in PTHP patients, but the causation is unclear (203, 204).

Clock genes in the parathyroid gland (PTG) were explored, and their disturbance caused parathyroid gland dysfunction (205). Normal circadian clock operation was confirmed in animal models with a periodicity of 24 hours and was significant for *Bmal1*, *Npas2*, *Per1,2,3*, *Cry1,2*, and *Rev-Erbα*. In hyperplastic PTG tissue, circadian genes were deregulated, with significant upregulation of *Per1,2* and *Rev-Erbα* and downregulation of *Npas2* (206). Egstrand et al. investigated the alteration of circadian genes during a 24-h cycle in murine PTG and found rhythmic expression of parathyroid signature genes, and this rhythm is essential for PTG function regulation. Mice with PTG-specific *Bmal1* knockdown (PTHcre;*Bmal1*$^{flox/flox}$) were created, and a global decrease in circadian genes was observed, including *Clock*, *Npas2*, *Cry1,2*, and *Per1,2,3*. Compared to WT, PTHcre; *Bmal1*$^{flox/flox}$ shows a higher parathyroid cell proliferation response and led to PTHP (205). The identification of transcriptional patterns in human PTG tissues presents that the transcript expression levels of *PER1* and *CRY1/2* are significantly lower in PHPT tissue than in healthy tissue (207).

Taken together, these studies indicate that circadian gene disturbance leads to PTG disorder and is a risk factor for KSD. However, more investigations are needed.

5.6.2 Vitamin D Deficiency

Vitamin D (vit D) participates in maintaining calcium homeostasis, and vit D deficiency (VDD) is defined as a serum 25-hydroxyvitamin-D [25(OH)D] concentration less than 30 ng/ml (208). VDD affects a large population globally and is more prevalent in KSD patients (209). The investigation by Elkoushy et al. showed that more than 80% of KSD patients have VDD, which is consistent with a multicenter study (210, 211). A case-control study in Spain including 366 participants found that calcium SFs had lower levels of Vit D (25.7 *vs*. 28.4 ng/ml, $p = 0.02$) and a higher percentage of VDD than non-SFs (28.0% *vs*. 15.7%, $p = 0.009$) (212). Vit D is a fundamental regulator of systemic inflammation, OS, and mitochondrial respiratory function. Based on current studies, cell injury by OS and inflammation by overproduced ROS are the cardinal pathogenic factors for KSD in VDD (213, 214).

Vit D status is significantly influenced by a circadian rhythm in which serum Vit D presents a significant diurnal rhythm with a nadir in the morning and is followed by a rapid increase to a plateau during the day (215). VDD is widespread in populations with

abnormal light exposure or insufficient sleep (216). Piovezan et al. indicated that short sleep duration showed an independent association with VDD (OR = 1.61, 95% CI: 1.25 to 2.26) (217). Then, shift workers are more likely to develop VDD. An investigation in Italy measured serum 25(OH)D in workers and observed that the level was lower in night shift workers than in daily workers (13.4 ± 5.3 ng/mL *vs.* 21.9 ± 10.7 ng/mL, $p < 0.001$) (218, 219).

Endogenous vit D is synthesized in the skin from the cholesterol-like precursor (7-dehydrocholesterol) present in epidermal cells by exposure to ultraviolet B (UV-B) from sunlight (220). Therefore, a lack of UV exposure reduces vit D levels and exacerbates OS in RTECs, which leads to KSD (129, 218). Moreover, research on the interaction between VDD and circadian genes is limited. Kwai et al. created an intestinal *Bmal1* KO model ($Bmal1_{Int}$-/- mice) and found that the vit D receptor (VDR) target genes in the intestine are disrupted. The expression of VDR and *Vdr* peaks at ZT8 (*zeitgeber* time [ZT]: light on, ZT0–ZT12; lights off, ZT12–ZT24) in the control group but disappears in $Bmal1_{Int}$-/- mice. The experiment in Caco-2 cell lines also reveals that the *BMAL1* KO reduced *VDR* and VDR expression (221). However, other circadian genes are still unclear in VDD, which is worth more analysis.

5.6.3 Renal Tubular Damage or Dysfunctions

The combination of urinary supersaturation and renal tubular damage is vital in stone formation. Renal tubular damage is related to sleep disorders, especially OSA (222, 223). The high oxygen demand of renal tubules makes them vulnerable to hypoxia by chronic IH in OSA and easily advanced to renal tubular injury (223). However, such a theory needs to be backed up by more research, and its relationship with KSD should be further elucidated based on direct evidence.

Distal renal tubular acidosis (dRTA) syndrome is a condition caused by the acidification defect in the collection tubule and the inability of the distal nephron to maximally increase the urinary secretion of protons ([H+]) in the presence of metabolic acidosis, characterized by a persistent hyperchloremia, normal plasma anion gap and metabolic acidosis with a relatively normal glomerular filtration rate. Patients with dRTA have elevated urinary calcium, recurrent CaOx or CaP stone formation, and nephrocalcinosis (224, 225). In addition to inherited dRTA, secondary dRTA is caused by numerous triggers, including autoimmune diseases, nephrotoxins, and miscellaneous aetiologies. Currently, there are insufficient epidemiological and basic studies to confirm the relationship between dRTA and circadian rhythm disturbance.

6 Future perspective

Growing evidence indicates that some therapeutic strategies enhancing circadian clock function or circadian gene expression may be beneficial for the prevention of KSD. First, feeding time is one of the most important external *Zeitgebers* in peripheral tissues, and unhealthy feeding time promotes diseases. A systematic review suggested that fasting results in altered urine metabolites and density, although this did not transfer to clinical outcomes. Safe fasting practices are vital for high-risk patients to prevent KSD (226). For OSA patients, continuous positive air treatment significantly reduces tubular damage, which may decrease the KSD risk (223). Moreover, melatonin (Mel), a hormone released from the pineal gland against SJL and sleep disorders with anti-inflammatory and antioxidative functions, can prevent crystalluria and kidney damage caused by crystal formation and aggregation (227, 228). Song et al. found that Mel has protective effects on oxalate-induced endoplasmic reticulum stress and apoptosis via the activation of the adenosine 5'-monophosphate-activated protein kinase pathway in HK-2 cells (229). In addition, *BMAL1* is a therapeutic target *in vitro*. *BMAL1* overexpression stimulated the OS-related NRF2/HO-1 pathway to reduce CaOx stone formation. This suggests that maintaining normal rhythms and properly intervening in some related circadian genes and downstream antioxidant pathways may benefit the prevention of CaOx stones (230). According to current limited studies, it can be speculated that artificial interventions in sleep status and circadian rhythm have the potential to prevent KSD. However, more clinical and basic research is needed.

7 Conclusions

Since KSD is a major challenge for global health, its potential mechanism should be investigated. Increasing convincing evidence has elucidated that a disordered circadian clock is a putative factor for KSD. This review summarizes the biological characteristics of the circadian rhythm, the mechanism of KSD, and the putative mechanism of the circadian rhythm disturbance in KSD. Existing clinical and basic studies have indicated that circadian rhythm-based interventions have potential clinical value in the management of KSD, but the specific and accurate mechanism of KSD caused by circadian rhythm disturbance is still unclear. KSD is not an isolated kidney disease, but a systemic disorder affected by various factors. Understanding the relationship between circadian rhythm and systemic multi-organ and multi-system health status is essential. In addition, both behavioral and pharmacological interventions related to rhythm modification deserve more research. A comprehensive and in-depth exploration of the mechanism of KSD caused by sleep and circadian rhythm disturbance and the efficacy of chronothrapies for KSD are necessary and can provide a new strategy for the clinical management of KSD.

Author contributions

SH: Conceptualization, Writing – original draft. JHW: Writing – original draft. TL: Writing – original draft. SY: Data curation, Writing – review & editing. JC: Writing – review & editing. YT: Supervision, Writing – review & editing. YX: Data curation, Writing – review & editing. JW: Conceptualization, Writing – review & editing. YB: Conceptualization, Writing – review & editing.

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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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References

1. Fagiani F, Di Marino D, Romagnoli A, Travelli C, Voltan D, Di Cesare Mannelli L, et al. Molecular regulations of circadian rhythm and implications for physiology and diseases. *Signal Transduct Target Ther* (2022) 7:41. doi: 10.1038/s41392-022-00899-y

2. Chan M-C, Spieth PM, Quinn K, Parotto M, Zhang H, Slutsky AS. Circadian rhythms: from basic mechanisms to the intensive care unit. *Crit Care Med* (2012) 40:246–53. doi: 10.1097/CCM.0b013e31822f0abe

3. Fatima N, Rana S. Metabolic implications of circadian disruption. *Pflugers Arch* (2020) 472:513–26. doi: 10.1007/s00424-020-02381-6

4. Estanislau TB, Kruger K, Rosa-Neto JC, Padilha CS, Gonçalves DC, Minuzzi LG, et al. Feeding pattern, circadian rhythm, and immune function: what do we know about? *Curr Pharm Des* (2022) 28(30):2478–87. doi: 10.2174/1381612828666220729091451

5. Rhodes V, Maguire M, Shetty M, McAloon C, Smeaton AF. Periodicity intensity of the 24 h circadian rhythm in newborn calves show indicators of herd welfare. *Sensors (Basel)* (2022) 22:5843. doi: 10.3390/s22155843

6. Yamaguchi ST, Kobayashi R, Tomita J, Kume K. The regulation of circadian rhythm by insulin signaling in Drosophila. *Neurosci Res* (2022) S0168-0102(22):00194–8. doi: 10.1016/j.neures.2022.07.005

7. Konishi T, Takahashi Y, Shiina Y, Oike H, Oishi K. Time-of-day effects of consumption of fish oil-enriched sausages on serum lipid parameters and fatty acid composition in normolipidemic adults: A randomized, double-blind, placebo-controlled, and parallel-group pilot study. *Nutrition* (2021) 90:111247. doi: 10.1016/j.nut.2021.111247

8. Walker WH, Bumgarner JR, Walton JC, Liu JA, Meléndez-Fernández OH, Nelson RJ, et al. Light pollution and cancer. *Int J Mol Sci* (2020) 21:9360. doi: 10.3390/ijms21249360

9. Reutrakul S, Van Cauter E. Interactions between sleep, circadian function, and glucose metabolism: implications for risk and severity of diabetes. *Ann N Y Acad Sci* (2014) 1311:151–73. doi: 10.1111/nyas.12355

10. McAlpine CS, Swirski FK. Circadian influence on metabolism and inflammation in atherosclerosis. *Circ Res* (2016) 119:131–41. doi: 10.1161/CIRCRESAHA.116.308034

11. Masri S, Sassone-Corsi P. The emerging link between cancer, metabolism, and circadian rhythms. *Nat Med* (2018) 24:1795–803. doi: 10.1038/s41591-018-0271-8

12. Li H, Ou J, Li Y, Xu N, Li Q, Wu P, et al. ISX-9 potentiates CaMKIIδ-mediated BMAL1 activation to enhance circadian amplitude. *Commun Biol* (2022) 5:750. doi: 10.1038/s42003-022-03725-x

13. Peerapen P, Thongboonkerd V. Kidney stone prevention. *Adv Nutr* (2023) 14:555–69. doi: 10.1016/j.advnut.2023.03.002

14. D'Ambrosio V, Moochhala S, Unwin RJ, Ferraro PM. Why is diagnosis, investigation, and improved management of kidney stone disease important? Non-pharmacological and pharmacological treatments for nephrolithiasis. *Expert Rev Clin Pharmacol* (2022) 15:407–14. doi: 10.1080/17512433.2022.2082943

15. Barghouthy Y, Corrales M, Doizi S, Somani BK, Traxer O. Tea and coffee consumption and the risk of urinary stones—a systematic review of the epidemiological data. *World J Urol* (2021) 39:2895–901. doi: 10.1007/s00345-020-03561-w

16. Wang Z, Zhang Y, Zhang J, Deng Q, Liang H. Recent advances on the mechanisms of kidney stone formation (Review). *Int J Mol Med* (2021) 48:149. doi: 10.3892/ijmm.2021.4982

17. van de Pol JAA, van den Brandt PA, Schouten LJ. Kidney stones and the risk of renal cell carcinoma and upper tract urothelial carcinoma: the Netherlands Cohort Study. *Br J Cancer* (2019) 120:368–74. doi: 10.1038/s41416-018-0356-7

18. Dhondup T, Kittanamongkolchai W, Vaughan LE, Mehta RA, Chhina JK, Enders FT, et al. Risk of ESRD and mortality in kidney and bladder stone formers. *Am J Kidney Dis* (2018) 72:790–7. doi: 10.1053/j.ajkd.2018.06.012

19. Luke M, Ankith M, Shabbir HM, Rukshana S, Manuel FP. Epidemiological and biological associations between cardiovascular disease and kidney stone formation: A systematic review and meta-analysis. *Nutrition Metab Cardiovasc Dis* (2023) S0939475323003691. doi: 10.1016/j.numecd.2023.09.011

20. Cheungpasitporn W, Thongprayoon C, O'Corragain OA, Edmonds PJ, Ungprasert P, Kittanamongkolchai W, et al. The risk of kidney cancer in patients with kidney stones: a systematic review and meta-analysis. *QJM* (2015) 108:205–12. doi: 10.1093/qjmed/hcu195

21. Rodríguez D, Sacco DE. Minimally invasive surgical treatment for kidney stone disease. *Adv Chronic Kidney Dis* (2015) 22:266–72. doi: 10.1053/j.ackd.2015.03.005

22. Costello HM, Johnston JG, Juffre A, Crislip GR, Gumz ML. Circadian clocks of the kidney: function, mechanism, and regulation. *Physiol Rev* (2022) 102:1669–701. doi: 10.1152/physrev.00045.2021

23. Xiao Y, Yin S, Bai Y, Yang Z, Wang J, Cui J, et al. Association between circadian syndrome and the prevalence of kidney stones in overweight adults: a cross-sectional analysis of NHANES 2007-2018. *BMC Public Health* (2023) 23:960. doi: 10.1186/s12889-023-15934-y

24. Lucas RJ, Freedman MS, Lupi D, Munoz M, David-Gray ZK, Foster RG. Identifying the photoreceptive inputs to the mammalian circadian system using transgenic and retinally degenerate mice. *Behav Brain Res* (2001) 125:97–102. doi: 10.1016/s0166-4328(01)00274-1

25. Li T, Bai Y, Jiang Y, Jiang K, Tian Y, Wang Z, et al. Potential effect of the circadian clock on erectile dysfunction. *Aging Dis* (2022) 13:8. doi: 10.14336/AD.2021.0728

26. Fan X, Chen Z, Li W, Qin H, Huang S, Lu Z, et al. Exploring the effects of large-area dorsal skin irradiation on locomotor activity and plasm melatonin level in C3H/He mice. *Chronobiol Int* (2021) 38:1776–85. doi: 10.1080/07420528.2021.1962904

27. Hergenhan S, Holtkamp S, Scheiermann C. Molecular interactions between components of the circadian clock and the immune system. *J Mol Biol* (2020) 432:3700–13. doi: 10.1016/j.jmb.2019.12.044

28. Xu H, Huang L, Zhao J, Chen S, Liu J, Li G. The circadian clock and inflammation: A new insight. *Clin Chim Acta* (2021) 512:12–7. doi: 10.1016/j.cca.2020.11.011

29. Zhang EE, Kay SA. Clocks not winding down: unravelling circadian networks. *Nat Rev Mol Cell Biol* (2010) 11:764–76. doi: 10.1038/nrm2995

30. Meijer JH, Michel S, Vanderleest HT, Rohling JHT. Daily and seasonal adaptation of the circadian clock requires plasticity of the SCN neuronal network. *Eur J Neurosci* (2010) 32:2143–51. doi: 10.1111/j.1460-9568.2010.07522.x

31. Deng F, Yang K, Zheng G. Period family of clock genes as novel predictors of survival in human cancer: A systematic review and meta-analysis. *Dis Markers* (2020) 2020:6486238. doi: 10.1155/2020/6486238

32. Munroe SH, Lazar MA. Inhibition of c-erbA mRNA splicing by a naturally occurring antisense RNA. *J Biol Chem* (1991) 266:22083–6. doi: 10.1016/S0021-9258(18)54535-X

33. Lazar MA, Hodin RA, Cardona G, Chin WW. Gene expression from the c-erbA alpha/Rev-ErbA alpha genomic locus. Potential regulation of alternative splicing by opposite strand transcription. *J Biol Chem* (1990) 265:12859–63. doi: 10.1016/S0021-9258(19)38238-9

34. Gupta S, Sk S. Kidney stones: Mechanism of formation, pathogenesis and possible treatments. (2018).

35. Paliouras C, Tsampikaki E, Alivanis P, Aperis G. Pathophysiology of nephrolithiasis. *Nephrol Res Rev* (2012) 4:58–65. doi: 10.4081/nr.2012.e14

36. Alelign T, Petros B. Kidney stone disease: an update on current concepts. *Adv Urol* (2018) 2018:1–12. doi: 10.1155/2018/3068365

37. Randall A. THE ORIGIN AND GROWTH OF RENAL CALCULI. *Ann Surg* (1937) 105:1009–27. doi: 10.1097/00000658-193706000-00014

38. Khan SR, Canales BK, Dominguez-Gutierrez PR. Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation. *Nat Rev Nephrol* (2021) 17:417–33. doi: 10.1038/s41581-020-00392-1

39. Ketha H, Singh RJ, Grebe SK, Bergstralh EJ, Rule AD, Lieske JC, et al. Altered calcium and vitamin D homeostasis in first-time calcium kidney stone-formers. *PloS One* (2015) 10:e0137350. doi: 10.1371/journal.pone.0137350

40. Chung J, Granja I, Taylor MG, Mpourmpakis G, Asplin JR, Rimer JD. Molecular modifiers reveal a mechanism of pathological crystal growth inhibition. *Nature* (2016) 536:446–50. doi: 10.1038/nature19062

41. Brainard J, Gobel M, Scott B, Koeppen M, Eckle T. Health implications of disrupted circadian rhythms and the potential for daylight as therapy. *Anesthesiology* (2015) 122:1170–5. doi: 10.1097/ALN.0000000000000596

42. Cho JW, Duffy JF. Sleep, sleep disorders, and sexual dysfunction. *World J Mens Health* (2019) 37:261–75. doi: 10.5534/wjmh.180045

43. Pallesen S, Bjorvatn B, Waage S, Harris A, Sagoe D. Prevalence of shift work disorder: A systematic review and meta-analysis. *Front Psychol* (2021) 12:638252. doi: 10.3389/fpsyg.2021.638252

44. Yin S, Wang J, Bai Y, Yang Z, Cui J, Wang J. Association between sleep duration and kidney stones in 34 190 American adults: A cross-sectional analysis of NHANES 2007-2018. *Sleep Health* (2022) 8:671–7. doi: 10.1016/j.sleh.2022.08.003

45. Wang H, Zhang YQ, Yu CQ, Guo Y, Pei P, Chen JS, et al. [Associations between sleep status and risk for kidney stones in Chinese adults: a prospective cohort study]. *Zhonghua Liu Xing Bing Xue Za Zhi* (2022) 43:1002–9. doi: 10.3760/cma.j.cn112338-20210930-00760

46. Fan M, Sun D, Zhou T, Heianza Y, Lv J, Li L, et al. Sleep patterns, genetic susceptibility, and incident cardiovascular disease: a prospective study of 385 292 UK biobank participants. *Eur Heart J* (2020) 41:1182–9. doi: 10.1093/eurheartj/ehz849

47. Wang S, Zhou X, Qiu S, Cai B, Li Y, Zhang C, et al. Association between sleep quality and urolithiasis among general population in Western China: a cross-sectional study. *BMC Public Health* (2022) 22:1787. doi: 10.1186/s12889-022-14187-5

48. Tsai S-H, Stoller ML, Sherer BA, Chao Z-H, Tung T-H. Risk of nephrolithiasis in patients with sleep apnea: A population-based cohort study. *J Clin Sleep Med* (2018) 14:767–73. doi: 10.5664/jcsm.7102

49. Tallman JE, Stone BV, Sui W, Miller NL, Hsi RS. Association between obstructive sleep apnea and 24-h urine chemistry risk factors for urinary stone disease. *Urolithiasis* (2023) 51:46. doi: 10.1007/s00240-023-01421-x

50. Khalili P, Jamali Z, Sadeghi T, Esmaeili-Nadimi A, Mohamadi M, Moghadam-Ahmadi A, et al. Risk factors of kidney stone disease: a cross-sectional study in the southeast of Iran. *BMC Urol* (2021) 21:141. doi: 10.1186/s12894-021-00905-5

51. Jiang YG, He LH, Luo GT, Zhang XD. Prevalence of kidney stones and associated risk factors in the Shunyi District of Beijing, China. *Hong Kong Med J* (2017) 23:462–9. doi: 10.12809/hkmj164904

52. Liu W, Wang M, Liu J, Yan Q, Liu M. Causal effects of modifiable risk factors on kidney stones: a bidirectional mendelian randomization study. *BMC Med Genomics* (2023) 16:82. doi: 10.1186/s12920-023-01520-z

53. Foster RG. Sleep, circadian rhythms and health. *Interface Focus* (2020) 10:20190098. doi: 10.1098/rsfs.2019.0098

54. Goldbeter A, Leloup J-C. From circadian clock mechanism to sleep disorders and jet lag: Insights from a computational approach. *Biochem Pharmacol* (2021) 191:114482. doi: 10.1016/j.bcp.2021.114482

55. Wulff K, Porcheret K, Cussans E, Foster RG. Sleep and circadian rhythm disturbances: multiple genes and multiple phenotypes. *Curr Opin Genet Dev* (2009) 19:237–46. doi: 10.1016/j.gde.2009.03.007

56. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* (2018) 20:12. doi: 10.1007/s11906-018-0812-z

57. Nolan PB, Carrick-Ranson G, Stinear JW, Reading SA, Dalleck LC. Prevalence of metabolic syndrome and metabolic syndrome components in young adults: A pooled analysis. *Prev Med Rep* (2017) 7:211–5. doi: 10.1016/j.pmedr.2017.07.004

58. Qiu F, Xu Y, Ji X, Pu J, Zhou J, Huang Y. Incidence and correlation of metabolic syndrome and kidney stones in a healthy screening population. *Transl Androl Urol* (2021) 10:3646–55. doi: 10.21037/tau-21-689

59. Chang C-W, Ke H-L, Lee J-I, Lee Y-C, Jhan J-H, Wang H-S, et al. Metabolic syndrome increases the risk of kidney stone disease: A cross-sectional and longitudinal cohort study. *JPM* (2021) 11:1154. doi: 10.3390/jpm11111154

60. Spatola L, Ferraro PM, Gambaro G, Badalamenti S, Dauriz M. Metabolic syndrome and uric acid nephrolithiasis: insulin resistance in focus. *Metabolism* (2018) 83:225–33. doi: 10.1016/j.metabol.2018.02.008

61. Zimmet P, Alberti KGMM, Stern N, Bilu C, El-Osta A, Einat H, et al. The Circadian Syndrome: is the Metabolic Syndrome and much more! *J Intern Med* (2019) 286:181–91. doi: 10.1111/joim.12924

62. Shi Z, Tuomilehto J, Kronfeld-Schor N, Alberti GK, Stern N, El-Osta A, et al. The circadian syndrome predicts cardiovascular disease better than metabolic syndrome in Chinese adults. *J Intern Med* (2021) 289:851–60. doi: 10.1111/joim.13204

63. Chaput J-P, McNeil J, Després J-P, Bouchard C, Tremblay A. Short sleep duration as a risk factor for the development of the metabolic syndrome in adults. *Prev Med* (2013) 57:872–7. doi: 10.1016/j.ypmed.2013.09.022

64. Bayon V, Berger M, Solelhac G, Haba-Rubio J, Marques-Vidal P, Strippoli M-P, et al. Impact of night and shift work on metabolic syndrome and its components: a cross-sectional study in an active middle-to-older-aged population-based sample. *BMJ Open* (2022) 12:e053591. doi: 10.1136/bmjopen-2021-053591

65. Koopman ADM, Rauh SP, van 't Riet E, Groeneveld L, van der Heijden AA, Elders PJ, et al. The association between social jetlag, the metabolic syndrome, and type 2 diabetes mellitus in the general population: the new hoorn study. *J Biol Rhythms* (2017) 32:359–68. doi: 10.1177/0748730417713572

66. Kim DH, Kim B, Han K, Kim SW. The relationship between metabolic syndrome and obstructive sleep apnea syndrome: a nationwide population-based study. *Sci Rep* (2021) 11:8751. doi: 10.1038/s41598-021-88233-4

67. Drager LF, Lopes HF, Maki-Nunes C, Trombetta IC, Toschi-Dias E, Alves MJNN, et al. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PloS One* (2010) 5:e12065. doi: 10.1371/journal.pone.0012065

68. Libório AB, Figueiredo PRL, Montenegro Junior RM, Montenegro RM, Martins MRA, Silva Junior GB, et al. Urinary calcium excretion and insulin resistance in patients with acromegaly. *Int Urol Nephrol* (2012) 44:1473–7. doi: 10.1007/s11255-011-0116-6

69. Meydan N, Barutca S, Caliskan S, Camsari T. Urinary stone disease in diabetes mellitus. *Scandinavian J Urol Nephrol* (2003) 37:64–70. doi: 10.1080/00365590310008730

70. Weinberg AE, Patel CJ, Chertow GM, Leppert JT. Diabetic severity and risk of kidney stone disease. *Eur Urol* (2014) 65:242–7. doi: 10.1016/j.eururo.2013.03.026

71. Khand TU, Channa NA, Khand F. Oxidative stress is the common causal link between renal stones and diabetes mellitus in adults. *J Pak Med Assoc* (2022) 72:1302–5. doi: 10.47391/JPMA.1188

72. Tan E, Scott EM. Circadian rhythms, insulin action, and glucose homeostasis. *Curr Opin Clin Nutr Metab Care* (2014) 17:343–8. doi: 10.1097/MCO.0000000000000061

73. Henry CJ, Kaur B, Quek RYC. Chrononutrition in the management of diabetes. *Nutr Diabetes* (2020) 10:6. doi: 10.1038/s41387-020-0109-6

74. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, et al. Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* (1988) 318:1231–9. doi: 10.1056/NEJM198805123181903

75. Song L, Shen J, Wang J, Zhang Y, Zhou Z, Sang L, et al. Shift patterns, physical exercise, and Type 2 diabetes mellitus (T2DM): a prospective cohort study in China. *Transl Behav Med* (2023) 13:183–91. doi: 10.1093/tbm/ibac089

76. Cui S, Li Y, Chen Y, Ren P, Fan M, Yang X, et al. Association of sleep duration with risk of type 2 diabetes mellitus in a rural Chinese population: a nested case-control study. *Sleep Breath* (2022) 26:2025–33. doi: 10.1007/s11325-021-02535-5

77. Pietroiusti A, Neri A, Somma G, Coppeta L, Iavicoli I, Bergamaschi A, et al. Incidence of metabolic syndrome among night-shift healthcare workers. *Occup Environ Med* (2010) 67:54–7. doi: 10.1136/oem.2009.046797

78. Gao Y, Gan T, Jiang L, Yu L, Tang D, Wang Y, et al. Association between shift work and risk of type 2 diabetes mellitus: a systematic review and dose-response meta-analysis of observational studies. *Chronobiol Int* (2020) 37:29–46. doi: 10.1080/07420528.2019.1683570

79. Chan K, Wong FS, Pearson JA. Circadian rhythms and pancreas physiology: A review. *Front Endocrinol (Lausanne)* (2022) 13:920261. doi: 10.3389/fendo.2022.920261

80. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* (2005) 308:1043–5. doi: 10.1126/science.1108750

81. Shi S, Ansari TS, McGuinness OP, Wasserman DH, Johnson CH. Circadian disruption leads to insulin resistance and obesity. *Curr Biol* (2013) 23:372–81. doi: 10.1016/j.cub.2013.01.048

82. Lee J, Moulik M, Fang Z, Saha P, Zou F, Xu Y, et al. Bmal1 and β-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced β-cell failure in mice. *Mol Cell Biol* (2013) 33:2327–38. doi: 10.1128/MCB.01421-12

83. Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* (2010) 466:627–31. doi: 10.1038/nature09253

84. Shi D, Chen J, Wang J, Yao J, Huang Y, Zhang G, et al. Circadian clock genes in the metabolism of non-alcoholic fatty liver disease. *Front Physiol* (2019) 10:423. doi: 10.3389/fphys.2019.00423

85. Baek M, Virgilio S, Lamb TM, Ibarra O, Andrade JM, Gonçalves RD, et al. Circadian clock regulation of the glycogen synthase (gsn) gene by WCC is critical for rhythmic glycogen metabolism in Neurospora crassa. *Proc Natl Acad Sci U.S.A.* (2019) 116:10435–40. doi: 10.1073/pnas.1815360116

86. Jouffe C, Weger BD, Martin E, Atger F, Weger M, Gobet C, et al. Disruption of the circadian clock component BMAL1 elicits an endocrine adaption impacting on insulin sensitivity and liver disease. *Proc Natl Acad Sci U.S.A.* (2022) 119:e2200083119. doi: 10.1073/pnas.2200083119

87. Zhang Z, Yu B, Wang X, Luo C, Zhou T, Zheng X, et al. Circadian rhythm and atherosclerosis (Review). *Exp Ther Med* (2020) 20:96. doi: 10.3892/etm.2020.9224

88. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, Jonker JW, et al. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* (2011) 480:552–6. doi: 10.1038/nature10700

89. Zhang EE, Liu Y, Dentin R, Pongsawakul PY, Liu AC, Hirota T, et al. Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat Med* (2010) 16:1152–6. doi: 10.1038/nm.2214

90. Qian J, Block GD, Colwell CS, Matveyenko AV. Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. *Diabetes* (2013) 62:3469–78. doi: 10.2337/db12-1543

91. Zani F, Breasson L, Becattini B, Vukolic A, Montani J-P, Albrecht U, et al. PER2 promotes glucose storage to liver glycogen during feeding and acute fasting by inducing Gys2 PTG and G L expression. *Mol Metab* (2013) 2:292–305. doi: 10.1016/j.molmet.2013.06.006

92. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, et al. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* (2012) 485:123–7. doi: 10.1038/nature11048

93. Delezie J, Dumont S, Dardente H, Oudart H, Gréchez-Cassiau A, Klosen P, et al. The nuclear receptor REV-ERBα is required for the daily balance of carbohydrate and lipid metabolism. *FASEB J* (2012) 26:3321–35. doi: 10.1096/fj.12-208751

94. Torricelli FCM, De SK, Gebreselassie S, Li I, Sarkissian C, Monga M. Dyslipidemia and kidney stone risk. *J Urol* (2014) 191:667–72. doi: 10.1016/j.juro.2013.09.022

95. Ding Q, Ouyang J, Fan B, Cao C, Fan Z, Ding L, et al. Association between dyslipidemia and nephrolithiasis risk in a chinese population. *Urol Int* (2019) 103:156–65. doi: 10.1159/000496208

96. Antonelli JA, Maalouf NM, Pearle MS, Lotan Y. Use of the National Health and Nutrition Examination Survey to calculate the impact of obesity and diabetes on cost and prevalence of urolithiasis in 2030. *Eur Urol* (2014) 66:724–9. doi: 10.1016/j.eururo.2014.06.036

97. Zhou T, Watts K, Agalliu I, DiVito J, Hoenig DM. Effects of visceral fat area and other metabolic parameters on stone composition in patients undergoing percutaneous nephrolithotomy. *J Urol* (2013) 190:1416–20. doi: 10.1016/j.juro.2013.05.016

98. Wu H, Ballantyne CM. Metabolic inflammation and insulin resistance in obesity. *Circ Res* (2020) 126:1549–64. doi: 10.1161/CIRCRESAHA.119.315896

99. Chua EC-P, Shui G, Lee IT-G, Lau P, Tan L-C, Yeo S-C, et al. Extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans. *Proc Natl Acad Sci U.S.A.* (2013) 110:14468–73. doi: 10.1073/pnas.1222647110

100. Gooley JJ. Circadian regulation of lipid metabolism. *Proc Nutr Soc* (2016) 75:440–50. doi: 10.1017/S0029665116000288

101. Seo SH, Shim YS. Association of sleep duration with obesity and cardiometabolic risk factors in children and adolescents: A population-based study. *Sci Rep* (2019) 9:9463. doi: 10.1038/s41598-019-45951-0

102. Brocato J, Wu F, Chen Y, Shamy M, Alghamdi MA, Khoder MI, et al. Association between sleeping hours and cardiometabolic risk factors for metabolic syndrome in a Saudi Arabian population. *BMJ Open* (2015) 5:e008590. doi: 10.1136/bmjopen-2015-008590

103. Parsons MJ, Moffitt TE, Gregory AM, Goldman-Mellor S, Nolan PM, Poulton R, et al. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. *Int: J Obes (Lond)* (2015) 39:842–8. doi: 10.1038/ijo.2014.201

104. Hirayama S, Soda S, Ito Y, Matsui H, Ueno T, Fukushima Y, et al. Circadian change of serum concentration of small dense LDL-cholesterol in type 2 diabetic patients. *Clin Chim Acta* (2010) 411:253–7. doi: 10.1016/j.cca.2009.11.017

105. Xing C, Huang X, Zhang Y, Zhang C, Wang W, Wu L, et al. Sleep disturbance induces increased cholesterol level by NR1D1 mediated CYP7A1 inhibition. *Front Genet* (2020) 11:610496. doi: 10.3389/fgene.2020.610496

106. Vieira E G, Ruano E, AL CF, Aranda G, Momblan D, Carmona F, et al. Altered clock gene expression in obese visceral adipose tissue is associated with metabolic syndrome. *PloS One* (2014) 9:e111678. doi: 10.1371/journal.pone.0111678

107. Valladares M, Obregón AM, Chaput J-P. Association between genetic variants of the clock gene and obesity and sleep duration. *J Physiol Biochem* (2015) 71:855–60. doi: 10.1007/s13105-015-0447-3

108. Pan X, Jiang X-C, Hussain MM. Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. *Circulation* (2013) 128:1758–69. doi: 10.1161/CIRCULATIONAHA.113.002885

109. Pérez-Mendoza M, Rivera-Zavala JB, Díaz-Muñoz M. Daytime restricted feeding modifies the daily variations of liver gluconeogenesis: adaptations in biochemical and endocrine regulators. *Chronobiol Int* (2014) 31:815–28. doi: 10.3109/07420528.2014.908898

110. Pan X, Bradfield CA, Hussain MM. Global and hepatocyte-specific ablation of Bmal1 induces hyperlipidaemia and enhances atherosclerosis. *Nat Commun* (2016) 7:13011. doi: 10.1038/ncomms13011

111. Dallmann R, Weaver DR. Altered body mass regulation in male mPeriod mutant mice on high-fat diet. *Chronobiol Int* (2010) 27:1317–28. doi: 10.3109/07420528.2010.489166

112. Ge W, Sun Q, Yang Y, Ding Z, Liu J, Zhang J. Circadian PER1 controls daily fat absorption with the regulation of PER1-PKA on phosphorylation of bile acid synthetase. *J Lipid Res* (2023) 64:100390. doi: 10.1016/j.jlr.2023.100390

113. Grimaldi B, Bellet MM, Katada S, Astarita G, Hirayama J, Amin RH, et al. PER2 controls lipid metabolism by direct regulation of PPARγ. *Cell Metab* (2010) 12:509–20. doi: 10.1016/j.cmet.2010.10.005

114. Yang S, Liu A, Weidenhammer A, Cooksey RC, McClain D, Kim MK, et al. The role of mPer2 clock gene in glucocorticoid and feeding rhythms. *Endocrinology* (2009) 150:2153–60. doi: 10.1210/en.2008-0705

115. Aggarwal A, Costa MJ, Rivero-Gutiérrez B, Ji L, Morgan SL, Feldman BJ. The circadian clock regulates adipogenesis by a per3 crosstalk pathway to klf15. *Cell Rep* (2017) 21:2367–75. doi: 10.1016/j.celrep.2017.11.004

116. Le Martelot G, Claudel T, Gatfield D, Schaad O, Kornmann B, Lo Sasso G, et al. REV-ERBalpha participates in circadian SREBP signaling and bile acid homeostasis. *PloS Biol* (2009) 7:e1000181. doi: 10.1371/journal.pbio.1000181

117. Wadei HM, Textor SC. The role of the kidney in regulating arterial blood pressure. *Nat Rev Nephrol* (2012) 8:602–9. doi: 10.1038/nrneph.2012.191

118. Hill AJ, Basourakos SP, Lewicki P, Wu X, Arenas-Gallo C, Chuang D, et al. Incidence of kidney stones in the United States: the continuous national health and nutrition examination survey. *J Urol* (2022) 207:851–6. doi: 10.1097/JU.0000000000002331

119. Borghi L, Meschi T, Guerra A, Briganti A, Schianchi T, Allegri F, et al. Essential arterial hypertension and stone disease. *Kidney Int* (1999) 55:2397–406. doi: 10.1046/j.1523-1755.1999.00483.x

120. Cappuccio FP, Siani A, Barba G, Mellone MC, Russo L, Farinaro E, et al. A: prospective study of hypertension and the incidence of kidney stones in men. *J: Hypertens* (1999) 17:1017–22. doi: 10.1097/00004872-199917070-00019

121. Kim Y-J, Park MS, Kim W-T, Yun S-J, Kim W-J, Lee S-C. Hypertension influences recurrent stone formation in nonobese stone formers. *Urology* (2011) 77:1059–63. doi: 10.1016/j.urology.2010.07.492

122. Madore F, Stampfer MJ, Rimm EB, Curhan GC. Nephrolithiasis and risk of hypertension. *Am J Hypertens* (1998) 11:46–53. doi: 10.1016/s0895-7061(97)00371-3

123. Madore F, Stampfer MJ, Willett WC, Speizer FE, Curhan GC. Nephrolithiasis and risk of hypertension in women. *Am J Kidney Dis* (1998) 32:802–7. doi: 10.1016/s0272-6386(98)70136-2

124. Cupisti A, D'Alessandro C, Samoni S, Meola M, Egidi MF. Nephrolithiasis and hypertension: possible links and clinical implications. *J Nephrol* (2014) 27:477–82. doi: 10.1007/s40620-014-0068-x

125. Taylor EN, Mount DB, Forman JP, Curhan GC. Association of prevalent hypertension with 24-hour urinary excretion of calcium, citrate, and other factors. *Am J Kidney Dis* (2006) 47:780–9. doi: 10.1053/j.ajkd.2006.01.024

126. Davalos M, Konno S, Eshghi M, Choudhury M. Oxidative renal cell injury induced by calcium oxalate crystal and renoprotection with antioxidants: a possible role of oxidative stress in nephrolithiasis. *J Endourol* (2010) 24:339–45. doi: 10.1089/end.2009.0205

127. Gumz ML, Shimbo D, Abdalla M, Balijepalli RC, Benedict C, Chen Y, et al. Toward precision medicine: circadian rhythm of blood pressure and chronotherapy for hypertension - 2021 NHLBI workshop report. *Hypertension* (2023) 80:503–22. doi: 10.1161/HYPERTENSIONAHA.122.19372

128. Douma LG, Gumz ML. Circadian clock-mediated regulation of blood pressure. *Free Radical Biol Med* (2018) 119:108–14. doi: 10.1016/j.freeradbiomed.2017.11.024

129. Smolensky MH, Sackett-Lundeen LL, Portaluppi F. Nocturnal light pollution and underexposure to daytime sunlight: Complementary mechanisms of circadian disruption and related diseases. *Chronobiol Int* (2015) 32:1029–48. doi: 10.3109/07420528.2015.1072002

130. Grandner M, Mullington JM, Hashmi SD, Redeker NS, Watson NF, Morgenthaler TI. Sleep duration and hypertension: analysis of > 700,000 adults by age and sex. *J Clin Sleep Med* (2018) 14:1031–9. doi: 10.5664/jcsm.7176

131. Li H, Ren Y, Wu Y, Zhao X. Correlation between sleep duration and hypertension: a dose-response meta-analysis. *J Hum Hypertens* (2019) 33:218–28. doi: 10.1038/s41371-018-0135-1

132. Hou H, Zhao Y, Yu W, Dong H, Xue X, Ding J, et al. Association of obstructive sleep apnea with hypertension: A systematic review and meta-analysis. *J Glob Health* (2018) 8:10405. doi: 10.7189/jogh.08.010405

133. Xiao Z, Xu C, Liu Q, Yan Q, Liang J, Weng Z, et al. Night shift work, genetic risk, and hypertension. *Mayo Clin Proc* (2022) 97:2016–27. doi: 10.1016/j.mayocp.2022.04.007

134. Makarem N, Zuraikat FM, Aggarwal B, Jelic S, St-Onge M-P. Variability in sleep patterns: an emerging risk factor for hypertension. *Curr Hypertens Rep* (2020) 22:19. doi: 10.1007/s11906-020-1025-9

135. Nakamura N, Akiyama H, Nishimura M, Zhu K, Suzuki K, Higuchi M, et al. Acute social jetlag augments morning blood pressure surge: a randomized crossover trial. *Hypertens Res* (2023) 46(9):2179–91. doi: 10.1038/s41440-023-01360-5

136. Dopp JM, Reichmuth KJ, Morgan BJ. Obstructive sleep apnea and hypertension: Mechanisms, evaluation, and management. *Curr Sci Inc* (2007) 9:529–34. doi: 10.1007/s11906-007-0095-2

137. Salman LA, Shulman R, Cohen JB. Obstructive sleep apnea, hypertension, and cardiovascular risk: epidemiology, pathophysiology, and management. *Curr Cardiol Rep* (2020) 22:6. doi: 10.1007/s11886-020-1257-y

138. Foster GE, Hanly PJ, Ahmed SB, Beaudin AE, Pialoux V, Poulin MJ. Intermittent hypoxia increases arterial blood pressure in humans through a Renin-Angiotensin system-dependent mechanism. *Hypertension* (2010) 56:369–77. doi: 10.1161/HYPERTENSIONAHA.110.152108

139. Nakashima A, Kawamoto T, Noshiro M, Ueno T, Doi S, Honda K, et al. Dec1 and CLOCK regulate na+/K+-ATPase β1 subunit expression and blood pressure. *Hypertension* (2018) 72:746–54. doi: 10.1161/HYPERTENSIONAHA.118.11075

140. Huo M, Cao X, Zhang H, Lau CW, Hong H, Chen FM, et al. Loss of myeloid Bmal1 exacerbates hypertensive vascular remodelling through interaction with STAT6 in mice. *Cardiovasc Res* (2022) 118:2859–74. doi: 10.1093/cvr/cvab336

141. Marques FZ, Campain AE, Tomaszewski M, Zukowska-Szczechowska E, Yang YHJ, Charchar FJ, et al. Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. *Hypertension* (2011) 58:1093–8. doi: 10.1161/HYPERTENSIONAHA.111.180729

142. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, et al. Salt-sensitive hypertension in circadian clock–deficient Cry-null mice involves dysregulated adrenal Hsd3b6. *Nat Med* (2010) 16:67–74. doi: 10.1038/nm.2061

143. Richards J, Diaz AN, Gumz ML. Clock genes in hypertension: novel insights from rodent models. *Blood Pressure Monit* (2014) 19:249–54. doi: 10.1097/MBP.0000000000000060

144. Mancusi C, Izzo R, di Gioia G, Losi MA, Barbato E, Morisco C. Insulin resistance the hinge between hypertension and type 2 diabetes. *High Blood Press Cardiovasc Prev* (2020) 27:515–26. doi: 10.1007/s40292-020-00408-8

145. Richards J, Ko B, All S, Cheng K-Y, Hoover RS, Gumz ML. A role for the circadian clock protein per1 in the regulation of the naCl co-transporter (NCC) and the with-no-lysine kinase (WNK) cascade in mouse distal convoluted tubule cells. *J Biol Chem* (2014) 289:11791–806. doi: 10.1074/jbc.M113.531095

146. Zuber AM, Centeno G, Pradervand S, Nikolaeva S, Maquelin L, Cardinaux L, et al. Molecular clock is involved in predictive circadian adjustment of renal function. *Proc Natl Acad Sci U.S.A.* (2009) 106:16523–8. doi: 10.1073/pnas.0904890106

147. Staub O, Debonneville A, Stifanelli M, Juffre A, Maillard MP, Gumz ML, et al. Renal tubular SGK1 is required to achieve blood pressure surge and circadian rhythm. *Am J Physiol Renal Physiol* (2023) 325:F629–37. doi: 10.1152/ajprenal.00211.2023

148. Chang JT. Pathophysiology of inflammatory bowel diseases. *N Engl J Med* (2020) 383:2652–64. doi: 10.1056/NEJMra2002697

149. Dincer MT, Dincer ZT, Bakkaloglu OK, Yalin SF, Trabulus S, Celik AF, et al. Renal manifestations in inflammatory bowel disease: A cohort study during the biologic era. *Med Sci Monit* (2022) 28:e936497-1–e936497-10. doi: 10.12659/MSM.936497

150. Fagagnini S, Heinrich H, Rossel J-B, Biedermann L, Frei P, Zeitz J, et al. Risk factors for gallstones and kidney stones in a cohort of patients with inflammatory bowel diseases. *PloS One* (2017) 12:e0185193. doi: 10.1371/journal.pone.0185193

151. Herbert J, Teeter E, Burstiner LS, Doka R, Royer A, Owings AH, et al. Urinary manifestations in African American and Caucasian inflammatory bowel disease patients: a retrospective cohort study. *BMC Urol* (2022) 22:1. doi: 10.1186/s12894-021-00951-z

152. Corica D, Romano C. Renal involvement in inflammatory bowel diseases. *ECCOJC* (2016) 10:226–35. doi: 10.1093/ecco-jcc/jjv138

153. Cury D, Moss A, Schor N. Nephrolithiasis in patients with inflammatory bowel disease in the community. *IJNRD* (2013) 6:139–42. doi: 10.2147/IJNRD.S45466

154. Ananthakrishnan AN, Khalili H, Konijeti GG, Higuchi LM, de Silva P, Fuchs CS, et al. Sleep duration affects risk for ulcerative colitis: a prospective cohort study. *Clin Gastroenterol Hepatol* (2014) 12:1879–86. doi: 10.1016/j.cgh.2014.04.021

155. Chakradeo PS, Keshavarzian A, Singh S, Dera AE, Esteban JPG, Lee AA, et al. Chronotype, social jet lag, sleep debt and food timing in inflammatory bowel disease. *Sleep Med* (2018) 52:188–95. doi: 10.1016/j.sleep.2018.08.002

156. Weintraub Y, Cohen S, Chapnik N, Ben-Tov A, Yerushalmy-Feler A, Dotan I, et al. Clock gene disruption is an initial manifestation of inflammatory bowel diseases. *Clin Gastroenterol Hepatol* (2020) 18:115–122.e1. doi: 10.1016/j.cgh.2019.04.013

157. Liu X, Yu R, Zhu L, Hou X, Zou K. Bidirectional regulation of circadian disturbance and inflammation in inflammatory bowel disease. *Inflammatory Bowel Dis* (2017) 23:1741–51. doi: 10.1097/MIB.0000000000001265

158. Weintraub Y, Cohen S, Anafy A, Chapnik N, Tsameret S, Ben-Tov A, et al. Inverse relationship between clock gene expression and inflammatory markers in ulcerative colitis patients undergoing remission. *Dig Dis Sci* (2023) 68:2454–62. doi: 10.1007/s10620-023-07847-y

159. Wang S, Lin Y, Yuan X, Li F, Guo L, Wu B. REV-ERBα integrates colon clock with experimental colitis through regulation of NF-κB/NLRP3 axis. *Nat Commun* (2018) 9:4246. doi: 10.1038/s41467-018-06568-5

160. Kyoko O, Kono H, Ishimaru K, Miyake K, Kubota T, Ogawa H, et al. Expressions of tight junction proteins Occludin and Claudin-1 are under the circadian control in the mouse large intestine: implications in intestinal permeability and susceptibility to colitis. *PloS One* (2014) 9:e98016. doi: 10.1371/journal.pone.0098016

161. Taleb Z, Carmona-Alcocer V, Stokes K, Haireek M, Wang H, Collins SM, et al. BMAL1 regulates the daily timing of colitis. *Front Cell Infect Microbiol* (2022) 12:773413. doi: 10.3389/fcimb.2022.773413

162. Oh SK, Kim D, Kim K, Boo K, Yu YS, Kim IS, et al. RORα is crucial for attenuated inflammatory response to maintain intestinal homeostasis. *Proc Natl Acad Sci U.S.A.* (2019) 116:21140–9. doi: 10.1073/pnas.1907595116

163. Wang S, Lin Y, Li F, Qin Z, Zhou Z, Gao L, et al. An NF-κB-driven lncRNA orchestrates colitis and circadian clock. *Sci Adv* (2020) 6:eabb5202. doi: 10.1126/sciadv.abb5202

164. Liu J-L, Wang C-Y, Cheng T-Y, Rixiati Y, Ji C, Deng M, et al. Circadian clock disruption suppresses PDL1+ Intraepithelial B cells in experimental colitis and colitis-associated colorectal cancer. *Cell Mol Gastroenterol Hepatol* (2021) 12:251–76. doi: 10.1016/j.jcmgh.2021.02.008

165. Pagel R, Bär F, Schröder T, Sünderhauf A, Künstner A, Ibrahim SM, et al. Circadian rhythm disruption impairs tissue homeostasis and exacerbates chronic inflammation in the intestine. *FASEB J* (2017) 31:4707–19. doi: 10.1096/fj.201700141RR

166. Miller AW, Penniston KL, Fitzpatrick K, Agudelo J, Tasian G, Lange D. Mechanisms of the intestinal and urinary microbiome in kidney stone disease. *Nat: Rev Urol* (2022) 19:695–707. doi: 10.1038/s41585-022-00647-5

167. Parizadeh M, Arrieta M-C. The global human gut microbiome: genes, lifestyles, and diet. *Trends Mol Med* (2023) S1471-4914(23):00152–1. doi: 10.1016/j.molmed.2023.07.002

168. Patel SR, Ingram C, Scovell JM, Link RE, Mayer WA. The microbiome and: urolithiasis: current advancements and future challenges. *Curr Urol Rep* (2022) 23:47–56. doi: 10.1007/s11934-022-01088-8

169. Kumar R, Mukherjee M, Bhandari M, Kumar A, Sidhu H, Mittal RD. Role of Oxalobacter formigenes in calcium oxalate stone disease: a study from North India. *Eur Urol* (2002) 41:318–22. doi: 10.1016/s0302-2838(02)00040-4

170. Kaufman DW, Kelly JP, Curhan GC, Anderson TE, Dretler SP, Preminger GM, et al. Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. *J: Am Soc Nephrol* (2008) 19:1197–203. doi: 10.1681/ASN.2007101058

171. Troxel SA, Sidhu H, Kaul P, Low RK. Intestinal Oxalobacter formigenes colonization in calcium oxalate stone formers and its relation to urinary oxalate. *J: Endourol* (2003) 17:173–6. doi: 10.1089/089277903321618743

172. Stern JM, Moazami S, Qiu Y, Kurland I, Chen Z, Agalliu I, et al. Evidence for a distinct gut microbiome in kidney stone formers compared to non-stone formers. *Urolithiasis* (2016) 44:399–407. doi: 10.1007/s00240-016-0882-9

173. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol* (2013) 3:41. doi: 10.3389/fcimb.2013.00041

174. Xie J, Huang J, Huang X, Peng J, Yu Z, Yuan Y, et al. Profiling the urinary microbiome in men with calcium-based kidney stones. *BMC Microbiol* (2020) 20:41. doi: 10.1186/s12866-020-01734-6

175. Yuan T, Xia Y, Li B, Yu W, Rao T, Ye Z, et al. Gut microbiota in patients with kidney stones: a systematic review and meta-analysis. *BMC Microbiol* (2023) 23:143. doi: 10.1186/s12866-023-02891-0

176. Amimanan P, Tavichakorntrakool R, Fong-Ngern K, Sribenjalux P, Lulitanond A, Prasongwatana V, et al. Elongation factor Tu on Escherichia coli isolated from urine of kidney stone patients promotes calcium oxalate crystal growth and aggregation. *Sci: Rep* (2017) 7:2953. doi: 10.1038/s41598-017-03213-x

177. Chutipongtanate S, Sutthimethakorn S, Chiangjong W, Thongboonkerd V. Bacteria can promote calcium oxalate crystal growth and aggregation. *J Biol Inorg Chem* (2013) 18:299–308. doi: 10.1007/s00775-012-0974-0

178. Gao H, Lin J, Xiong F, Yu Z, Pan S, Huang Y. Urinary microbial and metabolomic profiles in kidney stone disease. *Front Cell Infect Microbiol* (2022) 12:953392. doi: 10.3389/fcimb.2022.953392

179. Ticinesi A, Nouvenne A, Meschi T. Gut microbiome and kidney stone disease: not just an Oxalobacter story. *Kidney Int* (2019) 96:25–7. doi: 10.1016/j.kint.2019.03.020

180. Jones-Freeman B, Chonwerawong M, Marcelino VR, Deshpande AV, Forster SC, Starkey MR. The microbiome and host mucosal interactions in urinary tract diseases. *Mucosal Immunol* (2021) 14:779–92. doi: 10.1038/s41385-020-00372-5

181. Thaiss CA, Levy M, Korem T, Dohnalová L, Shapiro H, Jaitin DA, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* (2016) 167:1495–1510.e12. doi: 10.1016/j.cell.2016.11.003

182. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* (2014) 159:514–29. doi: 10.1016/j.cell.2014.09.048

183. Smith RP, Easson C, Lyle SM, Kapoor R, Donnelly CP, Davidson EJ, et al. Gut microbiome diversity is associated with sleep physiology in humans. *PloS One* (2019) 14:e0222394. doi: 10.1371/journal.pone.0222394

184. Agrawal R, Ajami NJ, Malhotra S, Chen L, White DL, Sharafkhaneh A, et al. Habitual sleep duration and the colonic mucosa-associated gut microbiota in humans-A pilot study. *Clocks Sleep* (2021) 3:387–97. doi: 10.3390/clockssleep3030025

185. Li Q, Wang B, Qiu H-Y, Yan X-J, Cheng L, Wang Q-Q, et al. Chronic jet lag exacerbates jejunal and colonic microenvironment in mice. *Front Cell Infect Microbiol* (2021) 11:648175. doi: 10.3389/fcimb.2021.648175

186. Voigt RM, Summa KC, Forsyth CB, Green SJ, Engen P, Naqib A, et al. The circadian *clock* mutation promotes intestinal dysbiosis. *Alcohol Clin Exp Res* (2016) 40:335–47. doi: 10.1111/acer.12943

187. Liang X, Bushman FD, FitzGerald GA. Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. *Proc Natl Acad Sci U.S.A.* (2015) 112:10479–84. doi: 10.1073/pnas.1501305112

188. Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock: function and metabolism. *Cell Host Microbe* (2015) 17:681–9. doi: 10.1016/j.chom.2015.03.006

189. Voigt RM, Forsyth CB, Green SJ, Mutlu E, Engen P, Vitaterna MH, et al. Circadian disorganization alters intestinal microbiota. *PloS One* (2014) 9:e97500. doi: 10.1371/journal.pone.0097500

190. Zarrinpar A, Chaix A, Yooseph S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab* (2014) 20:1006–17. doi: 10.1016/j.cmet.2014.11.008

191. Paulose JK, Cassone CV, Graniczkowska KB, Cassone VM. Entrainment of the circadian clock of the enteric bacterium klebsiella aerogenes by temperature cycles. *iScience* (2019) 19:1202–13. doi: 10.1016/j.isci.2019.09.007

192. Stone EF, Fulton BO, Ayres JS, Pham LN, Ziauddin J, Shirasu-Hiza MM. The: circadian clock protein timeless regulates phagocytosis of bacteria in Drosophila. *PloS Pathog* (2012) 8:e1002445. doi: 10.1371/journal.ppat.1002445

193. Shirasu-Hiza MM, Dionne MS, Pham LN, Ayres JS, Schneider DS. Interactions between circadian rhythm and immunity in Drosophila melanogaster. *Curr Biol* (2007) 17:R353–355. doi: 10.1016/j.cub.2007.03.049

194. Diallo AB, Mezouar S, Boumaza A, Fiammingo O, Coiffard B, Pontarotti P, et al. RadA, a key gene of the circadian rhythm of escherichia coli. *IJMS* (2022) 23:6136. doi: 10.3390/ijms23116136

195. Gomelsky M, Klug G. BLUF: a novel FAD-binding domain involved in sensory transduction in microorganisms. *Trends Biochem Sci* (2002) 27:497–500. doi: 10.1016/s0968-0004(02)02181-3

196. Goodman HO, Holmes RP, Assimos DG. Genetic factors in calcium oxalate stone disease. *J Urol* (1995) 153:301–7. doi: 10.1097/00005392-199502000-00003

197. Ducy P. A central regulation of PTH secretion and function. *Neuron* (2023) 111:1847–9. doi: 10.1016/j.neuron.2023.05.018

198. Ganesan C, Weia B, Thomas I-C, Song S, Velaer K, Seib CD, et al. Analysis of: primary hyperparathyroidism screening among US veterans with kidney stones. *JAMA Surg* (2020) 155:861–8. doi: 10.1001/jamasurg.2020.2423

199. Charles P-Y, Letavernier E, Périé S, Gauthé M, Daudon M, Haymann J-P. Effect of parathyroidectomy on renal stone recurrence. *Urolithiasis* (2021) 49:327–34. doi: 10.1007/s00240-020-01239-x

200. Perez AA, Schneider DF, Long KL, Pitt SC, Sippel RS. Timely evaluation and management of primary hyperparathyroidism in patients with kidney stones. *J Surg Res* (2018) 232:564–9. doi: 10.1016/j.jss.2018.07.028

201. Schou A, Jørgensen NR, Maro VP, Kilonzo K, Ramaiya K, Sironga J, et al. The circadian rhythm of calcium and bone homeostasis in Maasai. *Am J Hum Biol* (2022) 34:e23756. doi: 10.1002/ajhb.23756

202. Erden ES, Genc S, Motor S, Ustun I, Ulutas KT, Bilgic HK, et al. Investigation of serum bisphenol A, vitamin D, and parathyroid hormone levels in patients with obstructive sleep apnea syndrome. *Endocrine* (2014) 45:311–8. doi: 10.1007/s12020-013-0022-z

203. Murray SE, Pathak PR, Schaefer SC, Chen H, Sippel RS. Improvement of sleep disturbance and insomnia following parathyroidectomy for primary hyperparathyroidism. *World J Surg* (2014) 38:542–8. doi: 10.1007/s00268-013-2285-1

204. Chou F-F, Lee C-H, Chen J-B, Huang S-C, Lee C-T. Sleep disturbances before and after parathyroidectomy for secondary hyperparathyroidism. *Surgery* (2005) 137:426–30. doi: 10.1016/j.surg.2004.12.010

205. Egstrand S, Mace ML, Morevati M, Nordholm A, Engelholm LH, Thomsen JS, et al. Hypomorphic expression of parathyroid Bmal1 disrupts the internal parathyroid circadian clock and increases parathyroid cell proliferation in response to uremia. *Kidney Int* (2022) 101:1232–50. doi: 10.1016/j.kint.2022.02.018

206. Egstrand S, Nordholm A, Morevati M, Mace ML, Hassan A, Naveh-Many T, et al. A molecular circadian clock operates in the parathyroid gland and is disturbed in chronic kidney disease associated bone and mineral disorder. *Kidney Int* (2020) 98:1461–75. doi: 10.1016/j.kint.2020.06.034

207. Sadowski SM, Pusztaszeri M, Brulhart-Meynet M-C, Petrenko V, De Vito C, Sobel J, et al. Identification of differential transcriptional patterns in primary and secondary hyperparathyroidism. *J Clin Endocrinol Metab* (2018) 103:2189–98. doi: 10.1210/jc.2017-02506

208. Bouillon R, Carmeliet G. Vitamin D insufficiency: Definition, diagnosis and management. *Best Pract Res Clin Endocrinol Metab* (2018) 32:669–84. doi: 10.1016/j.beem.2018.09.014

209. Johri N, Jaeger P, Ferraro PM, Shavit L, Nair D, Robertson WG, et al. Vitamin D deficiency is prevalent among idiopathic stone formers, but does correction pose any risk? *Urolithiasis* (2017) 45:535–43. doi: 10.1007/s00240-016-0954-x

210. Elkoushy MA, Sabbagh R, Unikowsky B, Andonian S. Prevalence and metabolic abnormalities of vitamin D–inadequate patients presenting with urolithiasis to a tertiary stone clinic. *Urology* (2012) 79:781–5. doi: 10.1016/j.urology.2011.09.004

211. Eisner BH, Thavaseelan S, Sheth S, Haleblian G, Pareek G. Relationship between serum vitamin D and 24-hour urine calcium in patients with nephrolithiasis. *Urology* (2012) 80:1007–10. doi: 10.1016/j.urology.2012.04.041

212. Girón-Prieto MS, Del Carmen Cano-García M, Arrabal-Polo MÁ, Poyatos-Andujar A, Quesada-Charneco M, De Haro-Muñoz T, et al. Analysis of vitamin D deficiency in calcium stone-forming patients. *Int Urol Nephrol* (2016) 48:1243–6. doi: 10.1007/s11255-016-1290-3

213. Tavasoli S, Taheri M. Vitamin D and calcium kidney stones: a review and a proposal. *Int Urol Nephrol* (2019) 51:101–11. doi: 10.1007/s11255-018-1965-z

214. Wimalawansa SJ. Vitamin D deficiency: effects on oxidative stress, epigenetics, gene regulation, and aging. *Biol (Basel)* (2019) 8:30. doi: 10.3390/biology8020030

215. Masood T, Kushwaha RS, Singh R, Sailwal S, Pandey H, Varma A, et al. Circadian rhythm of serum 25 (OH) vitamin D, calcium and phosphorus levels in the treatment and management of type-2 diabetic patients. *Drug Discovery Ther* (2015) 9:70–4. doi: 10.5582/ddt.2015.01002

216. Alefishat E, Abu Farha R. Determinants of vitamin d status among Jordanian employees: Focus on the night shift effect. *Int J Occup Med Environ Health* (2016) 29:859–70. doi: 10.13075/ijomeh.1896.00657

217. Piovezan RD, Hirotsu C, Feres MC, Cintra FD, Andersen ML, Tufik S, et al. Obstructive sleep apnea and objective short sleep duration are independently associated with the risk of serum vitamin D deficiency. *PloS One* (2017) 12:e0180901. doi: 10.1371/journal.pone.0180901

218. Lehnert M, Beine A, Burek K, Putzke S, Schlösser S, Pallapies D, et al. Vitamin D supply in shift working nurses. *Chronobiol Int* (2018) 35:724–9. doi: 10.1080/07420528.2018.1424719

219. Romano A, Vigna L, Belluigi V, Conti DM, Barberi CE, Tomaino L, et al. Shift work and serum 25-OH vitamin D status among factory workers in Northern Italy: Cross-sectional study. *Chronobiol Int* (2015) 32:842–7. doi: 10.3109/07420528.2015.1048867

220. Martelli M, Salvio G, Santarelli L, Bracci M. Shift work and serum vitamin D levels: A systematic review and meta-analysis. *Int J Environ Res Public Health* (2022) 19:8919. doi: 10.3390/ijerph19158919

221. Kawai M, Kinoshita S, Yamazaki M, Yamamoto K, Rosen CJ, Shimba S, et al. Intestinal clock system regulates skeletal homeostasis. *JCI Insight* (2019) 4:e121798. doi: 10.1172/jci.insight.121798. 121798.

222. Pochetti P, Azzolina D, Ragnoli B, Tillio PA, Cantaluppi V, Malerba M. Interrelationship among obstructive sleep apnea, renal function and survival: A cohort study. *Int J Environ Res Public Health* (2020) 17:4922. doi: 10.3390/ijerph17144922

223. Moriya R, Hokari S, Ohshima Y, Suzuki R, Nagai A, Fujito N, et al. Continuous positive airway pressure treatment reduces renal tubular damage in patients with obstructive sleep apnea: A retrospective single-center cohort study. *Sleep Med* (2023) 106:106–15. doi: 10.1016/j.sleep.2023.03.028

224. Domrongkitchaiporn S, Stitchantrakul W. Renal tubular acidosis and stone formation. In: Talati JJ, Tiselius H-G, Albala DM, Ye Z, editors. *Urolithiasis*. London: Springer London (2012). p. 195–8. doi: 10.1007/978-1-4471-4387-1_23

225. Giglio S, Montini G, Trepiccione F, Gambaro G, Emma F. Distal renal tubular acidosis: a systematic approach from diagnosis to treatment. *J Nephrol* (2021) 34:2073–83. doi: 10.1007/s40620-021-01032-y

226. Kirubarajan A, Lam ACL, Khan S, Yau M, Golda N, Buckley R. The association between renal stones and fasting: A systematic review. *Canadian Urological Association Journal* (2021) 15(3):E169–74. doi: 10.5489/cuaj.6664

227. Sener TE, Sener G, Cevik O, Eker P, Cetinel S, Traxer O, et al. The effects of melatonin on ethylene glycol-induced nephrolithiasis: role on osteopontin mRNA gene expression. *Urology* (2017) 99:287.e9–287.e15. doi: 10.1016/j.urology.2016.09.032

228. Vasey C, McBride J, Penta K. Circadian rhythm dysregulation and restoration: the role of melatonin. *Nutrients* (2021) 13:3480. doi: 10.3390/nu13103480

229. Song Q, He Z, Li B, Liu J, Liu L, Liao W, et al. Melatonin inhibits oxalate-induced endoplasmic reticulum stress and apoptosis in HK-2 cells by activating the AMPK pathway. *Cell Cycle* (2020) 19:2600–10. doi: 10.1080/15384101.2020.1810401

230. Wang J, Bai Y, Yin S, Cui J, Zhang Y, Wang X, et al. Circadian clock gene BMAL1 reduces urinary calcium oxalate stones formation by regulating NRF2/HO-1 pathway. *Life Sci* (2021) 265:118853. doi: 10.1016/j.lfs.2020.118853

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Samir Patel,
United States Department of Veterans Affairs, United States
Tatsuo Shimosawa,
International University of Health and Welfare (IUHW), Japan

*CORRESPONDENCE
Zhiyuan Chen
Chiyuenchan2023@163.com

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Overview of research progress on the association of dietary potassium intake with serum potassium and survival in hemodialysis patients, does dietary potassium restriction really benefit hemodialysis patients?

Zuoya Sun[1], Jian Jiao[2], Gang Lu[1], Ruihong Liu[1], Zhuo Li[1], Yi Sun[3] and Zhiyuan Chen[1]*

[1]Department of Family Medicine, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China, [2]Department of Gastroenterology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shandong First Medical University, Jinan, Shandong, China, [3]Department of Nephrology, Beijing Huairou Hospital of University of Chinese Academy of Sciences, Beijing, China

For the general population, increasing potassium intake can reduce the incidence of cardiovascular and cerebrovascular diseases. However, since hyperkalemia is a common and life-threatening complication in maintenance hemodialysis patients, which can increase the risk of malignant arrhythmia and sudden death, the current mainstream of management for hemodialysis patients is dietary potassium restriction in order to prevent hyperkalemia. Hemodialysis patients are usually advised to reduce dietary potassium intake and limit potassium-rich fruits and vegetables, but there is limited evidence to support this approach can reduce mortality and improve quality of life. There is still no consistent conclusion on the association between dietary potassium intake and serum potassium and survival in hemodialysis patients. According to the current small observational studies, there was little or even no association between dietary potassium intake and serum potassium in hemodialysis patients when assurance of adequate dialysis and specific dietary patterns (such as the plant-based diet mentioned in the article) are being followed, and excessive dietary potassium restriction may not benefit the survival of hemodialysis patients. Additionally, when assessing the effect of diet on serum potassium, researchers should not only focus on the potassium content of foods, but also consider the type of food and the content of other nutrients. However, more large-scale, multi-center clinical trials are required to provide high-quality evidence support. Besides, further research is also needed to determine the optimal daily potassium intake and beneficial dietary patterns for hemodialysis patients.

KEYWORDS

hemodialysis patients, dietary potassium intake, serum potassium, dietary patterns, dietary potassium restriction

Introduction

Maintenance hemodialysis (MHD) is one of the primary alternatives to prolong the survival time and improve the quality of life of patients with end-stage renal disease. As renal function declines, the incidence of hyperkalemia progressively rises, which increases the risk of arrhythmias and sudden death (1–4). MHD patients mainly regulate potassium metabolism in the body through hemodialysis, restriction of potassium intake, potassium-lowering medications and potassium excretion by residual renal function (5, 6). Despite the fact that dialysis can effectively remove potassium ions from the body, the increase in pre-dialysis serum potassium caused by extended dialysis intervals poses a grave threat to patients' lives. Interventions to reduce pre-dialysis serum potassium may effectively reduce the mortality of hemodialysis patients (7). According to a study of 55,183 patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) multinational cohort, those with pre-dialysis serum potassium levels between 4-5.5 mEq/L had the lowest risk of mortality, whereas those with levels >5.6 mEq/L had a significantly higher risk of both death and arrhythmia (8). In their study of the influence of serum potassium and dialysate concentration on hemodialysis patient survival, Kovesdy et al. discovered that when pre-dialysis serum potassium was in the range of 4.6-5.3 mEq/L, the survival rate was highest, and with adequate nutrient intake, dietary strategies should be combined to prevent substantial fluctuations in serum potassium (9). Hemodialysis alone is insufficient to maintain normal serum potassium levels, and studies have indicated that controlling dietary potassium intake plays a crucial role in achieving stable serum potassium levels (10). A systematic review of changes in serum potassium (11), chronic kidney disease (CKD) progression, and mortality in patients with CKD following a low-potassium diet versus an unrestricted diet indicates that dietary potassium restriction, compared with higher potassium intake, may lower serum potassium in patients with normokalemia, and they discovered no evidence that dietary restriction is associated with decreased all-cause mortality in patients with CKD stages 3-5. For hemodialysis patients, dietary potassium restriction is currently the mainstream of management in order to prevent hyperkalemia, the latest Nutrition KDOQI Guidelines (12) propose an individualization of the dietary potassium intake recommendation. Other clinical practice guidelines in the field of renal nutrition recommend reducing dietary potassium intake to 2–2.5 g/day for patients undergoing hemodialysis, but evidence supporting such restriction independent of food source to improve morbidity, mortality, and quality of life in hemodialysis populations is limited and not supported by rigorous randomized controlled trials (11, 13–18).

Adequate and satisfying food is an essential human need and enjoyment before security, belonging, self-esteem, and self-actualization are satisfied. Moreover, for the general population, increasing dietary potassium intake can not only lower blood pressure (19–25) and the risk of cardiovascular and cerebrovascular disorders (26–30), but also have other protective effects, such as anti-inflammatory, anti-fibrosis and anti-oxidation effects, improve endothelial function and prevent atherosclerosis (31).

However, to prevent and manage hyperkalemia, many medical institutions have incorporated dietary instruction for hemodialysis patients advising them to limit their consumption of potassium-rich fruits and vegetables (32), more than half of hemodialysis patients report feeling deprived, feeling that the prescribed diet is bland (33). In addition, dietary restrictions that are too stringent might cause malnutrition and lower the quality of life for hemodialysis patients (34, 35).

Currently, there is no evidence from observational studies regarding the optimal daily potassium intake for hemodialysis patients, nor is there a consistent conclusion regarding the relationship between dietary potassium intake, serum potassium, and survival rate. This article is a narrative review to illustrate the relationship between dietary potassium intake and serum potassium and survival in hemodialysis patients by comparing the changes in serum potassium, the occurrence of hyperkalemia, and the impact on survival with dietary potassium restriction and non-dietary potassium restriction. We searched PubMed, Embase, and the Cochrane Central Register of Controlled Trials from inception to June 12, 2023, using the terms "Dietary Potassium or Potassium, Dietary and dialysis, renal disease, or kidney failure." All studies titles and abstracts were examined by two independent reviewers. A third investigator was consulted in the event of a disagreement to enable consensus-building. Finally, seven studies were included for analysis (Figure 1).

Overview of studies on the relationship between dietary potassium intake and serum potassium and its effect on hemodialysis patients

(The basic characteristics and outcomes of the clinical researches included in our study are shown in Table 1).

Khedr et al. (36) conducted a study with 400 MHD patients to investigate the prevalence of hyperkalemia in hemodialysis patients in Egypt. The study found that the pre-dialysis serum potassium of patients in the potassium-rich diet group (5.691 ± 0.462 mEq/L) was higher than that in the average potassium diet group (4.941 ± 0.376 mEq/L) and the low-potassium diet group (4.309 ± 0.321 mEq/L) ($p < 0.01$), and the incidence of hyperkalemia in hemodialysis patients was higher in the potassium-rich diet group than in the average or low-potassium diet group ($p < 0.01$) (36).

Abbreviations: MHD, Maintenance hemodialysis; DOPPS, the Dialysis Outcomes and Practice Patterns Study; CKD, Chronic kidney disease; NIED, the Nutritional and Inflammatory Evaluation in Dialysis; ESRD, End-stage renal disease; HD, hemodialysis; NDD-CKD, the nondialysis-dependent CKD; DASH, the dietary approaches to stop hypertension; HPD, a healthy plant-based diet; HPDS, a higher healthy plant-based diet score; ACEI/ARB, Angiotensin converting enzyme inhibitor/Angiotensin receptor blocker; RAAS, the renin-angiotensin-aldosterone system; MRA, the mineralocorticoid receptor antagonist.

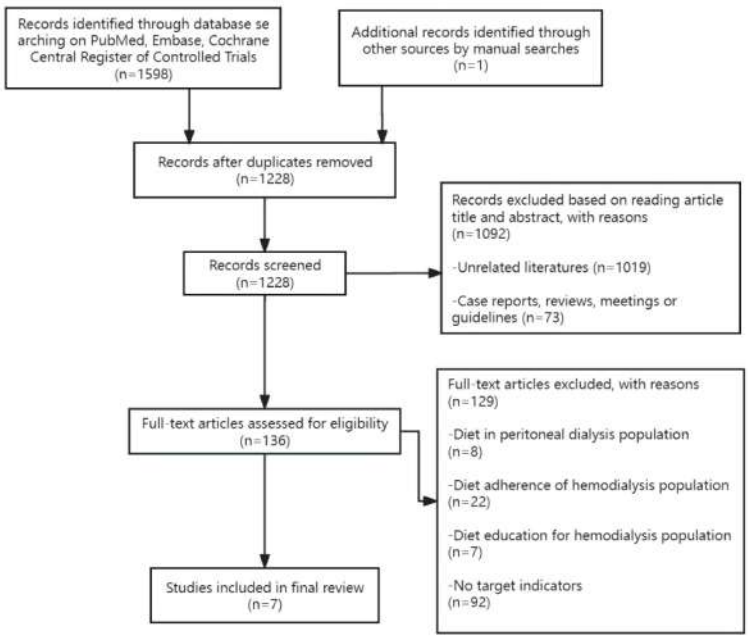

FIGURE 1
Study selection.

Similarly, in exploring the association between dietary potassium intake and mortality in hemodialysis patients, Noori et al. (37) observed a positive correlation between dietary potassium and predialysis serum levels (r=0.14, p<0.05), and the higher the potassium intake, the higher the risk of death.

However, in a study of dietary intake in 8043 adults with end-stage renal disease undergoing maintenance hemodialysis (DIET-HD) (38), dietary potassium intake was not associated with baseline serum potassium levels, dietary potassium was not associated with all-cause, cardiovascular, or noncardiovascular mortality, and consumption of fruits and vegetables was associated with a lower risk of all-cause death.

Likewise, Ramos et al. (39) investigated whether dietary potassium or specific food groups were linked with serum potassium in the presence of other risk factors, they discovered that potassium intake was directly related to fiber intake, as well as the intake of fruits, vegetables, and dairy products in the dialysis population's diet, with no association founded between dietary potassium and protein intake or beans, meat, and eggs. Furthermore, this study also revealed that dietary potassium did not correlate with serum potassium in either HD or nondialysis CKD patients (r=-0.06, P=0.46; r=0.01, P=0.98) (39).

Additionally, Garagarza et al. (40) conducted a multi-center, cross-sectional study to investigate the association between the dietary approaches to stop hypertension (DASH) dietary pattern, which is high in potassium-rich foods, and serum potassium in hemodialysis patients, including 582 patients from 37 dialysis centers. No statistically significant association was found between serum potassium and dietary potassium intake in this study (r=0.080; p=0.060), which was consistent with the conclusion of Ramos et al. (39). This study showed no association between serum potassium and dietary potassium intake in the low potassium intake (≤3000 mg/d) and high potassium intake(>3000 mg/d) groups, and no apparent differences in serum potassium value between the two groups (40). According to the study, serum potassium levels were favorably connected with milk, eggs, beef, pork, chicken liver, fatty fish, squid, octopus, banana, canned fruit, wine, and coffee (P<0.05, respectively) in terms of food types. Additionally, boiled potatoes, cow and pork meat, white cabbage, apples and pears, cherries, yogurt, oranges, beans, peaches, tomatoes, and milk had larger positive correlations (r≥0.300) with dietary potassium consumption (P<0.001, respectively) (40).

In the Malnutrition, Diet, and Racial Disparities in Chronic Kidney Disease (MADRAD) study (415 participants), a higher risk of death was shown to be connected with reduced dietary potassium consumption, which also suggested that severe dietary potassium restriction may be detrimental for hemodialysis patients (41). Furthermore, González-Ortiz et al. (42) also emphasized that in a healthy plant-based diet (HPD), a higher healthy plant-based diet score (HPDS) was not connected with serum potassium levels or hyperkalemia (potassium >5.5 mEq/L) in hemodialysis patients and a higher HPDS was associated with a lower malnutrition inflammation score (MIS), indicating improved nutritional status. They concluded that in their observational study challenged the routinely advice to avoid fruits and vegetables in dialysis patients and emphasize the significance of conducting interventional studies that investigate the potential benefits and harms of liberalizing the diet of dialysis patients in term of the consumption of plant foods (42).

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TABLE 1 Characteristics and the effects of dietary potassium intake on serum potassium of 7 clinical researches in hemodialysis patients.

Study/ year	Country	Study design	No. Patients	Study duration	Dietary potassium Intake	Serum Potassium	Outcome
Khedr.[36] 2009	Cairo, Egypt	Observational study	HD patients (n=400)	Not mentioned	The patients were classified into three groups: potassium-rich diet (n=214), low potassium diet (n=11), and average diet (n=129), but the specific intake was not mentioned.	Pre-dialysis serum potassium (low-potassium diet):4.309 ±0.321 mEq/L Pre-dialysis serum potassium (average diet): 4.941±0.376 mEq/L Pre-dialysis serum potassium (potassium-rich diet): 5.691±0.462 mEq/L	Patients on the potassium-rich diet had higher mean serum potassium at pre-dialysis, post-dialysis, and pre-next session than those on the average potassium diet and those on the low-potassium diet ($p < 0.01$).
Noori.[37] 2010	the South Bay Los Angeles, California, United States	Observational study	HD patients (n=224)	63 months follow-up	Quartiles of potassium intake: Quartile 1: 879 ±161 mg/d (n=56); Quartile 2: 1342 ±109 mg/d (n=56) ; Quartile 3: 1852 ±217 mg/d (n=56); Quartile 4: 3440 ±969 mg/ d (n=56)	Serum potassium (Quartile 1 of potassium intake): 5.1 ±0.5 mg/dl; Serum potassium (Quartile 2 of potassium intake): 5.0 ±0.5 mg/dl; Serum potassium (Quartile 3 of potassium intake): 5.1 ±0.5 mg/dl; Serum potassium (Quartile 4 of potassium intake): 5.2 ±0.5 mg/dl	potassium intake was positively correlated with the dietary energy, protein and phosphorus intake, and also marginally ($r=0.14$, $p<0.05$) with predialysis serum potassium.
Bernier-Jean.[38] 2021	Europe (France, Germany, Italy, Hungary, Poland, Portugal, Romania, Spain, Sweden, and Turkey) and South America (Argentina)	Prospective, multinational study	HD patients (n=8043)	4 years follow-up	Quartiles of potassium intake: Quartile 1: 1.84 g/ d (n=2011); Quartile 2: 3.00 g/ d (n=2011) Quartile 3: 4.19 g/ d (n=2010) Quartile 4: 7.18 g/ d (n=2011)	Serum potassium (Quartile 1 of potassium intake): 4.9 mEq/L; Serum potassium (Quartile 2 of potassium intake): 5.0 mEq/L; Serum potassium (Quartile 3 of potassium intake): 5.0 mEq/L; Serum potassium	1. Dietary potassium intake was not associated with allcause mortality (per 1 g/d higher dietary potassium intake: hazard ratio, 1.00; 95% CI, 0.95 to 1.05). 2. Potassium intake was not significantly associated with serum levels (0.03; 95% CI, -0.01 to 0.07 mEq/L per 1 g/d higher dietary potassium intake) or the prevalence of hyperkalemia (≥6.0 mEq/ L) at baseline (odds ratio, 1.11; 95% CI, 0.89 to 1.37 per 1 g/d higher dietary potassium intake). 3. Hyperkalemia was associated with cardiovascular death (hazard ratio, 1.23; 95% CI, 1.03 to 1.48). 3. Higher dietary potassium intake was associated with a lower risk of noncardiovascular mortality but not of all-cause or cardiovascular mortality. 4. The intake of fruitsand vegetables was

(Continued)

TABLE 1 Continued

Study/ year	Country	Study design	No. Patients	Study duration	Dietary potassium Intake	Serum Potassium	Outcome
						(Quartile 4 of potassium intake): 5.1 mEq/L;	associated with a lower risk of all-cause mortality.
Ramos.[39] 2021	Mexico City, Mexico	Cross-sectional study	HD patients (n=117)	Not mentioned	Potassium intake in the normokalemia group: 1.7 g/day (n=58); Potassium intake in the hyperkalemia group: 1.6 g/day (n=59);	Serum potassium in the normokalemia group: 4.3±0.5 mEq/L (n=58); Serum potassium in the hyperkalemia group: 5.8±0.6 mEq/L (n=59)	1. No association was found between serum potassium and potassium intake (r=-0.06, P=0.46). 2. Dietary potassium was not associated with serum potassium or hyperkalemia in HD patients.
Garagarza.[40] 2022	Portugal	Observational, cross-sectional, multicenter study	HD patients (n=582)	1 year follow-up	Mean dietary potassium intake: 2465±1005 mg/day	Mean serum potassium: 5.3 ± 0.67 mEq/L The lower potassium intake group (≤3000 mg/day): 5.2 ± 0.69 mEq/L(n = 418); The higher potassium intake group (>3000 mg/day): 5.4 ± 0.60 mEq/L(n = 126)	1. The potassium-rich DASH dietary pattern was not associated with elevated serum potassium levels in HD patients. 2. High adherence to the DASH dietary pattern predicted lower serum potassium levels (P=0.004).
Narasaki.[41] 2021	Southern California, United States	Prospective cohort study	HD patients (n=415) Tertile1 (dietary potassium intake of <903 mg/day, n=138) Tertile 2 (dietary potassium intake of 903-<1631 mg/day, n=138) Tertile 3 (dietary potassium intake of 1631- 7411 mg/day, n=139)	3 years follow-up	Tertile 1: 543±221 mg/day; Tertile 2: 1234 ±198 mg/day; Tertile 3: 2606 ±1166 mg/day	Tertile 1: 4.9 ±0.6 mEq/L; Tertile 2: 4.9 ±0.5 mEq/L; Tertile 3: 5.0 ±0.5 mEq/L	1. There was a monotonic increase in death risk with incrementally lower levels of dietary potassium intake. 2. No differences in serum potassium among groups with dietary potassium intake.
Gonza´lez-Ortiz.[42] 2021	Mexico City, Mexico	Observational single-centre cohort study	HD patients (n=150)	1 year follow-up	Median dietary potassium intake in the low HPD adherence group:	Median serum potassium in the low HPD adherence	1. There was no association between HPDS and serum potassium levels. 2. Higher HPD adherence was not associated with the odds of

(Continued)

TABLE 1 Continued

Study/ year	Country	Study design	No. Patients	Study duration	Dietary potassium Intake	Serum Potassium	Outcome
					993 mg/1000kcal/ day(n = 43); Median dietary potassium intake in the moderate HPD adherence group: 1039 mg/ 1000kcal/day(n = 53); Median dietary potassium intake in the high HPD adherence group: 1094 mg/ 1000kcal/day(n = 54)	group: 4.9 mmol/L(n = 43); Median serum potassium in the moderate HPD adherence group: 5.1 mmol/L(n = 53); Median serum potassium in the high HPD adherence group: 4.7 mmol/L(n = 54)	hyperkalaemia [OR 1.00 (95% CI 0.94– 1.07)].

HD, Hemodialysis; HPDS, healthy plant-based diet score; DASH, the dietary approaches to stop hypertension.

Discussion

The above several studies have no consistent conclusions on the correlation between dietary potassium intake and serum potassium. The reason for the absence of a consistent positive correlation between serum potassium and dietary potassium could be related to the type of potassium-containing food and its content in other nutrients (40). In the DIET-HD trial, Bernier-Jean et al. (38) found that higher potassium intake from only whole plant sources was linked with a lower mortality risk, and that this association vanished after controlling for dietary groups, such as consumption of fruits and vegetables. Noori et al. (37) observed a positive correlation between dietary potassium and pre-dialysis serum levels in hemodialysis patients, in this study, dietary potassium was mainly derived from beef, chicken, Mexican food, burgers, beans, fresh fruits, fruit juices, fried potatoes, cheeseburgers and canned fruit, whereas in the DIET-HD study, the majority of potassium sources were vegetables, fresh fruit, red meat, potatoes, milk, and bread. Garagarza et al. (40) also found no association in their study of dietary patterns of high-potassium foods (DASH) and serum potassium in hemodialysis patients, considering that although there is no positive correlation between serum potassium and dietary potassium, this does not imply that high-potassium foods do not cause hyperkalemia in patients, due to differences in potassium bioavailability, distinct sources of dietary potassium (animal, plant, and potassium-based food additives) could contribute to elevations in different ways. In this study, the DASH dietary pattern emphasized consumption of plant-based foods with low potassium bioavailability (43, 44), with the exception of bananas, canned fruit, wine, and coffee, foods positively associated with serum potassium levels are primarily animal sources (milk, beef, pork and chicken liver, fatty fish, squid, and octopus).

Besides, an article on plant-based diets by Carrero et al. published in Nature Reviews Nephrology stated that for patients with chronic kidney disease(CKD) (17), current evidence shows that encouraging the adoption of plant-based diets has few hazards and has potential benefits for the primary prevention of CKD, and delaying progression in patients with CKD G3-5. This article recommended that limiting plant-based foods as a strategy for preventing hyperkalemia or malnutrition be done on an individual basis to avoid depriving CKD patients of the possibly positive effects of a plant-based diet (17). According to a European survey, only 4% of 8,078 hemodialysis patients took in four or more servings of fruits and vegetables per day, which is the recommended quantity for the general population (45). Besides, increasing fruit and vegetable consumption is associated with a lower risk of all-cause mortality, and there is an inverse correlation between consumption and non-cardiovascular mortality, which could be related to a lower risk of cancer death in hemodialysis patients (45). In a study of 81,013 hemodialysis patients investigating the association between pre-dialysis serum potassium levels and all-cause and cardiovascular mortality, potassium concentrations between 4.6-5.3 mEq/L were related to the lowest all-cause mortality (9). After correcting for confounding variables related to comorbidities and nutritional status, potassium levels ≥5.6 mEq/L were most strongly associated with higher mortality, although potassium levels <4.0 mEq/L were associated with increased mortality when malnutrition owing to inadequate dietary intake was taken into account (9).

Other than that, nutritional interactions must be taken into account when controlling dietary potassium intake and serum potassium levels. Plant-based foods with high potassium content include melons, citrus juice, and potatoes, it should be noted, however, that some food sources with high potassium content also contain high carbohydrates, which could stimulate insulin

release and thus reduce the increase in plasma potassium concentration (6). Contrarily, animal products have higher potassium content but lower carbohydrate content, which may lead to higher plasma potassium levels after intake, therefore, not all potassium-rich foods are likely to result in similar increases in serum potassium concentrations (6). Garagarza et al. (40) noted that carbohydrate-rich and potassium-rich foods may have less effect on serum potassium than low-carbohydrate and high-potassium foods, because there are other nutrients in food, such as fiber, affect potassium distribution and excretion, and increased carbohydrate intake contributes to high fiber intake, which in turn causes potassium excretion (46). Besides, when increased potassium intake sufficiently increases plasma potassium concentrations, aldosterone also enhances potassium excretion in the distal colon (47), and adaptation of this function may be very crucial, especially when renal function is impaired (48). Hayes et al. (49) demonstrated that fecal potassium excretion was three times higher in hemodialysis compared with normal controls and could even reach 80% of dietary potassium in some cases, in addition to fecal potassium content being proportional to dietary potassium intake consumption and fecal weight. Since the digestive system is another route for excreting potassium (48, 50), it's crucial to consume enough fiber in the diet. For example, the DASH diet includes plenty of plant foods, which are high in fiber and can increase fecal excretion of potassium through stimulating intestinal peristalsis (46, 51, 52). On the other hand, considering the relatively high incidence of constipation in HD patients (53%) (53), infrequent bowel movements, rather than dietary potassium load, may be a major driver of hyperkalemia in HD patients (46). In a review of plant-based low-protein diets in the conservative management of patients with CKD, it was shown that meat consumption increases the production of nitride-containing end products, exacerbates uremia, and may lead to constipation due to inadequate fiber intake, thereby increasing the risk of hyperkalemia (54). In contrast, a plant-based diet may lower gut-derived uremic toxins by increasing intake of fiber and regulating the intestinal microbiota (55). In one study, increasing dietary fiber intake for 6 weeks in hemodialysis patients could reduce free plasma indoxyl sulfate (a uremic toxin produced by the breakdown of aromatic amino acids by intestinal microbiota) levels by 29% (56). On the other hand, a plant-based diet of wholesome fruits and vegetables reduces the likelihood of potassium-containing additives commonly found in meat products (57, 58). This dietary pattern also includes additional factors that may assist prevent serum potassium increases, such as a high consumption of alkaline foods (fruits and vegetables), which may enhance intracellular potassium transport, particularly in the context of metabolic acidosis (40).

Therefore, when evaluating the effect of diet on serum potassium, it is important to consider not only the potassium content of foods, but also the type of food and other nutrient content. Furthermore, excessive dietary potassium restriction may not be beneficial for survival in hemodialysis patients, and further research is required to explore the ideal dietary potassium intake for this population. Some studies have advocated for moderate potassium restriction in hemodialysis patients, or relaxation of potassium intake with the prescription of potassium binders (59, 60).

Analysis of other common influencing factors of predialysis serum potassium

Angiotensin converting enzyme inhibitor/Angiotensin receptor blocker (ACEI/ARB), Spironolactone, β-Receptor Blockers are common drugs that affect serum potassium fluctuations. Taking ACEI/ARB in patients with end-stage renal disease can not only cooperate with renin-angiotensin-aldosterone system (RAAS) related blood pressure increase, but also improve ventricular remodeling, which can effectively reduce the mortality of MHD patients by reducing blood pressure and reversing left ventricular hypertrophy (LVH) (61). ACEI/ARB drugs affect serum potassium levels by inhibiting aldosterone secretion, increasing prostaglandin or bradykinin synthesis, and decreasing potassium ion excretion. Knoll et al. (62) studied the risk of hyperkalemia in patients with renin-angiotensin system blockade and chronic hemodialysis, they found that the use of ACEIs/ARBs was significantly associated with an increased risk for hyperkalemia ($p < 0.05$). However, other clinical trials and meta-analysis evaluating RAAS blockers in hemodialysis populations have shown no difference in serum potassium or frequency of hyperkalemia between intervention and control groups (63–67). It has also been reported that the use of ACEIs/ARBs had no effect on hyperkalemia in MHD patients, where neither monotherapy (ACEIs or ARBs) nor combination therapy (ACEIs plus ARBs) was associated with an excess risk of hyperkalemia in MHD patients (68). Similarly, clinicians tend to worry that spironolactone would raise the risk of hyperkalemia in hemodialysis patients and limit its usage due to its "sodium excretion and potassium retention" (69, 70). The addition of low-dose of spironolactone or combined with conventional treatment is safe and will not significantly increase serum potassium levels, but more importantly, can improve LVH by lowering left ventricular mass index (LVMI) and raising left ventricular ejection fraction (LVEF), according to systematic reviews and meta-analyses of several studies analyzing the effects and safety of spironolactone on the cardiovascular system in the routine treatment of hemodialysis patients (71–76). In contrast to controls, spironolactone increased the frequency of moderate hyperkalemia (6.0-6.5 mmol/L), but not severe hyperkalemia (≥6.5 mmol/L), according to a study on the safety and efficacy of the drug in hemodialysis patients (77). With regards to β-receptor blockers, because nonselective β-receptor blockers (such as propranolol) can interfere with Na+-K+-ATPase on the cell membrane and prevent potassium ions from entering the cell, serum potassium levels rise (78). Some studies have demonstrated that β-blocker therapy could be used safely in hemodialysis patients, with severe hyperkalemia occurring in only a minority of patients (36, 79). In contrast, serum

potassium levels were observed to correlate with spironolactone, ACEIs, and β-blocker intake in a study by Muschart X et al. (80). Furthermore, insulin resistance, race, and gender variances can all have an impact on serum potassium levels (81–83). Among the studies included in this paper, Khedr et al. (36) found no significant association between serum potassium and ACEIs, β-blockers, or diabetes, and Ramos et al. (39) observed no notable distinction in serum potassium between the normokalemic and hyperkalemic groups in dialysis patients treated with β-blockers (P=0.79).

At present, there are still differing conclusions regarding whether aforementioned medications can affect the serum potassium level of hemodialysis patients. Since the renal potassium excretion function is nearly lost in MHD patients, dialysis is the primary means of controlling serum potassium excretion. Some researchers believe that hyperkalemia may not be a major concern in hemodialysis patients since dialysis, rather than renal tubular function, controls the factors affecting serum potassium in dialysis patients (84, 85). Thus, with the adequacy of dialysis, close monitoring of serum potassium, and timely adjustment of dialysate composition, whether taking ACEIs/ARBs, β-blockers, mineralocorticoid antagonists, or different residual renal function, may not have a significant effect on serum potassium levels in hemodialysis patients.

Conclusion

Based on the current small observational studies, we discovered that in cases where dialysis adequacy was ensured and specific dietary patterns (such as the above-mentioned plant-based diet) were followed, there was less or even no correlation between dietary potassium intake and serum potassium in hemodialysis patients; excessive dietary potassium restriction may not benefit the survival of hemodialysis patients. Additionally, when assessing the effect of diet on serum potassium, researchers should not only focus on the potassium content of foods, but also consider the type of food and the content of other nutrients. Meanwhile, we admit that publication bias may be present in this result attributed to the several positive small center series. Therefore, more large-scale, multi-center clinical trials are needed to provide high-quality evidence support, and we also need to further explore the dietary patterns and optimal daily dietary potassium intake that are beneficial to hemodialysis patients.

Author contributions

ZS: Methodology, Writing – original draft. JJ: Writing – original draft. GL: Methodology, Writing – review & editing. RL: Methodology, Writing – review & editing. ZL: Supervision, Writing – review & editing. YS: Supervision, Writing – review & editing. ZC: Supervision, Writing – review & editing.

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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. Collins AJ, Pitt B, Reaven N, Funk S, McGaughey K, Wilson D, et al. Association of Serum Potassium with All-Cause Mortality in Patients with and without Heart Failure, Chronic Kidney Disease, and/or Diabetes. *Am J Nephrol* (2017) 46(3):213–21. doi: 10.1159/000479802

2. Kovesdy CP, Rowan CG, Conrad A, Spiegel DM, Fogli J, Oestreicher N, et al. Real-world evaluation of patiromer for the treatment of hyperkalemia in hemodialysis patients. *Kidney Int Rep* (2019) 4(2):301–9. doi: 10.1016/j.ekir.2018.10.020

3. Montford JR, Linas S. How dangerous is hyperkalemia? *J Am Soc Nephrol* (2017) 28(11):3155–65. doi: 10.1681/ASN.2016121344

4. Yusuf AA, Hu Y, Singh B, Menoyo JA, Wetmore JB. Serum potassium levels and mortality in hemodialysis patients: A retrospective cohort study. *Am J Nephrol* (2016) 44(3):179–86. doi: 10.1159/000448341

5. Pun PH. Dialysate potassium concentration: Should mass balance trump electrophysiology? *Semin Dialysis* (2018) 31(6):569–75. doi: 10.1111/sdi.12738

6. Palmer BF, Clegg DJ. Physiology and pathophysiology of potassium homeostasis: core curriculum 2019. *Am J Kidney Dis* (2019) 74(5):682–95. doi: 10.1053/j.ajkd.2019.03.427

7. Sanghavi S, Whiting S, Uribarri J. Potassium balance in dialysis patients. *Semin Dialysis* (2013) 26(5):597–603. doi: 10.1111/sdi.12123

8. Karaboyas A, Zee J, Brunelli SM, Usvyat LA, Weiner DE, Maddux FW, et al. Dialysate potassium, serum potassium, mortality, and arrhythmia events in hemodialysis: results from the dialysis outcomes and practice patterns study (DOPPS). *Am J Kidney Dis* (2017) 69(2):266–77. doi: 10.1053/j.ajkd.2016.09.015

9. Kovesdy CP, Regidor DL, Mehrotra R, Jing J, McAllister CJ, Greenland S, et al. Serum and dialysate potassium concentrations and survival in hemodialysis patients. *Clin J Am Soc Nephrol* (2007) 2(5):999–1007. doi: 10.2215/CJN.04451206

10. Palmer SC, Ruospo M, Campbell KL, Garcia Larsen V, Saglimbene V, Natale P, et al. Nutrition and dietary intake and their association with mortality and hospitalisation in adults with chronic kidney disease treated with haemodialysis:

protocol for DIET-HD, a prospective multinational cohort study. *BMJ Open* (2015) 5 (3):e006897. doi: 10.1136/bmjopen-2014-006897

11. Morris A, Krishnan N, Kimani PK, Lycett D. Effect of dietary potassium restriction on serum potassium, disease progression, and mortality in chronic kidney disease: A systematic review and meta-analysis. *J Renal Nutr* (2020) 30(4):276–85. doi: 10.1053/j.jrn.2019.09.009

12. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis Off J Natl Kidney Foundation* (2020) 76(3 Suppl 1):S1–s107. doi: 10.1053/j.ajkd.2020.05.006

13. Kovesdy CP, Appel LJ, Grams ME, Gutekunst L, McCullough PA, Palmer BF, et al. Potassium homeostasis in health and disease: A scientific workshop cosponsored by the national kidney foundation and the american society of hypertension. *Am J Kidney Dis* (2017) 70(6):844–58. doi: 10.1053/j.ajkd.2017.09.003

14. Cupisti A, Kovesdy CP, D'Alessandro C, Kalantar-Zadeh K. Dietary approach to recurrent or chronic hyperkalaemia in patients with decreased kidney function. *Nutrients* (2018) 10(3):261. doi: 10.3390/nu10030261

15. James G, Jackson H. European guidelines for the nutritional care of adult renal patients. *EDTNA-ERCA J* (2003) 29(1):23–43. doi: 10.1111/j.1755-6686.2003.tb00270.x

16. Ash S, Campbell K, MacLaughlin H, McCoy E, Chan M, Anderson K, et al. Evidence based practice guidelines for the nutritional management of chronic kidney disease. *Nutr Dietetics* (2006) 63(s2):S33–45. doi: 10.1111/j.1747-0080.2006.00100.x

17. Carrero JJ, González-Ortiz A, Avesani CM, Bakker SJL, Bellizzi V, Chauveau P, et al. Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat Rev Nephrology.* (2020) 16(9):525–42. doi: 10.1038/s41581-020-0297-2

18. Clase CM, Carrero JJ, Ellison DH, Grams ME, Hemmelgarn BR, Jardine MJ, et al. Potassium homeostasis and management of dyskalemia in kidney diseases: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* (2020) 97(1):42–61. doi: 10.1016/j.kint.2019.09.018

19. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *Jama* (1997) 277(20):1624–32. doi: 10.1001/jama.1997.03540440058033

20. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. *J Hum hypertension.* (2003) 17(7):471–80. doi: 10.1038/sj.jhh.1001575

21. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. *BMJ (Clinical Res ed)* (2013) 346:f1326. doi: 10.1136/bmj.f1326

22. Filippini T, Violi F, D'Amico R, Vinceti M. The effect of potassium supplementation on blood pressure in hypertensive subjects: A systematic review and meta-analysis. *Int J Cardiol* (2017) 230:127–35. doi: 10.1016/j.ijcard.2016.12.048

23. Poorolajal J, Zeraati F, Soltanian AR, Sheikh V, Hooshmand E, Maleki A. Oral potassium supplementation for management of essential hypertension: A meta-analysis of randomized controlled trials. *PloS One* (2017) 12(4):e0174967. doi: 10.1371/journal.pone.0174967

24. Vinceti M, Filippini T, Crippa A, de Sesmaisons A, Wise LA, Orsini N. Meta-analysis of potassium intake and the risk of stroke. *J Am Heart Assoc* (2016) 5(10): e004210. doi: 10.1161/JAHA.116.004210

25. Carey RM, Muntner P, Bosworth HB, Whelton PK. Prevention and control of hypertension: JACC health promotion series. *J Am Coll Cardiol* (2018) 72(11):1278–93. doi: 10.1016/j.jacc.2018.07.008

26. Wei KY, Gritter M, Vogt L, de Borst MH, Rotmans JI, Hoorn EJ. Dietary potassium and the kidney: lifesaving physiology. *Clin Kidney J* (2020) 13(6):952–68. doi: 10.1093/ckj/sfaa157

27. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. *New Engl J Med* (2014) 371(7):601–11. doi: 10.1056/NEJMoa1311989

28. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. *BMJ (Clinical Res ed)* (2013) 346:f1378. doi: 10.1136/bmj.f1378

29. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. *New Engl J Med* (2014) 371(7):612–23. doi: 10.1056/NEJMoa1311889

30. Mente A, O'Donnell M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, et al. Urinary sodium excretion, blood pressure, cardiovascular disease, and mortality: a community-level prospective epidemiological cohort study. *Lancet (London England)* (2018) 392(10146):496–506. doi: 10.1016/S0140-6736(18)31376-X

31. Gritter M, Rotmans JI, Hoorn EJ. Role of dietary K(+) in natriuresis, blood pressure reduction, cardiovascular protection, and renoprotection. *Hypertension (Dallas Tex 1979)* (2019) 73(1):15–23. doi: 10.1161/HYPERTENSIONAHA.118.11209

32. Picard K, Griffiths M, Mager DR, Richard C. Handouts for low-potassium diets disproportionately restrict fruits and vegetables. *J Renal Nutr* (2021) 31(2):210–4. doi: 10.1053/j.jrn.2020.07.001

33. St-Jules DE, Woolf K, Pompeii ML, Sevick MA. Exploring problems in following the hemodialysis diet and their relation to energy and nutrient intakes: the balanceWise study. *J Renal Nutr* (2016) 26(2):118–24. doi: 10.1053/j.jrn.2015.10.002

34. Palmer SC, Hanson CS, Craig JC, Strippoli GF, Ruospo M, Campbell K, et al. Dietary and fluid restrictions in CKD: a thematic synthesis of patient views from qualitative studies. *Am J Kidney Dis* (2015) 65(4):559–73. doi: 10.1053/j.ajkd.2014.09.012

35. Kalantar-Zadeh K, Tortorici AR, Chen JL, Kamgar M, Lau WL, Moradi H, et al. Dietary restrictions in dialysis patients: is there anything left to eat? *Semin Dialysis* (2015) 28(2):159–68. doi: 10.1111/sdi.12348

36. Khedr E, Abdelwhab S, El-Sharkawy M, Ali M, El Said K. Prevalence of hyperkalemia among hemodialysis patients in Egypt. *Renal Failure* (2009) 31 (10):891–8. doi: 10.3109/08860220903216097

37. Noori N, Kalantar-Zadeh K, Kovesdy CP, Murali SB, Bross R, Nissenson AR, et al. Dietary potassium intake and mortality in long-term hemodialysis patients. *Am J Kidney Dis* (2010) 56(2):338–47. doi: 10.1053/j.ajkd.2010.03.022

38. Bernier-Jean A, Wong G, Saglimbene V, Ruospo M, Palmer SC, Natale P, et al. Dietary potassium intake and all-cause mortality in adults treated with hemodialysis. *Clin J Am Soc Nephrol* (2021) 16(12):1851–61. doi: 10.2215/CJN.08360621

39. Ramos CI, González-Ortiz A, Espinosa-Cuevas A, Avesani CM, Carrero JJ, Cuppari L. Does dietary potassium intake associate with hyperkalemia in patients with chronic kidney disease? *Nephrology Dialysis Transplant* (2021) 36(11):2049–57. doi: 10.1093/ndt/gfaa232

40. Garagarza C, Valente A, Caetano C, Ramos I, Sebastião J, Pinto M, et al. Potassium intake-(Un)Expected non-predictor of higher serum potassium levels in hemodialysis DASH diet consumers. *Nutrients* (2022) 14(10):2071. doi: 10.3390/nu14102071

41. Narasaki Y, Okuda Y, Kalantar SS, You AS, Novoa A, Nguyen T, et al. Dietary potassium intake and mortality in a prospective hemodialysis cohort. *J Renal Nutr* (2021) 31(4):411–20. doi: 10.1053/j.jrn.2020.05.008

42. González-Ortiz A, Xu H, Ramos-Acevedo S, Avesani CM, Lindholm B, Correa-Rotter R, et al. Nutritional status, hyperkalaemia and attainment of energy/protein intake targets in haemodialysis patients following plant-based diets: a longitudinal cohort study. *Nephrology dialysis Transplant* (2021) 36(4):681–8. doi: 10.1093/ndt/gfaa194

43. Naismith DJ, Braschi A. An investigation into the bioaccessibility of potassium in unprocessed fruits and vegetables. *Int J Food Sci Nutr* (2008) 59(5):438–50. doi: 10.1080/09637480701690519

44. Melse-Boonstra A. Bioavailability of micronutrients from nutrient-dense whole foods: zooming in on dairy, vegetables, and fruits. *Front Nutr* (2020) 7:101. doi: 10.3389/fnut.2020.00101

45. Saglimbene VM, Wong G, Ruospo M, Palmer SC, Garcia-Larsen V, Natale P, et al. Fruit and vegetable intake and mortality in adults undergoing maintenance hemodialysis. *Clin J Am Soc Nephrol* (2019) 14(2):250–60. doi: 10.2215/CJN.08580718

46. St-Jules DE, Goldfarb DS, Sevick MA. Nutrient non-equivalence: does restricting high-potassium plant foods help to prevent hyperkalemia in hemodialysis patients? *J Renal Nutr* (2016) 26(5):282–7. doi: 10.1053/j.jrn.2016.02.005

47. Giebisch G, Krapf R, Wagner C. Renal and extrarenal regulation of potassium. *Kidney Int* (2007) 72(4):397–410. doi: 10.1038/sj.ki.5002288

48. Epstein M, Lifschitz MD. The unappreciated role of extrarenal and gut sensors in modulating renal potassium handling: implications for diagnosis of dyskalemias and interpreting clinical trials. *Kidney Int Rep* (2016) 1(1):43–56. doi: 10.1016/j.ekir.2016.03.001

49. Hayes CP Jr., McLeod ME, Robinson RR. An extravenal mechanism for the maintenance of potassium balance in severe chronic renal failure. *Trans Assoc Am Physicians.* (1967) 80:207–16.

50. Batlle D, Boobés K, Manjee KG. The colon as the potassium target: entering the colonic age of hyperkalemia treatment? *EBioMedicine* (2015) 2(11):1562–3. doi: 10.1016/j.ebiom.2015.10.027

51. Cases A, Cigarrán-Guldrís S, Mas S, Gonzalez-Parra E. Vegetable-based diets for chronic kidney disease? *It is time to reconsider. Nutrients* (2019) 11(6):1263. doi: 10.3390/nu11061263

52. Goraya N, Simoni J, Jo CH, Wesson DE. A comparison of treating metabolic acidosis in CKD stage 4 hypertensive kidney disease with fruits and vegetables or sodium bicarbonate. *Clin J Am Soc Nephrol CJASN* (2013) 8(3):371–81. doi: 10.2215/CJN.02430312

53. Murtagh FE, Addington-Hall J, Higginson IJ. The prevalence of symptoms in end-stage renal disease: a systematic review. *Adv chronic Kidney Dis* (2007) 14(1):82–99. doi: 10.1053/j.ackd.2006.10.001

54. Kalantar-Zadeh K, Joshi S, Schlueter R, Cooke J, Brown-Tortorici A, Donnelly M, et al. Plant-dominant low-protein diet for conservative management of chronic kidney disease. *Nutrients* (2020) 12(7):1931. doi: 10.3390/nu12071931

55. Sakaguchi Y, Kaimori JY, Isaka Y. Plant-dominant low protein diet: A potential alternative dietary practice for patients with chronic kidney disease. *Nutrients* (2023) 15 (4):1002. doi: 10.3390/nu15041002

56. Sirich TL, Plummer NS, Gardner CD, Hostetter TH, Meyer TW. Effect of increasing dietary fiber on plasma levels of colon-derived solutes in hemodialysis patients. *Clin J Am Soc Nephrol* (2014) 9(9):1603–10. doi: 10.2215/CJN.00490114

57. Parpia AS, L'Abbé M, Goldstein M, Arcand J, Magnuson B, Darling PB. The impact of additives on the phosphorus, potassium, and sodium content of commonly

consumed meat, poultry, and fish products among patients with chronic kidney disease. *J Renal Nutr* (2018) 28(2):83–90. doi: 10.1053/j.jrn.2017.08.013

58. Picard K. Potassium additives and bioavailability: are we missing something in hyperkalemia management? *J Renal Nutr* (2019) 29(4):350–3. doi: 10.1053/j.jrn.2018.10.003

59. Packham DK, Rasmussen HS, Lavin PT, El-Shahawy MA, Roger SD, Block G, et al. Sodium zirconium cyclosilicate in hyperkalemia. *New Engl J Med* (2015) 372 (3):222–31. doi: 10.1056/NEJMoa1411487

60. Weir MR, Bakris GL, Bushinsky DA, Mayo MR, Garza D, Stasiv Y, et al. Patiromer in patients with kidney disease and hyperkalemia receiving RAAS inhibitors. *New Engl J Med* (2015) 372(3):211–21. doi: 10.1056/NEJMoa1410853

61. Efrati S, Zaidenstein R, Dishy V, Beberashvili I, Sharist M, Averbukh Z, et al. ACE inhibitors and survival of hemodialysis patients. *Am J Kidney Dis* (2002) 40 (5):1023–9. doi: 10.1053/ajkd.2002.36340

62. Knoll GA, Sahgal A, Nair RC, Graham J, van Walraven C, Burns KD. Renin-angiotensin system blockade and the risk of hyperkalemia in chronic hemodialysis patients. *Am J Med* (2002) 112(2):110–4. doi: 10.1016/S0002-9343(01)01068-3

63. Zannad F, Kessler M, Lehert P, Grünfeld JP, Thuilliez C, Leizorovicz A, et al. Prevention of cardiovascular events in end-stage renal disease: results of a randomized trial of fosinopril and implications for future studies. *Kidney Int* (2006) 70(7):1318–24. doi: 10.1038/sj.ki.5001657

64. Takahashi A, Takase H, Toriyama T, Sugiura T, Kurita Y, Ueda R, et al. Candesartan, an angiotensin II type-1 receptor blocker, reduces cardiovascular events in patients on chronic haemodialysis–a randomized study. *Nephrol Dialysis Transpl* (2006) 21(9):2507–12. doi: 10.1093/ndt/gfl293

65. Suzuki H, Kanno Y, Sugahara S, Ikeda N, Shoda J, Takenaka T, et al. Effect of angiotensin receptor blockers on cardiovascular events in patients undergoing hemodialysis: an open-label randomized controlled trial. *Am J Kidney Dis* (2008) 52 (3):501–6. doi: 10.1053/j.ajkd.2008.04.031

66. Liu Y, Ma X, Zheng J, Jia J, Yan T. Effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers on cardiovascular events and residual renal function in dialysis patients: a meta-analysis of randomised controlled trials. *BMC Nephrol* (2017) 18(1):206. doi: 10.1186/s12882-017-0605-7

67. Denker MG, Cohen DL. Antihypertensive medications in end-stage renal disease. *Semin Dialysis* (2015) 28(4):330–6. doi: 10.1111/sdi.12369

68. Han SW, Won YW, Yi JH, Kim HJ. No impact of hyperkalaemia with renin-angiotensin system blockades in maintenance haemodialysis patients. *Nephrology dialysis Transplant* (2007) 22(4):1150–5. doi: 10.1093/ndt/gfl752

69. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). *Rales Investigators. Circulation* (2000) 102(22):2700–6. doi: 10.1161/01.cir.102.22.2700

70. Hayashi M, Tsutamoto T, Wada A, Tsutsui T, Ishii C, Ohno K, et al. Immediate administration of mineralocorticoid receptor antagonist spironolactone prevents post-infarct left ventricular remodeling associated with suppression of a marker of myocardial collagen synthesis in patients with first anterior acute myocardial infarction. *Circulation* (2003) 107(20):2559–65. doi: 10.1161/01.CIR.0000068340.96506.0F

71. Rossignol P, Frimat L, Zannad F. The safety of mineralocorticoid antagonists in maintenance hemodialysis patients: two steps forward. *Kidney Int* (2019) 95(4):747–9. doi: 10.1016/j.kint.2018.12.006

72. Bhinder J, Patibandla S, Gupta CA, Levine A, Gass AL, Lanier GM. Mineralocorticoid receptor antagonist use in heart failure with reduced ejection fraction and end-stage renal disease patients on dialysis: A literature review. *Cardiol Rev* (2020) 28(3):107–15. doi: 10.1097/CRD.0000000000000286

73. Zeng Q, Zhou X, Xu G. Safety evaluation and cardiovascular effect of additional use of spironolactone in hemodialysis patients: a meta-analysis. *Drug design Dev Ther* (2019) 13:1487–99. doi: 10.2147/DDDT.S189454

74. Chen KT, Kang YN, Lin YC, Tsai IL, Chang WC, Fang TC, et al. Efficacy and safety of mineralocorticoid receptor antagonists in kidney failure patients treated with dialysis: A systematic review and meta-analysis. *Clin J Am Soc Nephrol* (2021) 16 (6):916–25. doi: 10.2215/CJN.15841020

75. Zhu Y, Liu Y, Cai R, Zheng D, Liang X, Tao M, et al. The safety and efficacy of low-dose mineralocorticoid receptor antagonists in dialysis patients: A meta-analysis. *Medicine* (2021) 100(8):e24882. doi: 10.1097/MD.0000000000024882

76. Sun Z, Chen Z, Liu R, Lu G, Li Z, Sun Y. Research progress on the efficacy and safety of spironolactone in reversing left ventricular hypertrophy in hemodialysis patients. *Drug Design Dev Ther* (2023) 17:181–90. doi: 10.2147/DDDT.S393480

77. Hammer F, Malzahn U, Donhauser J, Betz C, Schneider MP, Grupp C, et al. A randomized controlled trial of the effect of spironolactone on left ventricular mass in hemodialysis patients. *Kidney Int* (2019) 95(4):983–91. doi: 10.1016/j.kint.2018.11.025

78. Nowicki M, Miszczak-Kuban J. Nonselective Beta-adrenergic blockade augments fasting hyperkalemia in hemodialysis patients. *Nephron* (2002) 91(2):222–7. doi: 10.1159/000058396

79. Dunlay RW, Frock JT. Beta blockers and serum potassium levels in hemodialysis patients. *Am J Hypertension* (1999) 12(4, Supplement 1):134. doi: 10.1016/S0895-7061 (99)80473-7

80. Muschart X, Boulouffe C, Jamart J, Nougon G, Gérard V, de Cannière L, et al. A determination of the current causes of hyperkalaemia and whether they have changed over the past 25 years. *Acta Clinica Belgica* (2014) 69(4):280–4. doi: 10.1179/0001551214Z.00000000077

81. He Q, Heo M, Heshka S, Wang J, Pierson RN Jr., Albu J, et al. Total body potassium differs by sex and race across the adult age span. *Am J Clin Nutr* (2003) 78 (1):72–7. doi: 10.1093/ajcn/78.1.72

82. Nakhoul GN, Huang H, Arrigain S, Jolly SE, Schold JD, Nally JV Jr., et al. Serum potassium, end-stage renal disease and mortality in chronic kidney disease. *Am J Nephrol* (2015) 41(6):456–63. doi: 10.1159/000437151

83. Kim HW, Lee DH, Lee SA, Koh G. A relationship between serum potassium concentration and insulin resistance in patients with type 2 diabetes mellitus. *Int Urol Nephrol* (2015) 47(6):991–9. doi: 10.1007/s11255-015-1001-5

84. Lin C, Zhang Q, Zhang H, Lin A. Long-term effects of low-dose spironolactone on chronic dialysis patients: A randomized placebo-controlled study. *J Clin Hypertension (Greenwich Conn)* (2016) 18(2):121–8. doi: 10.1111/jch.12628

85. Chua D, Lo A, Lo C. Spironolactone use in heart failure patients with end-stage renal disease on hemodialysis: is it safe? *Clin Cardiol* (2010) 33(10):604–8. doi: 10.1002/clc.20838

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Gloria Formoso,
University of Studies G. d'Annunzio Chieti and Pescara, Italy
Miles Douglas Thompson,
University Health Network (UHN), Canada

*CORRESPONDENCE
Qin Wan
wanqin360@swmu.edu.cn

†These authors have contributed equally to this work and share first authorship

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Correlation of renal function indicators and vascular damage in T2DM patients with normal renal function

Yue-Yang Zhang[1,2,3,4,5†], Jing Gui[1,2,3,4,5†], Bing-Xue Chen[6] and Qin Wan[1,2,3,4,5*]

[1]Department of Endocrinology and Metabolism, the Affiliated Hospital of Southwest Medical University, Luzhou, China, [2]Metabolic Vascular Disease Key Laboratory of Sichuan Province, Luzhou, China, [3]Sichuan Clinical Research Center for Diabetes and Metabolism, Luzhou, China, [4]Sichuan Clinical Research Center for Nephropathy, Luzhou, China, [5]Cardiovascular and Metabolic Diseases Key Laboratory of Luzhou, Luzhou, China, [6]Department of Medical Imaging, Southwest Medical University, Luzhou, China

Background: This study aimed to assess the correlation between renal function-related indices and vascular damages among patients with type 2 diabetes mellitus (T2DM) and normal renal function.

Methods: We screened a cohort of eligible patients with T2DM, ultimately including 826 individuals. Utilizing multifactorial logistic regression, we conducted an in-depth analysis to explore the potential associations between renal function-related indices—specifically BUN, Cr, ALB, ACR, and eGFR—and the incidence of diabetic vascular damage. Additionally, to comprehensively understand the relationships, we employed Spearman correlation analysis to assess the connections between these indicators and the occurrence of vascular damage.

Results: In this cross-sectional study of 532 patients with carotid atherosclerosis (CA), the prevalence of CA was positively correlated with Cr (53.1%, 72.3%, 68.0%, P<0.05) and negatively correlated with eGFR (71.6%, 68.5%, 53.1%, P<0.05). the higher the Cr, the higher the predominance ratio of CA (T1: reference; T2:OR. 2.166,95%CI:1.454,3.225; T3:OR:1.677, 95%CI:1.075, 2.616; P<0.05), along with an eGFR of 66.9% and 52.0% in terms of sensitivity and specificity, with a 95% CI of 0.562-0.644.

Conclusion: Within our experimental sample, a noteworthy observation emerged: Creatinine (Cr) exhibited a positive correlation with the prevalence of individuals affected by carotid atherosclerosis (CA), underscoring a potential connection between Cr levels and CA incidence. Conversely, the estimated Glomerular Filtration Rate (eGFR) demonstrated a negative correlation with the occurrence of CA, implying that lower eGFR values might be associated with an increased likelihood of CA development.

KEYWORDS

type 2 diabetes mellitus, carotid atherosclerosis, renal function, eGFR, Cr

Introduction

Diabetes mellitus, a chronic and systemic metabolic disorder, stems from the intricate interplay of genetic and environmental factors over extended periods (1). Among the crucial disorders of the endocrine system, it stands as one of the most prevalent and significant (2). Statistics extracted from the 2020 Report on Nutrition and Chronic Disease Status of Chinese Residents underscore its prominence, revealing a diabetes prevalence rate of 11.9 percent among Chinese adults aged 18 and above, alongside a pre-diabetes detection rate of 35.2 percent. Notably, Type 2 diabetes exerts its most substantial impact on individuals aged 50 and above. This disease exhibits a disquieting trend toward earlier onset, prolonged duration, increased complications, amplified health risks, and augmented medical expenditures (3). The "IDF Diabetes Atlas (10th Edition)," published in 2021, forecasts that in 2021, approximately 537 million adults (aged 20-79 years) worldwide will grapple with diabetes—constituting 1 in 10 adults. This unsettling figure is anticipated to swell to 643 million by 2030 and further burgeon to 783 million by 2045. Simultaneously, as the global population is projected to grow by 20 percent within the same span, the estimated diabetes count is set to surge by 46 percent (4). These projections portend a future where more individuals are burdened by diabetes (5). Given that diabetes-associated vascular damage stands as the primary cause of mortality among T2DM patients, the imperative for enhanced predictive methodologies for these complications cannot be overstated (6).

Traditionally, risk factors linked to atherosclerosis encompass age, male gender, smoking, dyslipidemia, hypertension, and diabetes (7). Remarkably, individuals with chronic kidney disease demonstrate an elevated prevalence of atherosclerosis, a phenomenon subject to various academic propositions. These notions range from escalated oxidative stress and compromised endothelial function (8), heightened arterial rigidity (9), and compromised renal hemodynamics (10–12), to unfavorable conditions fostering plaque development and rupture (13, 14). Numerous investigations have established connections between glomerular filtration rate, serum creatinine levels, and atherosclerosis in otherwise healthy subjects before atherosclerotic onset. Intriguingly, however, no study to date has successfully identified parallels between renal function-related markers and atherosclerosis in T2DM patients with intact renal function (15).

Hence, the primary objective of this study was to meticulously assess the potential correlations between BUN, Cr, ALB, ACR, eGFR, and the occurrence of CA in patients diagnosed with T2DM, all while maintaining normal renal function.

Methods

Study subjects

The Division of Endocrinology and Metabolism at the Affiliated Hospital of Southwest Medical University undertook a retrospective cross-sectional investigation involving hospitalized individuals with type 2 diabetes, spanning the years 2017 to 2023. Inclusion criteria encompassed (1): Age exceeding 18 years; (2) Diagnosis by the American Diabetes Association's "Standards of Medical Care in Diabetes" (2019 version) (16); (3) Normal renal function defined by specific parameters: BUN within the range of 2.9-7.5 mmol/L, Cr ranging from 44-133 μmmol/L, Urine Albumin (ALB) levels below 20 mg/L, albumin-to-creatinine ratio (ACR) under 30 mg/g, and eGFR exceeding 90 mL/min/1.73m^2. On the other hand, exclusion criteria encompassed: (1) Aberrant results in kidney function tests; (2) History of kidney disease or kidney-related surgeries; (3) Usage of kidney function-impacting medications like cyclosporine.

Data acquisition for this study was meticulously executed by healthcare professionals, employing standard questionnaires and validated equipment. Each participant furnished demographic information alongside comprehensive medical records. The categorization of participants included identifying smokers and non-smokers, as well as distinguishing current drinkers from non-drinkers. During physical examinations, mean arterial blood pressure was continuously monitored for at least 30 minutes, with three consecutive measurements taken from the right arm arterial pressure.

In the realm of ethical considerations, this study strictly adhered to the principles outlined in the Helsinki Declaration of 2013 and obtained approval from the Ethics Committee of the Affiliated Hospital of Southwest Medical University (Ethics Approval Code: 2018017) (17). Informed consent was procured from all enrolled participants.

Calculation of eGFR

$$eGFR = 170 \times (Cr)^{-1.234} \times (Age)^{-0.179} \times 0.79(\text{if female})$$

Diagnosis of vascular damage in diabetes mellitus

Carotid artery intima-media thickness (CA-IMT) measurement has established itself as a secure and replicable technique to assess atherosclerosis severity. With guidance from ultrasound physicians, the bilateral carotid arteries of all participants underwent scanning using an identical color Doppler ultrasonic diagnostic apparatus. Throughout the data collection phase, a single operator carried out the measurements. The criterion for identifying atherosclerotic plaques hinged on a local carotid artery intima-media thickness (CIMT) ≥ 1.5 mm or a local CIMT surpassing 50% of the outer surface area. A diagnosis of carotid atherosclerosis was predicated on a CIMT increase ≥ 1.0 mm and/or the presence of carotid artery plaques (18–21).

Statistical analysis

Baseline clinical characteristics of CA patients with different genders were compared using descriptive statistics. For group

comparisons, one-way analysis of variance (ANOVA) was employed for normally distributed continuous variables, the Kruskal-Wallis H test for non-normally distributed continuous variables, and the Chi-square test (X^2 test) for categorical variables. The assessment of variables impacting diabetes vascular damages was accomplished using a Logistic regression analysis model, we additionally adjusted for age, sex, FBG, HbA1c, BMI, duration of diabetes, history of smoking, history of drinking, history of hypoglycemic drug usage, and history of insulin usage. To establish the relationship between renal function indicators and vascular damage, Spearman correlation analysis was employed. The predictive accuracy of renal function-related indicators for vascular damages was evaluated through Receiver Operating Characteristic (ROC) curves. All hypotheses were tested at a two-tailed significance level of 0.05. Risk factors were evaluated based on their odds ratio (OR) values. Forest plots were constructed using GraphPad Prism (version 9.0). The entire data analysis was conducted utilizing SPSS (version 26.0).

Results

Figure 1 shows a summary chart that visualizes the summary information of this article. A total of 826 T2DM patients participated in this study, comprising 440 males and 386 females. Males had an average age of 53.61 ± 10.35 years, while females averaged 58.33 ± 10.19 years. The mean duration of diabetes was 78.93 ± 76.73 months, and the study documented a total of 532 cases of cardiovascular damage. A comprehensive presentation of participants' demographic and biochemical information is detailed in Table 1. Comparative analysis revealed noteworthy distinctions between males and females. Specifically, males exhibited significantly higher values in height, weight, body mass index (BMI), waist circumference (WC), diastolic blood pressure (DBP), triglycerides (TG), fasting blood glucose (FBG), alanine transaminase (ALT), blood urea nitrogen (BUN), serum creatinine (Cr), urine microalbumin (ALB), current smoking rate, and current drinking rate (all with $P < 0.05$). Conversely, females displayed significantly higher mean values in average age, systolic blood pressure (SBP), high-density lipoprotein cholesterol (HDL), urine albumin-to-creatinine ratio (ACR), and estimated glomerular filtration rate (eGFR) (all with $P < 0.05$).

Table 2 illustrates the distribution of CA across the tertiles of BUN, Cr, ALB, ACR, and eGFR. A direct correlation was identified between the prevalence of CA and Cr(53.1%,72.3%,68.0%, P<0.05) and BUN(59.7%,65.5%,68.1%, P<0.05)levels, whereas an inverse relationship was observed with eGFR(71.6%, 68.5%,53.1%, P<0.05). Notably, the prevalence of CA remained nearly consistent across all tertiles of ALB, and ACR.

Odds ratios for CA were computed through a multivariate regression model, as depicted in Table 3. In Model 2, after gender adjustment, higher Cr levels were linked with amplified odds ratios for CA(T1: reference; T2:OR:2.158,95%CI: 1.473, 3.162; T3: or:1.696, 95%CI: 1.122, 2.565; P<0.05). This pattern persisted predominantly in Model 3, even after considering the influence of other potentially confounding variables (T1:reference; T2: OR:2.166,95%CI:1.454,3.225; T3:OR:1.677,95%CI:1.075, 2.616;

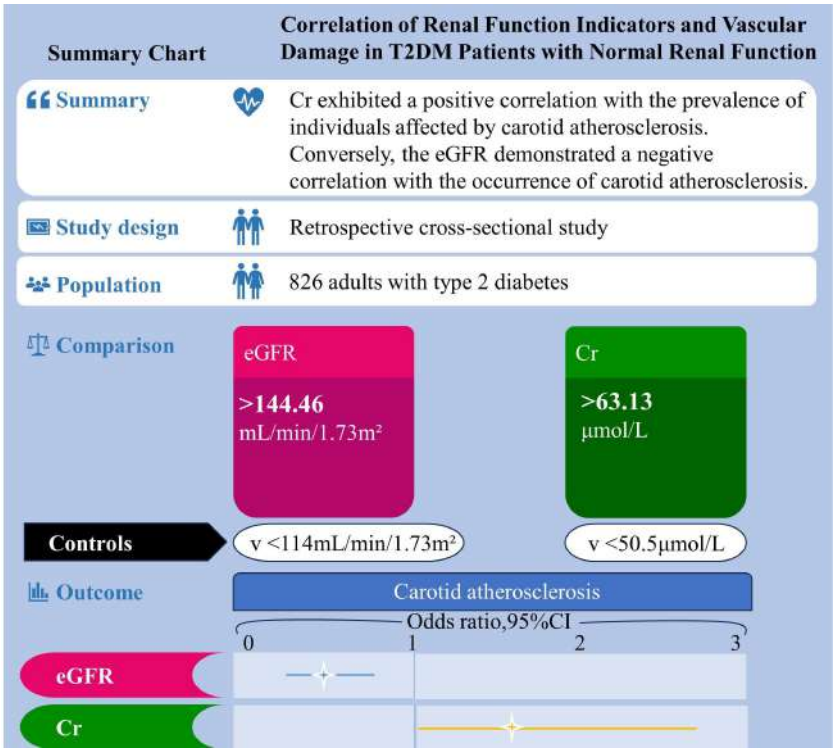

FIGURE 1
Summary chart. Correlation of renal function indicators and vascular damage in T2DM patients with normal renal function.

TABLE 1 Clinical characteristics according to gender.

Variables	Men (N=440)	Women (N=386)	P
Age,years old	53.61 ± 10.35	58.33 ± 10.19	<0.001*
Height,cm	166.44 ± 5.91	154.46 ± 10.17	<0.001*
Weight,kg	68.99 ± 11.05	58.15 ± 10.17	<0.001*
BMI,Kg/m^2	24.85 ± 3.38	24.32 ± 3.62	0.032*
WC,cm	88.36 ± 9.69	83.35 ± 9.62	<0.001*
SBP,mmHg	127.65 ± 16.63	131.66 ± 18.05	0.001*
DBP,mmHg	77.96 ± 10.40	75.42 ± 9.64	<0.001*
TG,mmol/L	2.45 ± 2.31	2.04 ± 1.89	0.006*
TC,mmol/L	4.57 ± 1.24	4.70 ± 1.10	0.125
LDL,mmol/L	2.70 ± 0.93	2.80 ± 0.93	0.128
HDL,mmol/L	1.09 ± 0.33	1.25 ± 0.35	<0.001*
FBG,mmol/L	9.20 ± 3.38	8.54 ± 3.09	0.006*
HbA1c,%	9.85 ± 2.52	9.37 ± 2.53	0.008*
ALT,mmol/L	35.42 ± 48.86	27.52 ± 23.3	0.004*
AST,mmol/L	26.13 ± 33.49	24.14 ± 18.39	0.299
BUN,mmol/L	5.43 ± 1.16	5.20 ± 1.13	0.004*
Cr,μmol/L	65.91 ± 14.00	50.39 ± 12.09	<0.001*
ALB,mg/L	6.45 ± 6.02	5.34 ± 5.52	0.006*
ACR,mg/g	10.32 ± 6.44	11.82 ± 6.80	0.001*
eGFR,mL/min/1.73m^2	127.55 ± 33.26	143.60 ± 84.50	<0.001*
Duration of diabetes,mouth	74.01 ± 73.03	84.74 ± 80.61	0.057
Current Drinking(No/Yes)	179/261	368/18	<0.001*
Current Smoking(No/Yes)	199/241	379/7	<0.001*
Hypoglycemic Drugs (No/Yes)	182/258	140/246	0.135
Insulin(No/Yes)	331/109	303/83	0.267

The values were expressed as the mean ± SD, n. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triacylglycerol; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, Creatinine; ALB, urine albumin; ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; *P<0.05.

TABLE 2 Prevalence of CA in different renal function indicator tertiles.

Events	Carotid atherosclerosis	P-value
BUN		0.039*
T1(<4.79)	166(59.7%)	
T2(4.79~5.90)	180(65.5%)	
T3(>5.90)	186(68.1%)	
Cr		<0.001*
T1(<50.50)	147(53.1%)	
T2(50.50~63.13)	198(72.3%)	
T3(>63.13)	187(68.0%)	
ALB		0.129
T1(<1.10)	181(65.6%)	
T2(1.10~8.10)	189(68.2%)	
T3(>8.10)	162(59.3%)	
ACR		0.588
T1(<7.20)	183(68.3%)	
T2(7.20~12.90)	174(62.8%)	
T3(>12.90)	175(64.1%)	
eGFR		<0.001*
T1(<114.10)	197(71.6%)	
T2(114.10~144.46)	189(68.5%)	
T3(>144.46)	146(53.1%)	

The values were expressed as n (%). *P<0.05.

P<0.05). A direct correlation emerged between Cr levels and CA risk, while eGFR displayed an inverse pattern. Regrettably, no discernible correlations materialized between BUN, ALB, ACR, and the prevalence of CA.

As depicted in Table 4, the connection between renal function indicators and CA in T2DM patients with normal kidney function was established through Spearman correlation analysis. This analysis unveiled a positive correlation between Cr(rs=0.144, P<0.001) and BUN(rs=0.068, P=0.049) with CA, while eGFR (rs=-0.170, P<0.001) exhibited a negative correlation. However, no substantial association was discerned between CA and ALB or ACR.

As illustrated in Figure 2, we conducted a multivariable regression analysis on variables that remain independent of diabetes-related vascular damage. The forest plot provided a visual representation, showcasing Cr(OR:1.021, 95%CI:1.011,1.031) and Duration of diabetes(OR:1.004, 95%CI:1.0020,1.006) as the notable risk factor for CA(all P < 0.001). Moreover, it was observed that eGFR(OR: 0.990, 95% CI:0.986, 0.994, P<0.001) played a protective role against CA development.

Finally, we evaluated the diagnostic efficacy of renal function-related indicators for CA through Receiver Operating Characteristic (ROC) curves (Figure 3). Remarkably, eGFR emerged as the most accurate indicator, boasting the highest Area Under the Curve (AUC) of 0.062 (95% CI: 0.562, 0.644, P<0.001). This was succeeded by Cr(AUC: 0.587, 95% CI: 0.54, 0628, P<0.001), BUN (AUC: 0.541, 95% CI:0.500, 0.583, P=0.049), ALB(AUC:0.530, 95% CI:0.488, 0.572, p=0.154), and ACR(AUC:0.520, 95% CI:0.479, 0.560, P=0.352). By utilizing the Youden index, we determined the optimal cutoff value for eGFR to be 138.2. The sensitivity of eGFR reached 66.9%, accompanied by a specificity of 52.0%.

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TABLE 3 Corrected OR and 95% CI for tertiles of renal function indicators.

Events	Model 1	P-value	Model 2	P-value	Model 3	P-value
BUN						
T1	1	0.108	1	0.166	1	0.405
T2	1.278(0.905,1.806)	0.163	1.248(0.882,1.766)	0.212	1.172(0.822,1.672)	0.381
T3	1.442(1.017,2.046)	0.040	1.394(0.980,1.982)	0.064	1.274(0.888,1.826)	0.188
Cr						
T1	1	<0.001*	1	<0.001*	1	0.001*
T2	2.304(1.616,3.284)	<0.001*	2.158(1.473,3.162)	<0.001*	2.166(1.454,3.225)	<0.001*
T3	1.879(1.329,2.657)	<0.001*	1.696(1.122,2.565)	0.012*	1.677(1.075,2.616)	0.023*
ALB						
T1	1	0.083	1	0.044	1	0.064
T2	1.127(0.791,1.607)	0.508	1.130(0.791,1.614)	0.502	1.049(0.729,1.510)	0.797
T3	0.766(0.542,1.083)	0.131	0.731(0.515,1.038)	0.080	0.707(0.494,1.013)	0.059
ACR						
T1	1	0.687	1	0.736	1	0.781
T2	0.859(0.606,1.217)	0.391	0.873(0.615,1.240)	0.449	0.880(0.616,1.225)	0.493
T3	0.907(0.639,1.290)	0.588	0.961(0.674,1.371)	0.827	0.936(0.652,1.344)	0.720
eGFR						
T1	1	<0.001*	1	<0.001*	1	<0.001*
T2	0.860(0.597,1.239)	0.419	0.865(0.600,1.248)	0.439	0.861(0.590,1.255)	0.436
T3	0.448(0.315,0.638)	<0.001*	0.474(0.332,0.678)	<0.001*	0.467(0.317,0.689)	<0.001*

Model 1: unadjusted; Model 2: adjusted for sex; Model 3: adjusted for sex, FBG, HbA1c, BMI, duration of diabetes, current smoking, current drinking, hypoglycemic drugs, insulin. OR, odds ratio; CI, confidence interval. *P<0.05.

Discussion

This cross-sectional study involved 826 T2DM patients and sought to investigate the interplay between CA prevalence and renal function-related indicators, specifically BUN, Cr, ALB, ACR, and eGFR, in T2DM patients possessing normal kidney function. Data analysis outcomes unveiled an escalating CA prevalence as Cr quartiles ascended ($P < 0.05$), while a distinct reduction in CA patients was discerned among the upper eGFR quartiles ($P < 0.05$).

TABLE 4 Association of renal function indicators with carotid atherosclerosis.

Events	CA, rs	P
BUN	0.068	0.049*
Cr	0.144	<0.001*
ALB	-0.050	0.153
ACR	-0.032	0.353
eGFR	-0.170	<0.001*

BUN, blood urea nitrogen; Cr, Creatinine; ALB, urine albumin; ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; CA, carotid atherosclerosis; rs, Spearman's correlation coefficient. *P<0.05.

Following the adjustment for confounding factors, including gender, a positive correlation emerged between Cr and CA ($P < 0.05$), while eGFR exhibited a negative association with CA ($P < 0.05$).

Collectively, kidney function demonstrated an adverse connection with CA, remaining unaffected by recognized risk factors such as age, gender, blood pressure, serum LDL cholesterol, and blood glucose. Remarkably, eGFR showcased the most robust performance in evaluating the correlation between renal function indicators and CA prevalence, closely trailed by Cr. Regrettably, up to this point, significant correlations between BUN, ALB, ACR, and CA prevalence have yet to be observed in T2DM patients boasting normal kidney function.

Furthermore, an intriguing yet perplexing observation surfaced during our analysis. While employing Spearman correlation analysis to discern the interrelation between renal function-related indicators and CA in T2DM patients with normal kidney function, BUN(rs=0.068, P=0.049) seemed to hint at a plausible positive correlation with CA occurrence. However, the outcomes derived from applying a multivariable regression model failed to demonstrate a significant association between the two. This discrepancy could potentially be attributed to the relatively

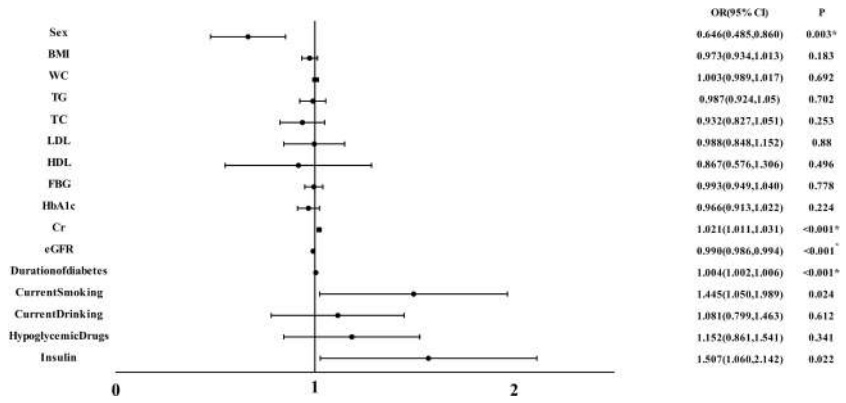

FIGURE 2
Multiple regression analysis of variables independently associated with CA in all participants. BMI, body mass index; WC, waist circumference; TG, triacylglycerol; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; Cr, Creatinine; eGFR, estimated glomerular filtration rate; CA, carotid atherosclerosis. *P<0.05.

modest size of our dataset or even potential limitations within our research methodology.

Currently, numerous research groups have delved into the intricate relationship between renal function and the onset of CA within diverse clinical contexts. For instance, Silvio Buscemi et al. (15) uncovered a distinct link between GFR and CA in individuals without renal insufficiency, substantiating a consistent negative correlation between renal function and CA, which aligns with our findings. Moreover, in patients grappling with chronic kidney failure, there has been validated affirmation of a closely intertwined association between compromised renal function and atherosclerosis. Multi-ethnic investigations involving a substantial cohort of Chronic Kidney Disease (CKD) patients have consistently reported a robust correlation between carotid intima-media thickness (c-IMT) and GFR, irrespective of patients' metabolic conditions (22–24). Notably, even during the incipient stages of chronic kidney failure, the prevalence of CA is augmented (25). Certain inquiries have spotlighted a nexus between atherosclerosis and diminishing GFR or Cr levels, irrespective of the patient's renal function status (12, 26)

Consequently, amalgamating the insights from our study with the existing body of research, a tentative inference can be drawn:

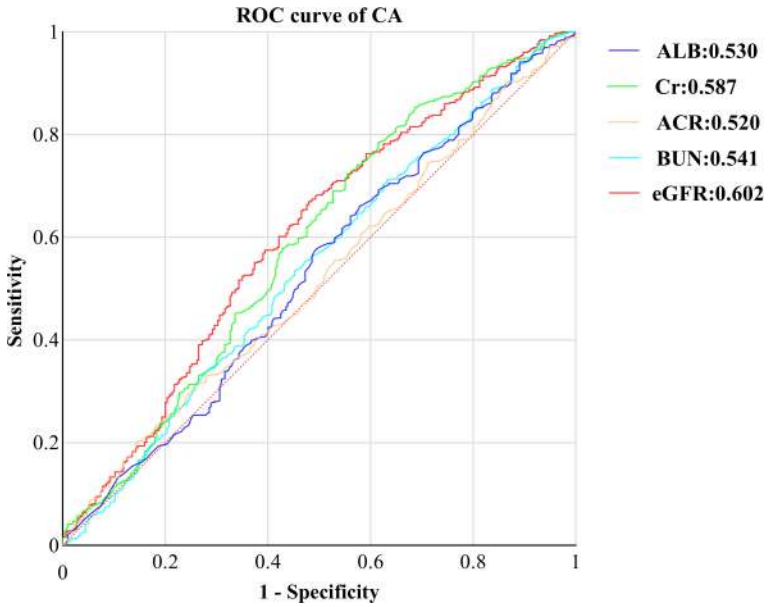

FIGURE 3
ROC curve of kidney function indicators CA in T2DM with normal kidney function. ROC, receiver operating characteristic; CA, carotid atherosclerosis; ALB, urine albumin; Cr, Creatinine; ACR, albumin-to-creatinine ration; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

sustaining GFR in proximity to the lower boundary of the normal range and maintaining Cr within the upper threshold of normal across the long term might potentially foster systemic atherosclerosis, exacerbate atherosclerotic damage, and potentially yield unfavorable consequences for kidney health. Furthermore, we have observed that the optimal eGFR threshold, calculated using the Youden index, is 138.2 ml/min/m^2. This value suggests that patients may be experiencing a state of hyperfiltration. While some studies have indicated an upward trend in eGFR with the progression of diabetes, it is worth noting that a high eGFR is recognized as one of the risk factors for diabetic kidney disease (27). Notably, the early onset of high filtration in diabetes is associated with more severe kidney damage in the advanced stages of the disease (28–31). However, the determination of the factors contributing to renal hyperfiltration involves intricate physiological and pathological mechanisms, making it challenging to pinpoint whether this state results from physiological or pathological factors. Additionally, there is currently a shortage of relevant research exploring the relationship between kidney function and vascular damage in diabetes patients who are experiencing hyperfiltration. To a certain extent, our study addresses this research gap. Of course, it's important to acknowledge a counter perspective presented by David Leander Rimmele et al., asserting that CA is independently associated with higher levels of NT-proBNP, through common risk factors and NT-proBNP with AF, and not with renal function (32).

Unlike our study and that of Silvio Buscemi, the divergent findings in the research by David Leander Rimmele et al. could plausibly emanate from varying population characteristics across different geographical regions or differences in methodological approaches.

Moreover, given that it stands as one of the most prevalent complications of Type 2 Diabetes Mellitus (T2DM), the significance of early intervention in addressing CA cannot be overstated, as it profoundly impacts patients' quality of life. Numerous studies have delved into the intricate web of associations between diverse indicators in T2DM patients and CA. For instance, the work of Jie Lin et al. (33) underscored a discernible correlation between thyroid-related hormones, diabetic peripheral neuropathy, and CA. Concurrently, Chenxi Wang et al. brought to the forefront the independent links between liver fat content index, fatty liver index, and CA within the landscape of T2DM. These indices could potentially serve as straightforward and invaluable markers, facilitating the evaluation of diabetic macrovascular damages and their progression (34).

The underlying biological mechanisms that tether Cr, GFR, and carotid atherosclerosis may be entwined with more profound factors. According to the perspective put forth by Kazuyuki Yahagi et al. (25), the pivotal driving forces behind diabetic atherosclerosis encompass a panorama of elements including oxidative stress, endothelial dysfunction, alterations in mineral metabolism, an overabundance of inflammatory cytokines, and even the mobilization of bone progenitor cells into the circulation. Nevertheless, it's important to acknowledge that due to the constrained exploration of these factors within our study, a comprehensive elucidation remains elusive, thereby underscoring the necessity for further animal studies to plumb the depths of this intricate subject.

Typically, other researchers tend to focus on exploring the correlation between a single indicator and CA. In contrast, this study adopted an innovative approach, delving into the correlation between five renal function indicators – namely eGFR, Cr, BUN, ALB, and ACR – and CA in patients with T2DM. This distinct methodology yields more comprehensive insights that hold immense value for clinical practitioners managing T2DM patients, regardless of their CA status. However, it's important to acknowledge the limitations inherent in this study. Chief among them is the relatively modest sample size, which could potentially introduce a degree of imprecision into the findings. Equally important, our study has certain limitations in diagnosing vascular damage in diabetes. According to the new expert consensus (35), flow-mediated dilation (FMD) and arterial stiffness are considered more valuable for assessing vascular damage, with FMD often regarded as the gold standard. However, in this study, we employed IMT as the diagnostic indicator for vascular damage, which is not widely recognized. This choice was made because FMD is not commonly used for diagnosing vascular damage in our study region, which may introduce some degree of error in the study results. Furthermore, the cross-sectional design employed in this research falls short of establishing definitive causality and fails to provide an intricate dissection of the mechanisms underpinning the connection between eGFR, Cr, and CA in T2DM patients. To substantiate the potential causal link between eGFR, Cr, and CA as unearthed in this study, subsequent longitudinal investigations are imperative.

Conclusions

In the context of T2DM patients possessing normal renal function, it becomes evident that those with lower eGFR levels and higher Cr levels are predisposed to a heightened likelihood of developing CA. Recognizing that elevated Cr and diminished eGFR serve as risk factors for CA, it's noteworthy that even when patients' eGFR and Cr values fall within the normal range, proactively managing and maintaining optimal eGFR and Cr levels within the T2DM population could yield substantial benefits in terms of CA prevention.

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 the ethics committee of the Affiliated Hospital of Southwest Medical

University. 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

Y-YZ: Writing – original draft. JG: Writing – original draft. B-XC: Data curation, Writing – original draft. QW: Writing – review & editing.

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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. Kato M, Natarajan R. Diabetic nephropathy–emerging epigenetic mechanisms. *Nat Rev Nephrol.* (2014) 10(9):517–30. doi: 10.1038/nrneph.2014.116

2. Mukhtar Y, Galalain A, Yunusa U. A modern overview on diabetes mellitus: a chronic endocrine disorder. *Eur J Biol* (2020) 5(2):1–14. doi: 10.47672/ejb.409

3. National Health and Health Commission of the People's Republic of China. Guidelines for diabetic diet in adults (2023 edition). *Clin Educ Family Med* (2023) 21 (05):388–91. doi: 10.13558/j.cnki.issn1672-3686.2023.005.002

4. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

5. Rowley WR, Bezold C, Arikan Y, Byrne E, Krohe S. Diabetes 2030: insights from yesterday, today, and future trends. *Popul Health Manage* (2017) 20(1):6–12. doi: 10.1089/pop.2015.0181

6. Bhamidipati T, Kumar M, Verma SS, Mohanty SK, Kacar S, Reese D, et al. Epigenetic basis of diabetic vasculopathy. *Front Endocrinol (Lausanne).* (2022) 13:989844. doi: 10.3389/fendo.2022.989844

7. Budoff MJ, Nasir K, Mao S, Tseng PH, Chau A, Liu ST, et al. Ethnic differences of the presence and severity of coronary atherosclerosis. *Atherosclerosis.* (2006) 187 (2):343–50. doi: 10.1016/j.atherosclerosis.2005.09.013

8. Cottone S, Lorito MC, Riccobene R, Nardi E, Mulè G, Buscemi S, et al. Oxidative stress, inflammation and cardiovascular disease in chronic renal failure. *J Nephrol.* (2008) 21(2):175–9.

9. Salvi P, Parati G. Chronic kidney disease: Arterial stiffness and renal function–a complex relationship. *Nat Rev Nephrol.* (2015) 11(1):11–3. doi: 10.1038/nrneph.2014.226

10. Buscemi S, Verga S, Batsis JA, Cottone S, Mattina A, Re A, et al. Intra-renal hemodynamics and carotid intima-media thickness in the metabolic syndrome. *Diabetes Res Clin Pract* (2009) 86(3):177–85. doi: 10.1016/j.diabres.2009.09.015

11. Geraci G, Mulè G, Mogavero M, Geraci C, D'Ignoti D, Guglielmo C, et al. Renal haemodynamics and severity of carotid atherosclerosis in hypertensive patients with and without impaired renal function. *Nutr Metab Cardiovasc Dis* (2015) 25(2):160–6. doi: 10.1016/j.numecd.2014.10.008

12. Geraci G, Mulè G, Costanza G, Mogavero M, Geraci C, Cottone S. Relationship between carotid atherosclerosis and pulse pressure with renal hemodynamics in hypertensive patients. *Am J Hypertens* (2016) 29(4):519–27. doi: 10.1093/ajh/hpv130

13. Lamprea-Montealegre JA, Astor BC, McClelland RL, de Boer IH, Burke GL, Sibley CT, et al. CKD, plasma lipids, and common carotid intima-media thickness: results from the multi-ethnic study of atherosclerosis. *Clin J Am Soc Nephrol.* (2012) 7 (11):1777–85. doi: 10.2215/CJN.02090212

14. Preston E, Ellis MR, Kulinskaya E, Davies AH, Brown EA. Association between carotid artery intima-media thickness and cardiovascular risk factors in CKD. *Am J Kidney Dis* (2005) 46(5):856–62. doi: 10.1053/j.ajkd.2005.07.048

15. Buscemi S, Geraci G, Massenti FM, Buscemi C, Costa F, D'Orio C, et al. Renal function and carotid atherosclerosis in adults with no known kidney disease. *Nutr Metab Cardiovasc Dis* (2017) 27(3):267–73. doi: 10.1016/j.numecd.2016.09.013

16. American Diabetes Association. 2. classification and diagnosis of diabetes. *Diabetes Care* (2019) 42(Suppl 1):S13–28. doi: 10.2337/dc19-S002

17. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* (2013) 310 (20):2191–4. doi: 10.1001/jama.2013.281053

18. Katakami N, Matsuoka TA, Shimomura I. Clinical utility of carotid ultrasonography: Application for the management of patients with diabetes. *J Diabetes Investig* (2019) 10(4):883–98. doi: 10.1111/jdi.13042

19. Scoutt LM, Gunabushanam G. Carotid ultrasound. *Radiol Clin North Am* (2019) 57(3):501–18. doi: 10.1016/j.rcl.2019.01.008

20. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* (2008) 21(2):93–190. doi: 10.1016/j.echo.2007.11.011

21. Johri AM, Nambi V, Naqvi TZ, Feinstein TZ, Kim TZ, Park TZ, et al. Recommendations for the assessment of carotid arterial plaque by ultrasound for the characterization of atherosclerosis and evaluation of cardiovascular risk: from the American society of echocardiography. *J Am Soc Echocardiogr* (2020) 33(8):917–33. doi: 10.1016/j.echo.2020.04.021

22. Okwuosa TM, Greenland P, Burke GL, Eng J, Cushman M, Michos ED, et al. Prediction of coronary artery calcium progression in individuals with low Framingham Risk Score: the Multi-Ethnic Study of Atherosclerosis. *JACC Cardiovasc Imaging.* (2012) 5(2):144–53. doi: 10.1016/j.jcmg.2011.11.008

23. Saydam CD. Subclinical cardiovascular disease and utility of coronary artery calcium score. *Int J Cardiol Heart Vasc* (2021) 37:100909. doi: 10.1016/j.ijcha.2021.100909

24. Grau-Perez M, Caballero-Mateos MJ, Domingo-Relloso A, Navas-Acien A, Gomez-Ariza JL, Garcia-Barrera T, et al. Toxic metals and subclinical atherosclerosis in carotid, femoral, and coronary vascular territories: the aragon workers health study. *Arterioscler Thromb Vasc Biol* (2022) 42(1):87–99. doi: 10.1161/ATVBAHA.121.316358

25. Yahagi K, Kolodgie FD, Lutter C, Mori H, Romero ME, Finn AV, et al. Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. *Arterioscler Thromb Vasc Biol* (2017) 37(2):191–204. doi: 10.1161/ATVBAHA.116.306256

26. van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, et al. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. *Stroke.* (2001) 32(2):454–60. doi: 10.1161/01.str.32.2.454

27. Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nat Rev Nephrol.* (2020) 16(6):317–36. doi: 10.1038/s41581-020-0256-y

28. Vallon V, Komers R. Pathophysiology of the diabetic kidney. *Compr Physiol* (2011) 1(3):1175–232. doi: 10.1002/cphy.c100049

29. Kanwar YS, Sun L, Xie P, Liu FY, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. *Annu Rev Pathol* (2011) 6:395–423. doi: 10.1146/annurev.pathol.4.110807.092150

30. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. *Diabetologia.* (2009) 52(4):691–7. doi: 10.1007/s00125-009-1268-0

31. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. *J Am Soc Nephrol.* (2017) 28(4):1023–39. doi: 10.1681/ASN.2016060666

32. Rimmele DL, Borof K, Jensen M, Behrendt CA, Cheng B, Debus ES, et al. Association between carotid atherosclerosis and atrial fibrillation, cardiac, and renal function. *Eur J Vasc Endovasc Surg* (2022) 63(4):641–7. doi: 10.1016/j.ejvs.2022.01.010

33. Lin J, Xiang X, Qin Y, Gui J, Wan Q. Correlation of thyroid-related hormones with vascular complications in type 2 diabetes patients with euthyroid. *Front Endocrinol (Lausanne).* (2022) 13:1037969. doi: 10.3389/fendo.2022.1037969

34. Wang C, Cai Z, Deng X, Li H, Zhao Z, Guo C, et al. Association of hepatic steatosis index and fatty liver index with carotid atherosclerosis in type 2 diabetes. *Int J Med Sci* (2021) 18(14):3280–9. doi: 10.7150/ijms.62010

35. Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* (2019) 40(30):2534–47. doi: 10.1093/eurheartj/ehz350

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Yuan-Hung Wang,
Taipei Medical University, Taiwan
Yingyong Zhao,
Northwest University, China
Weijun Peng,
Central South University, China

*CORRESPONDENCE
Rong Yu
yurong196905@163.com

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A Mendelian randomization study of the effect of serum 25-hydroxyvitamin D levels on autoimmune thyroid disease

Yunfeng Yu[1], Xinyu Yang[2], Jingyi Wu[3], Xueli Shangguan[1], Siyang Bai[2] and Rong Yu[1*]

[1]The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan, China, [2]College of Chinese Medicine, Hunan University of Chinese Medicine, Changsha, Hunan, China, [3]The Third School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China

Objective: The influence of vitamin D on autoimmune thyroid disease (AITD) remains a subject of ongoing debate. This study employs Mendelian randomization (MR) to investigate the causal correlations of serum 25-hydroxyvitamin D (25[OH]D) levels with autoimmune thyroiditis (AIT), autoimmune hyperthyroidism (AIH), and Graves disease (GD).

Methods: Data on single nucleotide polymorphisms related to serum 25(OH)D levels, AIT, AIH, and GD were sourced from UK Biobank and FinnGen. Inverse variance weighted, MR-Egger, and weighted median were employed to test the exposure-outcome causal relationship. Assessments of horizontal pleiotropy, heterogeneity, and stability were performed using the MR-Egger intercept, Cochran's Q test, and leave-one-out sensitivity analysis, respectively.

Results: The results of MR analysis showed increased serum 25(OH)D levels was associated with a reduced risk of AIT (OR 0.499, 95% CI 0.289 to 0.860, p = 0.012) but not causal associated with AIH (OR 0.935, 95% CI 0.695 to 1.256, p = 0.654) and GD (OR 0.813, 95% CI 0.635 to 1.040, p = 0.100). Intercept analysis showed no horizontal pleiotropy (p > 0.05), and Cochran's Q test showed no heterogeneity (p > 0.05). Sensitivity analysis suggested that these results were robust.

Conclusion: An increased serum 25(OH)D level is associated with AIT risk reduction but unrelated to AIH and GD. This finding suggests that vitamin D supplementation can be valuable for preventing and treating AIT.

KEYWORDS

vitamin D, autoimmune thyroid disease, autoimmune thyroiditis, Graves disease, Mendelian randomization

Abbreviations: AIH, Autoimmune hyperthyroidism; AITD, Autoimmune thyroid disease; AIT, Autoimmune thyroiditis; GD, Graves disease; GWAS, Genome-wide association studies; HT, Hashimoto's thyroiditis; IVW, Inverse variance weighting; MR, Mendelian randomization; SNP, Single nucleotide polymorphism; TGAb, Thyroglobulin antibodies; TPOAb, Thyroid peroxidase antibodies; TRAb, Thyroid stimulating hormone receptor antibodies; TSH, Thyroid stimulating hormone; 25[OH]D, 25-hydroxyvitamin D.

1 Introduction

Autoimmune thyroid disease (AITD), an autoimmune disorder characterized by the breakdown of tolerance in the immune system towards thyroid antigens (1), is one of the most common autoimmune diseases. In recent years, the incidence of AITD has been increasing (2). Reports indicate that AITD affects about 5% of the general population, with a higher prevalence among females (2). The pathogenesis of AITD remains incompletely understood but is generally attributed to immune system dysregulation mediated by genetic and environmental triggering factors, with T cells and B cells infiltration into the thyroid glands being its typical manifestation (3). Hashimoto's thyroiditis (HT) and Graves disease (GD) are the two most frequently discussed AITD types. HT is the most common autoimmune thyroiditis (AIT), which causes damage to thyroid follicular cells, leading to hypothyroidism (4). GD is the most prevalent form of hyperthyroidism, causing thyroid cell proliferation and excessive thyroid hormone synthesis, resulting in hyperthyroidism (5). Currently, levothyroxine is the primary treatment for HT (6), while antithyroid drugs, radioactive iodine therapy, and surgical treatment are the main strategies for GD (7). Nutrients such as vitamin D and selenium have also been considered to positively impact AITD (8).

Vitamin D is a type of steroid hormone produced from the skin or absorbed from the diet, and its primary function is to regulate calcium phosphate metabolism and promote bone homeostasis (9). A relevant study has shown that vitamin D is related to immune regulation functions, which affects monocyte-mediated innate immune responses and regulates adaptive immune responses by inhibiting antigen-presenting cell functions (10). Vitamin D deficiency is believed to be associated with an increased risk of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and others (11). However, its role in AITD is not yet clear. It has been reported that serum 25-hydroxyvitamin D (25[OH]D) levels are closely correlated with autoimmune antibody titers such as thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), and thyroid stimulating hormone receptor antibodies (TRAb) (12, 13). Vitamin D supplementation therapy may potentially enhance AITD prognosis (14). However, some studies have refuted the benefits of vitamin D supplementation for AITD patients (15, 16). The influence of vitamin D on AITD remains controversial, and the causal relationship between the two needs to be further elucidated.

Mendelian randomization (MR) is an epidemiological method used to study causal relationships between exposure factors and outcome variables (17). MR effectively avoids the influence of confounding factors due to the random nature of the genes (18). This study aims to investigate the causal correlations of serum 25 (OH)D levels with AIT, autoimmune hyperthyroidism (AIH), and GD from genetic prediction by MR.

2 Materials and methods

2.1 Study design

MR relied on three basic assumptions: (1) The relevance assumption: Single nucleotide polymorphisms (SNPs) were closely associated with the exposure factor. (2) The independence assumption: SNPs were independent of confounding factors. (3) The exclusion restriction assumption: SNPs could not affect the outcome variable through pathways other than the exposure factor. The study used a two-sample MR design. "Two-sample" refers to the fact that the exposure factor and the outcome variable came from two different datasets. In this MR analysis, the dataset for serum 25(OH)D levels as an exposure factor was obtained from UK-Biobank, while the datasets for AIT, AIH, and GD as outcome variables were obtained from FinnGen. This approach helped avoid data overfitting problems, horizontal pleiotropy and weak instrumental bias. The MR design process is shown in Figure 1.

2.2 Data sources

Data on serum 25(OH)D levels, AIT, AIH, and GD were sourced from UK Biobank (www.nealelab.is/uk-biobank) and FinnGen (www.finngen.fi/en). The criteria for defining the status of serum 25 (OH)D levels are as follows: Desirable ≥ 75 nmol/L, Sufficient 50 to 74.9 nmol/L, Insufficient 25 to 49.9 nmol/L, Deficient< 25 nmol/L. All data were sourced from publicly available databases, eliminating the need for additional ethical approval.

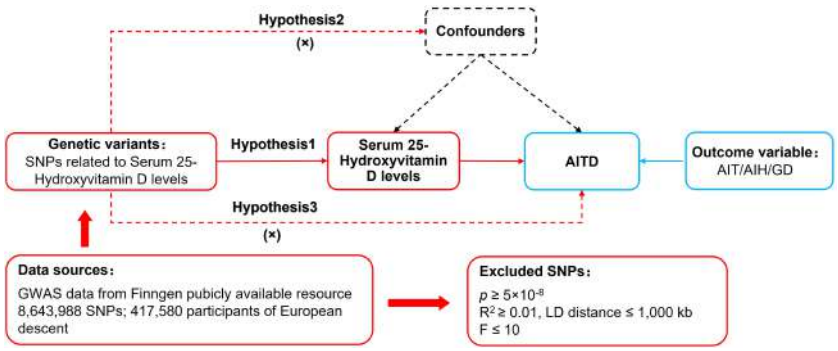

FIGURE 1
MR design for causal analysis of serum 25(OH)D levels and AITD.

2.3 Selection of genetic instrumental variables

First, SNPs closely associated with exposure factors were screened in the genome-wide association studies (GWAS) database according to a threshold of $p< 5\times10^{-8}$ to fulfill assumption 1. Second, independent SNPs were screened according to R2< 0.001 and kb = 10,000 to mitigate potential linkage disequilibrium bias. Third, the F-statistics of each SNP were calculated, and the SNPs were further screened according to the parameter of F > 10. The calculation of the F-statistic is as follows: F= $[R^2/(1-R^2)]*[(N-K-1)/k]$. $R^2 = 2*(1-MAF)*MAF*\beta^2$. R^2: The cumulative explained variance of the selected instrumental variables on exposure; MAF: The effect of minor allele frequency; β: The estimated effect of SNP; N: The sample size of the GWAS. Forth, SNPs potentially related to AITD were removed based on PhenoScanner (www.phenoscanner.medschl.cam.ac.uk) and relevant literature to satisfy assumption 2. Finally, duplicates and mismatched SNPs were excluded based on EAF values while harmonizing the allelic orientation of exposure-SNPs and outcome-SNPs. The remaining SNPs were used to perform MR analysis.

2.4 Data analysis

The study followed the STROBE-MR guidelines (19). The two-sample MR analysis used the "TwoSampleMR (0.5.7)" package in R 4.3.1 (Lucent Technologies, MurrayHill, State of New Jersey, USA). The primary methods for assessing causal relationships were inverse variance weighting (IVW), MR-Egger, and weighted median. IVW, the main analytical method (20), provides unbiased causal estimates without horizontal pleiotropy and is considered the most informative. MR-Egger and the weighted median are used as complementary methods to MR analysis. MR-Egger can yield effective causal estimates in the presence of some horizontal pleiotropy, while weighted median has a lower sensitivity to outliers and measurement errors.

MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) was used to remove outlier ($p < 1$) SNPs. The remaining SNPs were used to re-perform the MR analysis and obtain the final results. Horizontal pleiotropy was assessed using MR-Egger's intercept analysis, with $p \geq 0.05$ indicating the absence of horizontal pleiotropy, satisfying assumption 3. Heterogeneity was assessed using Cochran's Q, with $p \geq 0.05$ indicating the absence of heterogeneity. Sensitivity analysis was performed using the leave-one-out method to assess the robustness of the MR results and identify individual SNPs that significantly affect the combined results.

3 Results

3.1 GWAS data for serum 25(OH)D levels

Data on serum 25(OH)D levels was obtained from UK-Biobank, which included GWAS data from 417,580 individuals of European descent. UK Biobank provided 113 SNPs closely associated with serum 25(OH)D levels. Among these, 8 SNPs were excluded due to their association with known confounding factors, leaving 105 SNPs included in this study, as shown in Supplementary Table S1. Duplicates and mismatched SNPs were excluded based on EAF values while harmonizing the allelic orientation of exposure-SNPs and outcome-SNPs, and the final included SNPs are shown in Supplementary Tables S2-S4.

3.2 GWAS data from AITD

Comprehensive details of the GWAS datasets utilized in this study are presented in Table 1. Data for AIT was obtained from the FinnGen database, including 321,192 individuals of European descent (dataset ID: finngen_R9_E4_THYROIDITAUTOIM). Data for AIH was also sourced from FinnGen, including 281,683 European individuals (dataset ID: finngen_R9_AUTOIMMUNE_HYPERTHYROIDISM). Data for GD was obtained from FinnGen, including 377,277 individuals of European descent (dataset ID: finngen_R9_E4_GRAVES_STRICT).

3.3 Two-Sample MR Analysis Results

This study conducted MR to analyze the causal correlations of serum 25(OH)D levels with AIT, AIH, and GD. The forest plot of the MR analysis is shown in Figure 2, and the effect estimates for each SNP are displayed in Figure 3. MR-Egger intercept analysis is presented in Supplementary Table S5, heterogeneity test results are in Figure 4; Supplementary Table S6, and sensitivity analysis is in Figure 5.

3.3.1 AIT

IVW (OR 0.499, 95% CI 0.289 to 0.860, $p = 0.012$) and weighted median (OR 0.327, 95% CI 0.131 to 0.817, $p = 0.017$) revealed that increased serum 25(OH)D levels are associated with a reduced risk of AIT. At the same time, MR-Egger (OR 0.639, 95% CI 0.274 to 1.493, $p = 0.304$) did not observe such a causal relationship. Intercept analysis indicated the absence of horizontal pleiotropy ($p = 0.455$), heterogeneity test showed no significant heterogeneity ($p = 0.734$), and sensitivity analysis suggested the results were robust.

3.3.2 AIH

All three analytical methods showed no significant causal relationship between serum 25(OH)D levels and AIH: IVW (OR 0.935, 95% CI 0.695 to 1.256, $p = 0.654$), MR-Egger (OR 1.240, 95% CI 0.786 to 1.956, p 0.358) and weighted median (OR 1.173, 95% CI 0.761 to 1.806, $p = 0.470$). Intercept analysis indicated the absence of horizontal pleiotropy ($p = 0.116$), heterogeneity test showed no significant heterogeneity (p=0.264), and sensitivity analysis suggested the results were robust.

3.3.3 GD

All three analytical methods showed no significant causal relationship between serum 25(OH)D levels and GD: IVW (OR

TABLE 1 Details of the GWAS studies included in the Mendelian randomization.

Year	Trait	Population	Sample size	Web source
2020	Serum 25-Hydroxyvitamin D levels	European	417,580	www.nealelab.is/uk-biobank
2023	Autoimmune thyroiditis	European	321,192	www.finngen.fi/en
2023	Autoimmune hyperthyroidism	European	281,683	www.finngen.fi/en
2023	Graves disease	European	377,277	www.finngen.fi/en

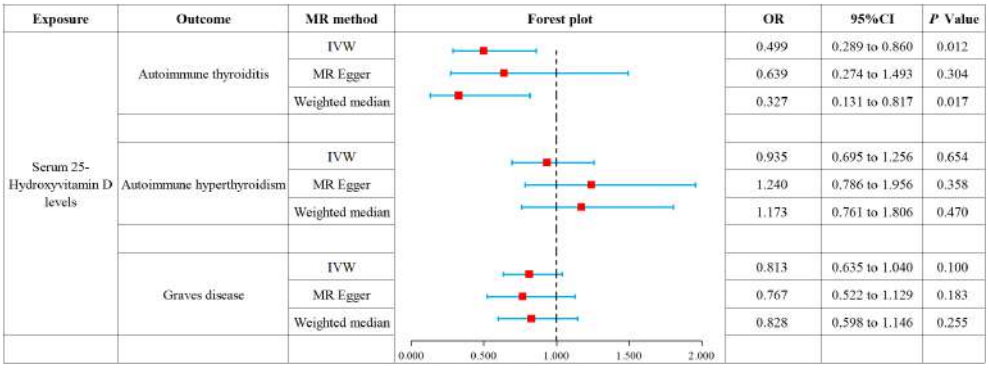

FIGURE 2
Forest plot of MR analysis on the causal relationship between serum 25(OH)D levels and AITD.

0.813, 95% CI 0.635 to 1.040, $p = 0.100$), MR-Egger (OR 0.767, 95% CI 0.522 to 1.129, $p = 0.183$) and weighted median (OR 0.828, 95% CI 0.598 to 1.146, $p = 0.255$). Intercept analysis indicated the absence of horizontal pleiotropy ($p = 0.706$), heterogeneity test showed no significant heterogeneity ($p = 0.112$), and sensitivity analysis suggested the results were robust.

4 Discussion

AITD is a significant risk factor for thyroid cancer (21). Recent studies have shown that vitamin D deficiency increases the risk and difficulty in treating AITD (22), which may be related to its role in modulating the adaptive immune response (23). However, other reports suggest that vitamin D levels are not associated with AITD (15, 16), and supraphysiological levels of vitamin D supplementation may even increase the risk of death (24). To gain a clearer understanding of the role of vitamin D on AITD, this study used MR to analyze the causal relationship of serum 25(OH)D levels with AIT, AIH, and GD.

Our study results indicated increased serum 25(OH)D levels was associated with a reduced risk of AIT but not causal associated with AIH and GD. These results were free of horizontal pleiotropy and heterogeneity, and sensitivity analysis suggested they were robust. Since all our data were derived from Europeans, this study primarily illustrated the link between elevated serum 25(OH)D levels and reduced AIT risk in Europeans. Additionally, we found that increased serum 25(OH)D levels were associated with a lower risk of AIT but not GD, probably due to the different pathogenesis and specific antibodies of the two.

HT is one of the main types of ATID and the most common form of AIT (25). Our study results indicated an association

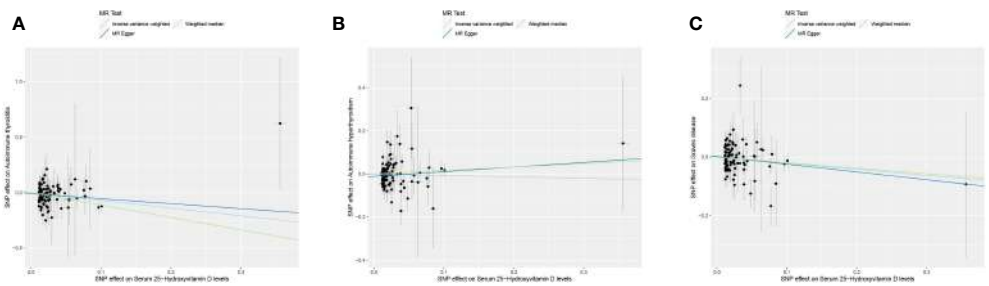

FIGURE 3
Scatter plot of MR analysis on the causal relationship between serum 25(OH)D levels and AITD. **(A)** AIT, **(B)** AIH, **(C)** GD. *AITD, autoimmune thyroid disease; AIT, autoimmune thyroiditis; AIH, autoimmune hyperthyroidism; GD, Graves disease.

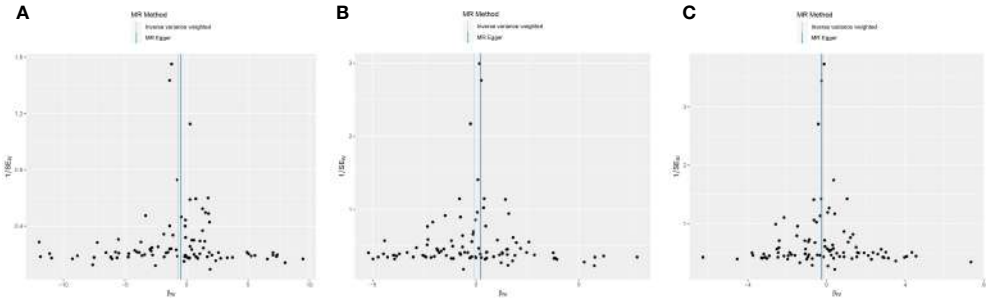

FIGURE 4
Funnel plot of heterogeneity analysis on serum 25(OH)D levels and AITD. **(A)** AIT, **(B)** AIH, **(C)** GD. *AITD, autoimmune thyroid disease; AIT, autoimmune thyroiditis; AIH, autoimmune hyperthyroidism; GD, Graves disease.

between elevated serum 25(OH)D levels and a diminished AIT risk. Unal AD et al. (26) conducted a cross-sectional investigation, revealing markedly lower serum 25(OH)D levels in HT patients compared to healthy individuals. At the same time, they found serum 25(OH)D levels exhibited a negative correlation with TPOAb and TGAb titers in HT patients. A subsequent study confirmed this conclusion and reported a substantial association between vitamin D deficiency and notable thyroid dysfunction among individuals with HT (27). A study of Polish women showed a strong negative correlation between thyroid stimulating hormone (TSH) and vitamin D levels across healthy individuals, HT patients, and hypothyroid patients, while TPOAb and TGAb exhibited a weak negative correlation with vitamin D levels (12). Bozkurt NC et al. (28) highlighted that the severity of vitamin D deficiency was not only related to antibody levels but also to the HT duration and thyroid volume. Therefore, vitamin D deficiency may be a potential risk factor for AIT, and vitamin D supplementation may benefit patients with AIT.

A clinical study by Ucan B et al. (29) found that vitamin D supplementation therapy at 50,000 IU per week for eight weeks can slow down thyroid dysfunction in HT patients and reduce their cardiovascular risk. In a double-blind randomized controlled trial, Chahardoli R et al. (30) reported that weekly supplementation with 50,000 IU of vitamin D reduced TSH and TGAb levels in female HT patients, attenuating their disease activity. Meta-analysis suggested that vitamin D supplementation ≤3 months effectively reduced TGAb titers in HT patients but had no benefit in TPOAb titers, while supplementation ≥3 months reduced both TGAb and TPOAb titers, indicating that long-term vitamin D intake may have more significant benefits to HT patients (31).

However, some studies believe that HT is unrelated to higher vitamin D deficiency rates (32). Pani MA et al. (33), through a study on polymorphic vitamin D binding protein (DBP), found that DBP allelic variation did not confer susceptibility to HT in people of Caucasian ancestry. Cvek M et al. (16), through observation of CROHT biobank, found that vitamin D levels showed no correlation with the presence of HT. Anaraki PV et al. (34) reported that vitamin D supplementation therapy (50,000 IU per week for 12 weeks) did not improve metabolism-related parameters such as glucose, lipids, albumin, and electrolytes in HT patients with vitamin D deficiency. Although the causal relationship between vitamin D and AIT remains controversial, most studies support the

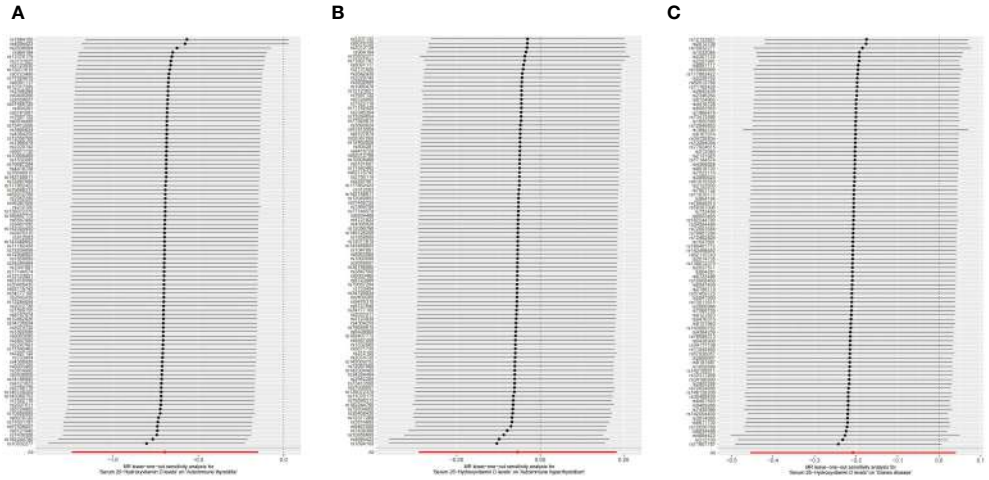

FIGURE 5
Leave-one-out sensitivity analysis on serum 25(OH)D levels and AITD. **(A)** AIT, **(B)** AIH, **(C)** GD. *AITD, autoimmune thyroid disease; AIT, autoimmune thyroiditis; AIH, autoimmune hyperthyroidism; GD, Graves disease.

connection between vitamin D deficiency and an elevated AIT risk. These findings align with our study results, suggesting that vitamin D supplementation may mitigate the risk and improve the prognosis of AIT.

The effect of vitamin D on AIT may be related to the inhibition of T and B lymphocytes. Vitamin D inhibits dendritic cell-dependent T-cell activation and reduces HLA II gene expression to suppress pro-inflammatory factor expression (10). The vitamin also increases the number of Tregs while inhibiting the differentiation of naive T cells into Th17 (35).

GD is one of the main AITD types and is the most common form of hyperthyroidism (36). Our study found that serum 25(OH)D levels are not associated with AIH and GD risk, similar to previous reports. A clinical study in China showed that serum 25(OH)D levels were relatively deficient in HT patients but similar to healthy individuals in GD patients (37). Although cross-sectional studies in India by Mangaraj S et al. (38) and Planck T et al. (39) reported lower serum 25(OH)D levels in GD patients, they did not support a correlation between these levels and thyroid hormones or TRAb. Research by Yasuda T et al. (40) further confirmed that serum 25(OH)D levels affected only thyroid volume but were not associated with thyroid function or TRAb. Conversely, Zhang H et al. (13) found that although vitamin D levels were unrelated to FT3, FT4, TSH, TGAb, TPOAb, and other indicators in GD patients, they were closely related to TRAb titers. They suggested that vitamin D deficiency might be linked to heightened autoimmune responses in GD patients (13). Veneti S et al. (41) pointed out from a genetic perspective that the vitamin D receptor gene polymorphism was associated with GD, and the TT subtype of TaqI polymorphism was linked to heightened GD susceptibility. Xu MY et al.'s (42) meta-analysis suggested that low vitamin D status may increase the risk of developing GD. These pieces of evidence point to a possible association of serum 25(OH)D levels with GD risk.

However, previous studies have suggested that vitamin D supplementation is ineffective in treating GD. The DAGMAR Trial (15) showed that supplementing vitamin D did not significantly benefit GD patients with normal or insufficient vitamin D levels. Cho YY et al.'s (43) report showed that vitamin D supplementation did not significantly affect the recurrence rate of GD within one year of discontinuing antithyroid drugs. Clinical trials by Grove-Laugesen D et al. (44, 45) found that vitamin D supplementation of 2,800 IU per day for nine months did not achieve a positive effect on pulse wave velocity and even had adverse effects on the recovery of muscle performance. In summary, the association between vitamin D and GD remains controversial and needs to be explored in more studies in the future. There is insufficient evidence to suggest that vitamin D supplementation benefits GD, so we do not recommend it for GD patients without vitamin D deficiency.

Interestingly, we found that increased serum vitamin D levels were associated with a reduced risk of AIT but not with a GD risk. This difference may be related to the different pathogenesis of the two. Studies have shown that AIT is dominated by an autoimmune response mediated by Th1 cells (46), whereas GD is dominated by a humoral response mediated by Th2 cells (47). Vitamin D can inhibit the proliferation and differentiation of Th1 cells, and it can reduce the risk of AIT by suppressing the Th1-dominated immune response (48).

Of the 105 SNPs for serum 25(OH)D levels included in this study, only ten SNPs have been reported in the literature, as shown in Supplementary Table S7. Of these, rs801872, located in the SEC23A gene, was reported to be significantly associated with 25(OH)D levels (49), and the remaining nine SNPs were not reported to be associated with vitamin D and its receptor. Moreover, the published literature did not report the relevance of these SNPs to AITD. More studies are needed in the future to explore the role of these SNPs in the pathogenesis of AIT and to search for the key loci of vitamin D regulation of AIT.

Our study also has certain limitations. First, because the GWAS database only contains datasets for AIT, AIH, and GD, this study can only explain how serum 25(OH)D levels relate to them and does not apply to all types of AITD. Second, because the database lacks matched data on Asian ancestry and African ancestry, the entirety of our data was derived from Europeans, which leads to the possibility that the results of this study may not apply to other races. Third, this study was conducted based on the exposure factor serum 25(OH)D levels for the outcome variables AIT, AIH, and GD. Therefore, it can only describe the effect of serum 25(OH)D levels on disease risk and does not apply to antibody levels.

Given these limitations, we look forward to future research improvements: First, conducting some stratified experiments. In these experiments, relevant variables should be controlled to investigate the effects of different doses and durations of vitamin D supplementation on thyroid hormone levels and autoimmune antibody levels in different AITD patients. Second, establishing research centers in multiple continents and countries to investigate the effects of vitamin D levels on AITD patients of different races to provide a more comprehensive data source for MR studies.

5 Conclusion

This MR analysis suggests that elevated serum 25(OH)D levels correlate with a decreased AIT risk but are not associated with AIH and GD. Vitamin D supplementation may help mitigate the risk and enhance the prognosis of AIT. In forthcoming studies, further investigation is warranted to delve into the causal relationship and underlying mechanisms linking vitamin D to AITD.

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

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional

requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

YY: Conceptualization, Data curation, Supervision, Writing – original draft. XY: Methodology, Supervision, Writing – original draft. JW: Data curation, Methodology, Writing – original draft. XS: Data curation, Formal analysis, Writing – original draft. SB: Formal analysis, Writing – original draft. RY: Formal analysis, Writing – review & editing.

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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/fimmu.2023.1298708/full#supplementary-material

References

1. Ferrari SM, Fallahi P, Elia G, Ragusa F, Camastra S, Paparo SR, et al. Novel therapies for thyroid autoimmune diseases: an update. *Best Pract Res Clin Endocrinol Metab* (2020) 34:101366. doi: 10.1016/j.beem.2019.101366

2. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. *Autoimmun Rev* (2015) 14:174–80. doi: 10.1016/j.autrev.2014.10.016

3. Orgiazzi J. Thyroid autoimmunity. *Presse Med* (2012) 41:e611–25. doi: 10.1016/j.lpm.2012.10.002

4. Ferrari SM, Ragusa F, Elia G, Paparo SR, Mazzi V, Baldini E, et al. Precision medicine in autoimmune thyroiditis and hypothyroidism. *Front Pharmacol* (2021) 12:750380. doi: 10.3389/fphar.2021.750380

5. Elia G, Fallahi P, Ragusa F, Paparo SR, Mazzi V, Benvenga S, et al. Precision medicine in graves disease and ophthalmopathy. *Front Pharmacol* (2021) 12:754386. doi: 10.3389/fphar.2021.754386

6. Ralli M, Angeletti D, Fiore M, D'Aguanno V, Lambiase A, Artico M, et al. Hashimoto's thyroiditis: an update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential Malignant transformation. *Autoimmun Rev* (2020) 19:102649. doi: 10.1016/j.autrev.2020.102649

7. Yoo WS, Chung HK. Recent advances in autoimmune thyroid diseases. *Endocrinol Metab (Seoul).* (2016) 31:379–85. doi: 10.3803/EnM.2016.31.3.379

8. Liontiris MI, Mazokopakis EE. A concise review of Hashimoto thyroiditis (HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients.Points that need more investigation. *Hell J Nucl Med* (2017) 20:51–6. doi: 10.1967/s002449910507

9. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol* (2014) 21:319–29. doi: 10.1016/j.chembiol.2013.12.016

10. Zhao R, Zhang W, Ma C, Zhao Y, Xiong R, Wang H, et al. Immunomodulatory function of vitamin D and its role in autoimmune thyroid disease. *Front Immunol* (2021) 12:574967. doi: 10.3389/fimmu.2021.574967

11. Ao T, Kikuta J, Ishii M. The effects of vitamin D on immune system and inflammatory diseases. *biomolecules.* (2021) 11:1624. doi: 10.3390/biom11111624

12. Gierach M, Junik R. The role of vitamin D in women with Hashimoto's thyroiditis. *Endokrynol Pol* (2023) 74:176–80. doi: 10.5603/EP.a2022.0095

13. Zhang H, Liang L, Xie Z. Low vitamin D status is associated with increased thyrotropin-receptor antibody titer in graves disease. *Endocr Pract* (2015) 21:258–63. doi: 10.4158/EP14191.OR

14. Czarnywojtek A, Florek E, Pietrończyk K, Sawicka-Gutaj N, Ruchała M, Ronen O, et al. The role of vitamin D in autoimmune thyroid diseases: a narrative review. *J Clin Med* (2023) 12:1452. doi: 10.3390/jcm12041452

15. Grove-Laugesen D, Ebbehoj E, Watt T, Riis AL, Østergård T, Bruun BJ, et al. Effect of vitamin D supplementation on graves disease: the DAGMAR trial. *Thyroid* (2023) 33(9):1110–18. doi: 10.1089/thy.2023.0111 [Epub ahead of print].

16. Cvek M, Kaličanin D, Barić A, Vuletić M, Gunjača I, Torlak Lovrić V, et al. Vitamin D and hashimoto's thyroiditis: observations from CROHT biobank. *Nutrients.* (2021) 13:2793. doi: 10.3390/nu13082793

17. Sanderson E. Multivariable mendelian randomization and mediation. *Cold Spring Harb Perspect Med* (202) 11(2):a038984. doi: 10.1101/cshperspect.a038984

18. Julian TH, Boddy S, Islam M, Kurz J, Whittaker KJ, Moll T, et al. A review of Mendelian randomization in amyotrophic lateral sclerosis. *Brain.* (2022) 145(3):832–42. doi: 10.1093/brain/awab420

19. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* (2013) 37 (7):658–65. doi: 10.1002/gepi.21758

20. 1000 Genomes Project Consortium, Auton A, LD B, RM D, EP G, HM K, et al. A global reference for human genetic variation. *nature* (2015) 526(7571):68–74. doi: 10.1038/nature15393

21. Dias Lopes NM, Mendonça Lens HH, Armani A, Marinello PC, Cecchini AL. Thyroid cancer and thyroid autoimmune disease: a review of molecular aspects and clinical outcomes. *Pathol Res Pract* (2020) 216:153098. doi: 10.1016/j.prp.2020.153098

22. Vieira IH, Rodrigues D, Paiva I. Vitamin D and autoimmune thyroid disease-cause, consequence, or a vicious cycle? *Nutrients* (2020) 12:2791. doi: 10.3390/nu12092791

23. Bellan M, Andreoli L, Mele C, Sainaghi PP, Rigamonti C, Piantoni S, et al. Pathophysiological role and therapeutic implications of vitamin D in autoimmunity: focus on chronic autoimmune diseases. *Nutrients.* (2020) 12:789. doi: 10.3390/nu12030789

24. Durup D, Jørgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B. A reverse J-shaped association of all-cause mortality with serum 25- hydroxyvitamin D in general practice: the CopD study. *J Clin Endocrinol Metab* (2012) 97:2644–52. doi: 10.1210/jc.2012-1176

25. Rayman MP. Multiple nutritional factors and thyroid disease, with particular reference to autoimmune thyroid disease. *Proc Nutr Soc* (2019) 78:34–44. doi: 10.1017/S0029665118001192

26. Unal AD, Tarcin O, Parildar H, Cigerli O, Eroglu H, Demirag NG. Vitamin D deficiency is related to thyroid antibodies in autoimmune thyroiditis. *cent Eur J Immunol* (2014) 39:493–7. doi: 10.5114/ceji.2014.47735

27. Kim D. Low vitamin D status is associated with hypothyroid Hashimoto's thyroiditis. *Hormones (Athens).* (2016) 15:385–93. doi: 10.14310/horm.2002.1681

28. Bozkurt NC, Karbek B, Ucan B, Sahin M, Cakal E, Ozbek M, et al. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. *Endocr Pract* (2013) 19:479–84. doi: 10.4158/EP12376.OR

29. Ucan B, Sahin M, Sayki Arslan M, Colak Bozkurt N, Kizilgul M, Güngünes A, et al. Vitamin D treatment in patients with hashimoto's thyroiditis may decrease the development of hypothyroidism. *Int J Vitam Nutr Res* (2016) 86:9–17. doi: 10.1024/0300-9831/a000269

30. Chahardoli R, Saboor-Yaraghi AA, Amouzegar A, Khalili D, Vakili AZ, Azizi F. Can supplementation with vitamin D modify thyroid autoantibodies (Anti- TPO ab, anti-tg ab) and thyroid profile (T3, T4, TSH) in hashimoto's thyroiditis? A double blind, randomized clinical trial. *Horm Metab Res* (2019) 51:296–301. doi: 10.1055/a-0856-1044

31. Zhang J, Chen Y, Li H, Li H. Effects of vitamin D on thyroid autoimmunity markers in Hashimoto's thyroiditis: systematic review and meta-analysis. *J Int Med Res* (2021) 49:3000605211060675. doi: 10.1177/03000605211060675

32. Yasmeh J, Farpour F, Rizzo V, Kheradnam S, Sachmechi I. Hashimoto thyroiditis not associated with vitamin D deficiency. *Endocr Pract* (2016) 22:809–13. doi: 10.4158/EP15934.OR

33. Pani MA, Regulla K, Segni M, Hofmann S, Hüfner M, Pasquino AM, et al. A polymorphism within the vitamin D-binding protein gene is associated with Graves disease but not with Hashimoto's thyroiditis. *J Clin Endocrinol Metab* (2002) 87:2564–7. doi: 10.1210/jcem.87.6.8562

34. Anaraki PV, Aminorroaya A, Amini M, Feizi A, Iraj B, Tabatabaei A. Effects of Vitamin D deficiency treatment on metabolic markers in Hashimoto thyroiditis patients. *J Res Med Sci* (2017) 22:5. doi: 10.4103/1735-1995.199090

35. Dankers W, Davelaar N, van Hamburg JP, van de Peppel J, Colin EM, Lubberts E. Human memory th17 cell populations change into anti-inflammatory cells with regulatory capacity upon exposure to active vitamin D. *Front Immunol* (2019) 10:1504. doi: 10.3389/fimmu.2019.01504

36. Antonelli A, Ferrari SM, Ragusa F, Elia G, Paparo SR, Ruffilli I, et al. Graves disease: epidemiology, genetic and environmental risk factors and viruses. *Best Pract Res Clin Endocrinol Metab* (2020) 34:101387. doi: 10.1016/j.beem.2020.101387

37. Ke W, Sun T, Zhang Y, He L, Wu Q, Liu J, et al. 25-Hydroxyvitamin D serum level in Hashimoto's thyroiditis, but not Graves disease is relatively deficient. *Endocr J* (2017) 64:581–7. doi: 10.1507/endocrj.EJ16-0547

38. Mangaraj S, Choudhury AK, Swain BM, Sarangi PK, Mohanty BK, Baliarsinha AK. Evaluation of Vitamin D Status and its Impact on Thyroid Related Parameters in New Onset Graves disease- A Cross-sectional Observational Study. *Indian J Endocrinol Metab* (2019) 23:35–9. doi: 10.4103/ijem.IJEM_183_18

39. Planck T, Shahida B, Malm J, Manjer J. Vitamin D in graves disease: levels, correlation with laboratory and clinical parameters, and genetics. *Eur Thyroid J* (2018) 7:27–33. doi: 10.1159/000484521

40. Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al. Serum vitamin D levels are decreased and associated with thyroid volume in female patients with newly onset Graves disease. *Endocrine.* (2012) 42:739–41. doi: 10.1007/s12020-012-9679-y

41. Veneti S, Anagnostis P, Adamidou F, Artzouchaltzi AM, Boboridis K, Kita M. Association between vitamin D receptor gene polymorphisms and Graves disease: a systematic review and meta-analysis. *Endocrine.* (2019) 65:244–51. doi: 10.1007/s12020-019-01902-3

42. Xu MY, Cao B, Yin J, Wang DF, Chen KL, Lu QB. Vitamin D and Graves disease: a meta-analysis update. *nutrients.* (2015) 7:3813–27. doi: 10.3390/nu7053813

43. Cho YY, Chung YJ. Vitamin D supplementation does not prevent the recurrence of Graves disease. *Sci Rep* (2020) 10:16. doi: 10.1038/s41598-019-55107-9

44. Grove-Laugesen D, Malmstroem S, Ebbehoj E, Riis AL, Watt T, Hansen KW, et al. Effect of 9 months of vitamin D supplementation on arterial stiffness and blood pressure in Graves disease: a randomized clinical trial. *Endocrine.* (2019) 66:386–97. doi: 10.1007/s12020-019-01997-8

45. Grove-Laugesen D, Cramon PK, Malmstroem S, Ebbehoj E, Watt T, Hansen KW, et al. Effects of supplemental vitamin D on muscle performance and quality of life in graves disease: a randomized clinical trial. *Thyroid.* (2020) 30:661–71. doi: 10.1089/thy.2019.0634

46. Klecha AJ, Barreiro Arcos ML, Frick L, Genaro AM, Cremaschi G. Immune-endocrine interactions in autoimmune thyroid diseases. *Neuroimmunomodulation.* (2008) 15(1):68–75. doi: 10.1159/000135626

47. Phenekos C, Vryonidou A, Gritzapis AD, Baxevanis CN, Goula M, Papamichail M. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). *Neuroimmunomodulation.* (2004) 11(4):209–13. doi: 10.1159/000078438

48. Kim D. The role of vitamin D in thyroid diseases. *Int J Mol Sci* (2017) 18(9):1949. doi: 10.3390/ijms18091949

49. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun* (2018) 9(1):260. doi: 10.1038/s41467-017-02662-2

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Ricardo Adrian Nugraha,
Airlangga University, Indonesia
Giuseppe Murdaca,
University of Genoa, Italy

*CORRESPONDENCE
Minghui Yang
yangminghui301@163.com
Jianye Li
15910617543@139.com

†These authors have contributed equally to this work and share first authorship

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A two-sample mendelian randomization analysis excludes causal relationships between non-alcoholic fatty liver disease and kidney stones

Xintao Li[1,2,3†], Yongpeng Xie[4†], Lu Tang[2†], Di Li[3], Jun Wang[3], Haibo Sheng[3], Kaikai Chen[3], Shuwei Xiao[3], Jianye Li[3*] and Minghui Yang[1*]

[1]Department of Traditional Chinese Medicine, The Sixth Medical Centre, Chinese People's Liberation Army General Hospital, Beijing, China, [2]Department of Urology, The Third Medical Center of Chinese People's Liberation Army (PLA) General Hospital, Beijing, China, [3]Department of Urology, Air Force Medical Center, Air Force Medical University, Beijing, China, [4]Department of Urology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Objectives: Non-alcoholic fatty liver disease (NAFLD) has been linked to an increased risk of kidney stones in prior observational studies, However, the results are inconsistent, and the causality remains to be established. We aimed to investigate the potential causal relationship between NAFLD and kidney stones using two-sample Mendelian randomization (MR).

Methods: Genetic instruments were used as proxies for NAFLD. Summary-level data for the associations of exposure-associated SNPs with kidney stones were obtained from the UK Biobank study (6536 cases and 388,508 controls) and the FinnGen consortium (9713 cases and 366,693 non-cases). MR methods were conducted, including inverse variance weighted method (IVW), MR-Egger, weighted median, and MR-PRESSO. MR-Egger Regression Intercept and Cochran's Q test were used to assess the directional pleiotropy and heterogeneity.

Results: cALT-associated NAFLD did not exhibit an association with kidney stones in the Inverse variance weighted (IVW) methods, in both the FinnGen consortium (OR: 1.02, 95%CI: 0.94-1.11, p = 0.632) and the UKBB study (OR: 1.000, 95%CI: 0.998-1.002, p = 0.852). The results were consistent in European ancestry (FinnGen OR: 1.05, 95%CI: 0.98-1.14, p = 0.144, UKBB OR: 1.000, 95%CI: 0.998-1.002, p = 0.859). IVW MR analysis also did not reveal a significant causal relationship between NAFLD and the risk of kidney stone for the other three NAFLD-related traits, including imaging-based, biopsy-confirmed NAFLD, and more stringent biopsy-confirmed NAFLD. The results remained consistent and robust in the sensitivity analysis.

Conclusions: The MR study did not provide sufficient evidence to support the causal associations of NAFLD with kidney stones.

KEYWORDS

NAFLD, kidney stone, Mendelian randomization, causality, genome wide association study

1 Introduction

Kidney stones, diverse in type and composition, affect approximately 15% of the population and have a high recurrence rate, with 50% of patients experiencing a recurrence within the first 5 years after the initial stone episode (1). This prevalence and recurrence impose a significant burden on healthcare resources and public health, with the total annual healthcare resources and public health, with the total annual healthcare expenditure for kidney stone treatment exceeding 2 billion dollars in the USA (2). The main cause of kidney stone disease lies in an imbalance of promoters and inhibitors of crystallization (3). Kidney stones can be classified based on their composition, with common types including calcium oxalate (65%), calcium phosphate (10%), uric acid (15%), magnesium ammonium phosphate (10%) and cystine stones (1%) (4). Comprehending the distinct characteristics of these stones is crucial for devising effective preventive and management strategies. Genetic variation, nutritional factors, and metabolic disorders play crucial roles in the pathogenesis of kidney stones.

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disease consisting of simple steatosis, non-alcoholic steatohepatitis, fibrosis and cirrhosis (5). It ranks among the most prevalent causes of chronic liver disease globally, impacting approximately 25% of the population worldwide (6, 7).

Several cross-sectional and prospective studies have consistently revealed a substantial rise in the prevalence of kidney stones among patients with NAFLD (8–12). Two meta-analyses have further consolidated the association between NAFLD and an elevated risk of urolithiasis (13, 14). Several potential mechanisms linking NAFLD to kidney stone formation have been proposed, primarily concerning hepatic steatosis, insulin resistance, and oxidative stress (15–18).

The observed links between NAFLD and kidney stones, as highlighted by previous epidemiological studies, are undoubtedly noteworthy. However, the question of whether these associations represent causal relationships remains undetermined. This uncertainty can be attributed to several potential limitations in the existing body of observational research, including residual confounding and other biases. NAFLD shares strong connections with risk factors for kidney stones, such as obesity and type 2 diabetes (19). These overlapping risk factors could confound the relationship between NAFLD and kidney stones in observational studies.

Mendelian randomization (MR) is a powerful tool for inferring causality in observational research. As individuals are randomized at conception to receive genetic variants that either predispose to or protect from the exposure of interest, these variants can be used as instruments to study for a causal relationship with a clinically relevant outcome (20). MR is considered less susceptible to biases stemming from confounding factors and reverse causality compared to traditional observational studies (21).

In our study, we employ a two-sample MR approach to explore the potential causal links between NAFLD and the risk of developing kidney stones. This method offers a robust framework for examining these associations, minimizing the impact of common biases encountered in observational research.

2 Materials and methods

2.1 Study design

Our study adopts a two-sample MR approach, as depicted in Figure 1, employing single nucleotide polymorphisms (SNPs) as instrumental variables (IVs). The primary objective is to assess the causal link between NAFLD and the risk of kidney stones. This method relies on three key assumptions (1): the SNPs must exhibit a robust association with NAFLD (2), the SNPs should not exert an influence on confounding factors that could affect the association between the exposure (NAFLD) and the outcome (kidney stones),

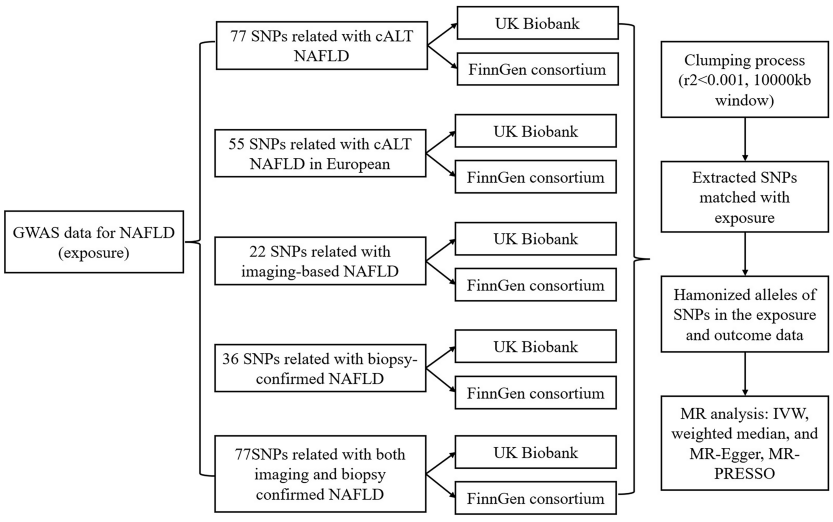

FIGURE 1
Workflow of Mendelian randomization study revealing causality from NAFLD on kidney stones. NAFLD, Non-alcoholic fatty liver disease; SNP, single-nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; IVW, inverse variance weighted; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual Sum and outlier.

and (3) the SNPs should solely impact the outcome through their effect on the exposure and not through any other pathways.

2.2 The data source for NAFLD and the selection of IVs

All the databases utilized for gene-exposure and gene-outcome data were shown in Table 1. The data source for NAFLD and the selection of IVs were derived from the Million Veteran Program (MVP) consortium, which commenced participant recruitment in 2011 and has evolved into one of the world's largest biobanks (26). Gene-exposure data were obtained from a recent genome-wide association study (GWAS) within the MVP consortium, where NAFLD was defined by (1): elevated ALT>40 U liter^{-1} for men or >30 U liter^{-1} for women during at least two time points at least 6 months apart within a 2-year window at any point prior to enrollment and (2) exclusion of other causes of liver disease, chronic liver diseases or systemic conditions and/or alcohol use disorders (22). This comprehensive study identified 77 independent SNPs with genome-wide significance (p < 5×10^-8) in the discovery cohort, which included 90,408 cases of chronically elevated ALT (cALT) and 128,187 controls. In the European ancestry analysis including 68725 cases and 95472 controls, 55 independent significant SNPs were identified. Of all the 77 SNPs, 22 and 36 SNPs from this initial set were further validated in two external cohorts. The first cohort comprised individuals with liver fat quantified via imaging (either computed tomography or magnetic resonance imaging), totaling 44,289 participants. The second cohort consisted of individuals with biopsy-confirmed NAFLD, comprising 7,397 cases and 56,785 controls. Impressively, 17 of the 77 cALT SNPs demonstrated nominal significance in both the imaging and biopsy-confirmed NAFLD cohorts.

Five sets of IVs were extracted for consideration (1): All cALT-associated SNPs (n=77, p<5×10^−8): This set included all SNPs that exhibited a strong association with cALT levels, surpassing the genome-wide significance threshold (2). cALT-associated SNPs (n=55, p<5×10^−8) in European ancestry discovery analysis (3). cALT-associated SNPs with nominal significance and directional concordance in the imaging cohorts (n=22, p<0.05): This subset comprised SNPs that not only displayed nominal significance in relation to cALT but also exhibited a consistent directional association in the imaging cohorts. Importantly, the effect estimates for the imaging data (expressed as Z-scores) were employed for this analysis (4). cALT-associated SNPs with Nominal Significance and Directional Concordance in the Biopsy Cohorts (n=36, p<0.05): This group consisted of SNPs that achieved nominal significance with cALT and maintained consistent directional concordance in the biopsy cohorts. Here, the effect estimates were represented as biopsy-confirmed NAFLD (yes/no) (5). cALT-associated SNPs with nominal significance and directional concordance in both the imaging and biopsy cohorts (n=17, p<0.05): SNPs in this category satisfied the criteria of nominal significance and directional agreement with both imaging and biopsy cohorts. The effect estimates for this analysis were also expressed as biopsy-confirmed NAFLD (yes/no).

SNPs were disregarded if they exhibited linkage disequilibrium (r^2>0.001 and clump_distance<10,000kb), were palindromic with intermediate allele frequencies, or were unavailable in the outcome GWAS data. Furthermore, proxy SNPs were not included in the analysis. To assess the strength of the IVs, F statistics were calculated, with only SNPs possessing an F statistic exceeding 10 being deemed valid and reliable IVs for NAFLD.

2.3 Outcome data

The outcome data for the associations of NAFLD-associated was derived from the UK Biobank study (23) and the FinnGen consortium (25). In UK Biobank, cases with kidney stones were

TABLE 1 Databases utilized for gene-exposure and gene-outcome data.

GWAS dataset	Phenotype	Sample size	Adjustment variables	Ethnicity
GWAS with the MVP consortium (22)	cALT (yes/no)	90,408 cases and 128,187 controls	Age, gender, audit-C and first 10 principal components.	European-American, African-American, Hispanic-American and Asian-American
	cALT (yes/no)	68725 cases and 95472 controls	Age, gender, audit-C and first 10 principal components of ancestry	European-American
	Imaging-based NAFLD (Z-scores)	44,289	Age, gender, and first 10 principal components.	European-American, African-American and Hispanic American
	biopsy-confirmed NAFLD (yes/no)	7,397 cases and 56,785 controls	Age, gender, and first 10 principal components.	European-American and Hispanic American
UK Biobank (23, 24)	Kidney stones	6,536 cases and 388,508 controls	Age, sex, and the genotyping platform	European ancestry
FinnGen consortium (25)	Kidney stones	9,713 cases and 376,406 controls	Age, sex, genetic principal components, and genotyping batch.	European ancestry

GWAS, genome-wide association study; MVP, Million Veteran Program; cALT, chronically elevated alanine transaminase; NAFLD, Non-alcoholic fatty liver disease.

defined by the International Classification of Diseases, 10th Revision (ICD-10), Office of Population and Censuses Surveys, and self-reported operation codes. GWAS was performed on 6,536 cases and 388,508 controls of European ancestry with the adjustment for sex, age, and the genotyping platform (24).

The FinnGen consortium provided the second source of outcome data. In the latest release 9 (https://r9.finngen.fi/), this dataset KSD (N14_CALCUKIDUR) comprised a remarkable 9,713 individuals who had experienced kidney stone formation, as well as 376,406 healthy controls, all of European ancestry. The dataset underwent association tests that were meticulously adjusted for various factors, including age, sex, genetic principal components, and genotyping batch. It is noteworthy that individuals who had chosen to withdraw their consent were thoughtfully excluded from the dataset.

2.4 Statistical analysis

After harmonization of the effect alleles of NAFLD and kidney stones, we used the following MR approaches to determine MR estimates of NAFLD for kidney stones: the Inverse variance weighted (IVW), weighted median, and MR-Egger (1). IVW meta-analysis: This method was employed as the primary approach to estimate the causal relationship between NAFLD and kidney stones. For exposures instrumented by at least 3 SNPs, the IVW method under a multiplicative random-effects model was used as the primary statistical method; otherwise, the IVW fixed-effects method was applied. It utilizes the Wald ratio for individual SNPs and assumes that IVs only influence the outcome (kidney stones) through the exposure of interest (NAFLD) (27) (2). Weighted median methods: In addition to the IVW, the weighted median method was used to provide more robust estimates in a broader range of scenarios, even though it might yield wider confidence intervals (28).

Sensitivity analysis is an essential component of MR analysis to detect pleiotropy and ensure the reliability of the results. We conducted several sensitivity tests, including (1): Cochran Q derived p value threshold: A threshold of less than 0.05 was used from the IVW method to assess the heterogeneity among estimates of SNPs in each analysis (2). MR-Egger Regression Intercept: This was used to detect horizontal pleiotropy, with a threshold of less than 0.05 indicating the presence of pleiotropy (29) (3). MR-Pleiotropy Residual Sum and Outlier Methods (MR-PRESSO): MR-PRESSO was employed to identify and correct horizontal pleiotropy through outlier removal, and MR-PRESSO global test was used to detect horizontal pleiotropy (28). It is known for its accuracy when the proportion of horizontal pleiotropy variants is less than 10% (30).

We estimated R^2, representing the proportion of IVs that could explain each kidney stone event. Statistical power was calculated using an online tool (https://shiny.cnsgenomics.com/mRnd/) (31). The results and calculation methods are listed in Supplementary Table 2. The TwoSample MR package (version 0.5.7) in the R software (version 4.3.1) was employed to conduct all the analyses.

3 Results

The selection process of all IVs in each group was detailed in Supplementary Table 1. The F-statistic range for the association between NAFLD and the GWAS conducted in the MVP consortium was robust, ranging from 26.4 to 1113.9, signifying the excellent strength of the IVs (Supplementary Table 2).

As shown in Figure 2 and Supplementary Table 3, for the cALT-associated SNPs, IVW MR analysis with a random-effects model demonstrated no significant causal relationship between NAFLD and the risk of kidney stones in both the FinnGen consortium and the UK Biobank (UKBB) study (FinnGen: OR: 1.02, 95% CI: 0.94-1.11, p = 0.632; UKBB: OR: 1.000, 95% CI: 0.998-1.002, p = 0.852). While for participants of European ancestry, the results remained consistent (FinnGen: OR: 1.05, 95% CI: 0.98-1.14, p = 0.144; UKBB: OR: 1.000, 95% CI: 0.998-1.002, p = 0.859). Consistent results were observed with the weighted median and MR-Egger methods. MR-Egger regression analysis indicated no significant intercept in either the FinnGen consortium (All ancestries: p-value 0.618, European ancestries: p-value 0.365) or the UKBB study (All ancestries: p-value 0.252, European ancestries: p-value 0.322), suggesting no evidence of pleiotropy. Cochran's Q test revealed potential SNP heterogeneity using the IVW method in both outcome databases. For participants with all ancestries, MR-PRESSO analyses identified one outlier in the FinnGen consortium and one in the UKBB study, respectively. Notably, the association remained robust even after the removal of these outliers (FinnGen: OR: 1.04, 95% CI: 0.96-1.12, p = 0.372; UKBB: OR: 1.000, 95% CI: 0.999-1.002, p = 0.662). For participants with European ancestry, MR-PRESSO analyses identified one outlier in the UKBB study. The association persisted robustly upon excluding the outliers (OR: 1.000, 95% CI: 0.999-1.002, p = 0.642).

For the other three groups of NAFLD-related SNPs, IVW MR analysis also showed no significant causal relationship between NAFLD and the risk of kidney stones in both the FinnGen consortium and the UKBB study. Weighted median and MR-Egger methods yielded consistent results. MR-Egger regression analysis showed no statistically significant intercept, except for the subgroup analysis between biopsy-confirmed NAFLD and the risk of kidney stones in the UKBB study (p = 0.005), rendering the result invalid in this subgroup. In the FinnGen consortium, Cochran's Q test indicated significant SNP heterogeneity for all three group analyses. MR-PRESSO analyses identified one outlier for each MR analysis using imaging-based NAFLD IVs, Biopsy-confirmed NAFLD IVs, and imaging and biopsy-confirmed NAFLD IVs. The MR-PRESSO global test outcomes revealed controlled false positive rates of approximately 5% in the majority of analyses. However, an exception was noted in the analysis that incorporated SNPs associated with biopsy-confirmed NAFLD and those related to imaging and biopsy-confirmed NAFLD in the UKBB database. Remarkably, the association remained consistent even after outlier removal. In the UKBB study, Cochran's Q test revealed mild SNP heterogeneity in MR analysis using imaging-based NAFLD IVs, but no outliers were identified using MR-PRESSO analysis.

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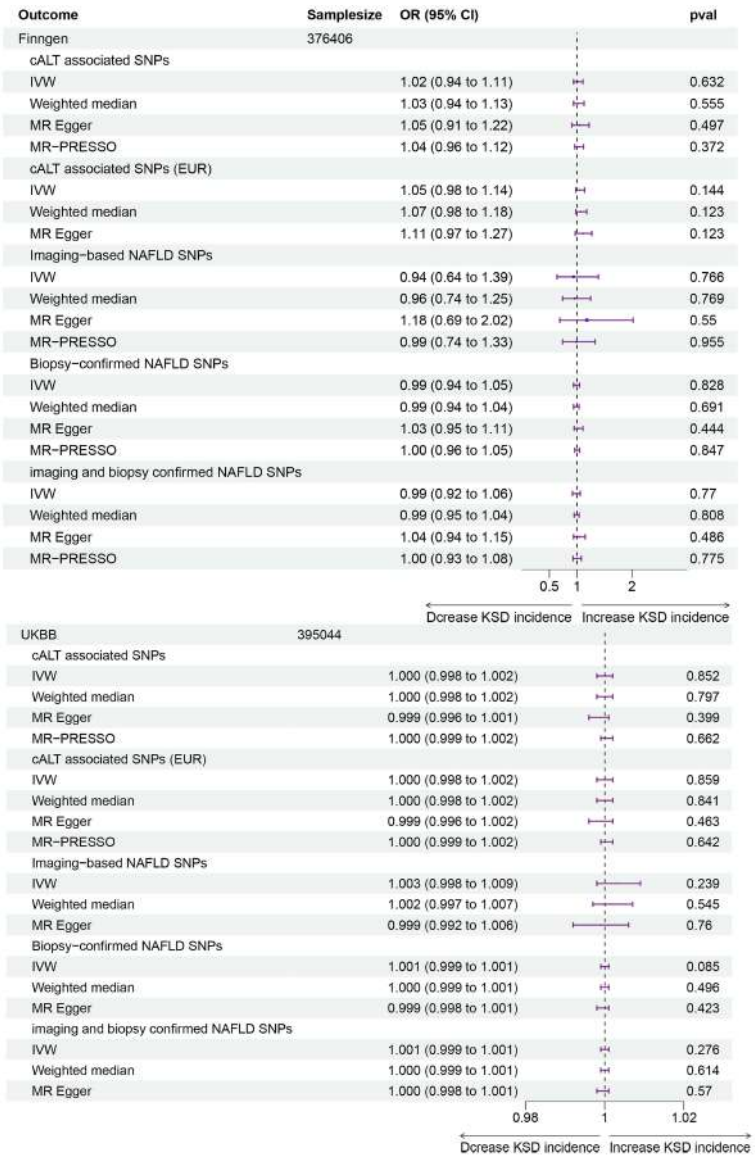

FIGURE 2
Forest plot for associations of NAFLD and kidney stones in the FinnGen consortium and UK Biobank study. NAFLD, Non-alcoholic fatty liver disease; OR, Odds ratio; CI, Confidence interval; SNP, single-nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; IVW, inverse variance weighted; MR, medelian randomization; MR-PRESSO, MR pleiotropy residual Sum and outlier.

All the forest plots, scatter plots, funnel plots and leave-one-out plots were shown in Supplementary Figures 1-10. The leave-one-out sensitivity analysis revealed that no single SNP notably challenged the overall impact of NAFLD on urolithiasis.

4 Discussion

Our MR study analysis do not provide sufficient evidence of significant associations of genetically predicted NAFLD and risk of kidney stones, which was different from most but not all observational studies. In a previous systematic review and meta-analysis, large-scale, population-based studies found that NAFLD was associated with an increased risk of kidney stones (13). Additionally, another two meta-analysis reached similar conclusions, and subgroup analysis suggested a stronger association when diagnostic criteria based on computed tomography were used (14, 32). However, discrepancies remained in the literature. One prospective cohort study with a large sample size concluded that NAFLD was associated with an increased incidence of nephrolithiasis in men but not in women (11). Conversely, a recent study utilizing NHANES data reported an association between NAFLD and an increased risk of nephrolithiasis, but this association was observed only in women (33). In a noteworthy parallel, Zeina's and Wei's study, they reported that the association between fatty liver and nephrolithiasis remained significant, albeit with a reduced effect size, after adjusting for other confounding factors (8, 9). This

finding suggests that while the direct causal link between NAFLD and kidney stones might not be strong, there could still be a nuanced connection influenced by multiple factors.

It is worth noting that several mechanisms have been proposed to explain the potential link between NAFLD and kidney stone formation. On one hand, it has been reported that NAFLD may lead to changes in urinary constituents, potentially increasing the risk of stone formation. Studies suggest that metabolic defects associated with NAFLD, such as impaired glyoxylate detoxification, could contribute to the development of hyperoxaluria, a known risk factor for kidney stones (34, 35). On the other hand, NAFLD is characterized by increased levels of proinflammatory molecules and lipotoxicity, which might also play a role in kidney stone formation. Inflammatory processes and lipotoxic effects could potentially contribute to the pathogenesis of calcium oxalate nephrolithiasis (36, 37).

However, it is essential to consider several potential factors that might account for the discrepancies observed in previous observational studies. First and foremost, the majority of these studies employed a cross-sectional study design, which inherently lacks the capacity to establish causal relationships. Moreover, many previous systematic reviews and meta-analyses also relied heavily on cross-sectional data when reporting an increased risk of kidney stones in individuals with NAFLD. This reliance on cross-sectional studies could introduce a level of bias and complicate the interpretation of causality, given the limitations of such study designs. Another crucial aspect to consider is the presence of classic metabolic risk factors for NAFLD, including obesity, hypertension, diabetes mellitus, and metabolic syndrome. These factors have gained recognition as predisposing elements for urolithiasis in their own right (38). This shared association between these metabolic risk factors and kidney stone formation raises the possibility of confounding variables in observational studies. Furthermore, a significant limitation in many previous studies is the lack of comprehensive multivariable analyses that adjust for all relevant confounding factors. Finally, retrospective study designs and the potential for selection bias in these studies may further impact the accuracy and reliability of the results.

Our study possesses several noteworthy strengths that merit discussion. The foremost strength lies in the MR design employed, which enhances the capacity for causal inference in examining the associations between NAFLD and the risk of kidney stones. MR leverages genetic IVs, minimizing the potential for reverse causality and unmeasured confounding, thereby strengthening the validity of our findings. Second, our study benefited from the use of large-scale, summary-level data from the GWAS within the MVP consortium, ensuring sufficient IV strength. The range of F statistics, a measure of IV strength, for the association between NAFLD and kidney stones (ranging from 26.4 to 1113.9) underscores the robustness of the chosen IVs. Third, we incorporated gene-exposure data for four distinct NAFLD-related traits, including cALT-confirmed NAFLD, imaging-related NAFLD, biopsy-confirmed NAFLD, and imaging and biopsy-confirmed NAFLD. The gene-exposure data for cALT-confirmed NAFLD in European ancestry was analyzed separately. Notably, the consistent lack of significant causal associations between NAFLD, as defined by these alternative genetic instruments, and the risk of kidney stones reinforces the conclusion that NAFLD is not causally linked to kidney stone development. Finally, we examined these associations on two independent populations, and the consistent results guaranteed the robustness of findings.

However, our study is not without its limitations. One limitation pertains to the restriction of the studied population to individuals of European ancestry in the outcome database. While this choice facilitated the internal validity of our study, it might limit the generalizability of our findings to other populations. To enhance the external validity of our conclusions, future research should explore the relationship between NAFLD and kidney stones in diverse populations. Another notable limitation is the lack of distinction between different histological stages of NAFLD in the original GWAS data. This differentiation is of significance, as certain histological features, particularly fibrosis, have been specifically associated with kidney stone formation (39, 40). Future research might benefit from a more nuanced analysis that considers the histological heterogeneity of NAFLD in the context of kidney stone risk. Furthermore, the outcome database lacked information about the types of kidney stones, limiting the analysis of the causal relationship between NAFLD and kidney stones of specific compositions. Finally, our study relied on summary-level data, precluding the performance of subgroup analyses, such as stratification by sex or ethnicity. Such subgroup analyses have been conducted in previous observational studies and could provide valuable insights into potential variations in the association (11, 33).

The interpretation of our MR study warrants careful consideration. While our findings suggest no significant causal association between NAFLD and kidney stones, it is imperative to acknowledge the limitations inherent in our study design, such as the reliance on summary-level data and the absence of distinction between different histological stages of NAFLD. In addition, the gene-environment equivalence assumption must be approached with caution. The validity of our MR estimates relies on the assumption that genetic variants used as instruments influence the outcome (kidney stones) solely through their impact on the exposure (NAFLD). While this assumption is theoretically sound, it is crucial to recognize that the inherent complexity of biological processes may introduce nuances not fully captured by our genetic instruments. Therefore, cautious interpretation is warranted. We incorporated MR-PRESSO global test results into our analysis, providing insights into the performance of MR methods in detecting horizontal pleiotropy. The controlled false positive rates of approximately 5%, observed in most analyses, enhance the robustness of our conclusions. However, the deviation noted in the analysis involving SNPs related to Imaging and biopsy-confirmed NAFLD in the UKBB database emphasizes the importance of cautious interpretation in this specific context. From a clinical perspective, our findings underscore the importance of a comprehensive patient assessment when evaluating the risk of kidney stones in individuals with NAFLD. Healthcare providers should consider a wide range of risk factors, including metabolic, dietary, and genetic factors.

In conclusion, the comprehensive MR analysis conducted in this study fails to provide compelling evidence of a causal

association between NAFLD and an increased risk of kidney stones. The solidity of our IVs, the absence of pleiotropy, and the persistence of our results after the removal of outliers collectively underscore the strength and stability of our conclusions. This study challenges conventional assumptions and substantially contributes to our comprehension of the complex interplay between NAFLD and kidney stones. While our findings do not substantiate a direct causal link, they prompt further exploration of the multifaceted factors involved in the relationship between NAFLD and kidney stones.

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

All studies included in cited genome-wide association studies had been approved by a relevant review board. The MVP received ethical and study protocol approval from the VA Central Institutional Review Board (IRB) in accordance with the principles outlined in the Declaration of Helsinki. UK Biobank has approval from the North West Multi-Centre Research Ethics Committee (11/NW/0382), and the study (Epidemiology of Kidney Stone Disease), which provided the GWAS data in the present study, has UK Biobank study ID 885. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) approved the FinnGen study protocol (number HUS/990/2017). Participants in FinnGen provided informed consent for biobank research on basis of the Finnish Biobank Act.

Author contributions

XL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. YX: Conceptualization, Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. LT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. DL: Conceptualization, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. JW: Conceptualization, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. HS: Conceptualization, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. KC: Conceptualization, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. SX: Conceptualization, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. JL: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. MY: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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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.2023.1343367/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Mendelian randomization plots of SNPs associated with cALT-associated NAFLD and kidney stones in the FinnGen consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 2
Mendelian randomization plots of SNPs associated with cALT-associated NAFLD and kidney stones in the UK Biobank consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-

nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 3
Mendelian randomization plots of SNPs associated with European cALT-associated NAFLD and kidney stones in the FinnGen consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 4
Mendelian randomization plots of SNPs associated with European cALT-associated NAFLD and kidney stones in the UK Biobank consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; cALT, chronically elevated serum alanine aminotransferase levels; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 5
Mendelian randomization plots of SNPs associated with biopsy-confirmed NAFLD and kidney stones in the FinnGen consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 6
Mendelian randomization plots of SNPs associated with biopsy-confirmed NAFLD and kidney stones in the UK Biobank consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 7
Mendelian randomization plots of SNPs associated with imaging-based NAFLD and kidney stones in the FinnGen consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 8
Mendelian randomization plots of SNPs associated with imaging-based NAFLD and kidney stones in the UK Biobank consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 9
Mendelian randomization plots of SNPs associated with imaging and biopsy-confirmed NAFLD and kidney stones in the FinnGen consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

SUPPLEMENTARY FIGURE 10
Mendelian randomization plots of SNPs associated with imaging and biopsy-confirmed NAFLD and kidney stones in the UK Biobank consortium. **(A)** Forest plot; **(B)** Scatter plot; **(C)** Funnel plot; **(D)** leave-one-out forest plot. SNP, single-nucleotide polymorphisms; NAFLD, Non-alcoholic fatty liver disease.

References

1. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. *Nat Rev Dis Primers* (2016) 2:16008. doi: 10.1038/nrdp.2016.8

2. Thongprayoon C, Krambeck AE, Rule AD. Determining the true burden of kidney stone disease. *Nat Rev Nephrol* (2020) 16(12):736–46. doi: 10.1038/s41581-020-0320-7

3. Li H, Shkolyar E, Wang J, Conti S, Pao AC, Liao JC, et al. SLIPS-LAB-A bioinspired bioanalysis system for metabolic evaluation of urinary stone disease. *Sci Adv* (2020) 6(21):eaba8535. doi: 10.1126/sciadv.aba8535

4. Howles SA, Thakker RV. Genetics of kidney stone disease. *Nat Rev Urol* (2020) 17 (7):407–21. doi: 10.1038/s41585-020-0332-x

5. European Association for the Study of the L, European Association for the Study of D and European Association for the Study of O.. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* (2016) 64(6):1388–402. doi: 10.1016/j.jhep.2015.11.004

6. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* (2018) 67(1):328–57. doi: 10.1002/hep.29367

7. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* (2018) 15(1):11–20. doi: 10.1038/nrgastro.2017.109

8. Wei YP, Lin XG, He RQ, Shen J, Sun SL, Chen G, et al. Epidemiologic association of nonalcoholic fatty liver disease and urinary calculi: a population-based cross-sectional study in southern China. *Iran J Kidney Dis* (2018) 12(2):112–9.

9. Zeina AR, Goldenberg L, Nachtigal A, Hasadia R, Saliba W. Association between nephrolithiasis and fatty liver detected on non-enhanced CT for clinically suspected renal colic. *Clin Imaging* (2017) 43:148–52. doi: 10.1016/j.clinimag.2017.03.011

10. Nam IC. Association of non-alcoholic fatty liver disease with renal stone disease detected on computed tomography. *Eur J Radiol Open* (2016) 3:195–9. doi: 10.1016/j.ejro.2016.07.004

11. Kim S, Chang Y, Sung E, Kim CH, Yun KE, Jung HS, et al. Non-alcoholic fatty liver disease and the development of nephrolithiasis: A cohort study. *PloS One* (2017) 12(10):e0184506. doi: 10.1371/journal.pone.0184506

12. Einollahi B, Naghii MR, Sepandi M. Association of nonalcoholic fatty liver disease (NAFLD) with urolithiasis. *Endocr Regul* (2013) 47(1):27–32. doi: 10.4149/endo_2013_01_27

13. Qin S, Wang S, Wang X, Wang J. Non-alcoholic fatty liver disease and the risk of urolithiasis: A systematic review and meta-analysis. *Med (Baltimore)* (2018) 97(35): e12092. doi: 10.1097/MD.0000000000012092

14. Wijarnpreecha K, Lou S, Panjawatanan P, Sanguankeo A, Pungpapong S, Lukens FJ, et al. Nonalcoholic fatty liver disease and urolithiasis. A systematic review and meta-analysis. *J Gastrointestin Liver Dis* (2018) 27(4):427–32. doi: 10.15403/jgld.2014.1121.274.nac

15. Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* (2013) 14(10):20704–28. doi: 10.3390/ijms141020704

16. Tariq Z, Green CJ, Hodson L. Are oxidative stress mechanisms the common denominator in the progression from hepatic steatosis towards non-alcoholic steatohepatitis (NASH)? *Liver Int* (2014) 34(7):e180–90. doi: 10.1111/liv.12523

17. Sakaida I, Okita K. The role of oxidative stress in NASH and fatty liver model. *Hepatol Res* (2005) 33(2):128–31. doi: 10.1016/j.hepres.2005.09.019

18. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nat Rev Endocrinol* (2017) 13(9):509–20. doi: 10.1038/nrendo.2017.56

19. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* (2017) 66(6):1138–53. doi: 10.1136/gutjnl-2017-313884

20. Burgess S, Foley CN, Zuber V. Inferring causal relationships between risk factors and outcomes from genome-wide association study data. *Annu Rev Genomics Hum Genet* (2018) 19:303–27. doi: 10.1146/annurev-genom-083117-021731

21. van Kippersluis H, Rietveld CA. Pleiotropy-robust mendelian randomization. *Int J Epidemiol* (2018) 47(4):1279–88. doi: 10.1093/ije/dyx002

22. Vujkovic M, Ramdas S, Lorenz KM, Guo X, Darlay R, Cordell HJ, et al. A multiancestry genome-wide association study of unexplained chronic ALT elevation as a proxy for nonalcoholic fatty liver disease with histological and radiological validation. *Nat Genet* (2022) 54(6):761–71. doi: 10.1038/s41588-022-01078-z

23. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PloS Med* (2015) 12(3):e1001779. doi: 10.1371/journal.pmed.1001779

24. Howles SA, Wiberg A, Goldsworthy M, Bayliss AL, Gluck AK, Ng M, et al. Genetic variants of calcium and vitamin D metabolism in kidney stone disease. *Nat Commun* (2019) 10(1):5175. doi: 10.1038/s41467-019-13145-x

25. Kurki MI, Karjalainen J, Palta P, Sipila TP, Kristiansson K, Donner KM, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature* (2023) 613(7944):508–18. doi: 10.1038/s41586-022-05473-8

26. Gaziano JM, Concato J, Brophy M, Fiore L, Pyarajan S, Breeling J, et al. Million Veteran Program: A mega-biobank to study genetic influences on health and disease. *J Clin Epidemiol* (2016) 70:214–23. doi: 10.1016/j.jclinepi.2015.09.016

27. 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(2):512–25. doi: 10.1093/ije/dyv080

28. Ong JS, MacGregor S. Implementing MR-PRESSO and GCTA-GSMR for pleiotropy assessment in Mendelian randomization studies from a practitioner's perspective. *Genet Epidemiol* (2019) 43(6):609–16. doi: 10.1002/gepi.22207

29. 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

30. Verbanck M, Chen CY, 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(5):693–8. doi: 10.1038/s41588-018-0099-7

31. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol* (2013) 42(5):1497–501. doi: 10.1093/ije/dyt179

32. Yi M, Peng W, Feng X, Teng F, Tang Y, Kong Q, et al. Extrahepatic morbidities and mortality of NAFLD: an umbrella review of meta-analyses. *Aliment Pharmacol Ther* (2022) 56(7):1119–30. doi: 10.1111/apt.17165

33. Decker RD, Ghiraldi EM, Weiss AH, Gaughan JP, Friedlander JI. Nonalcoholic fatty liver disease is an independent risk factor for nephrolithiasis in women: findings from NHANES III. *J Endourol* (2020) 34(12):1258–62. doi: 10.1089/end.2020.0486

34. Tasca A, Ammendola C. [Nephrolithiasis: metabolic defects and terapeutic implications]. *Urologia* (2014) 81(1):1–11. doi: 10.5301/uro.5000058

35. Gianmoena K, Gasparoni N, Jashari A, Gabrys P, Grgas K, Ghallab A, et al. Epigenomic and transcriptional profiling identifies impaired glyoxylate detoxification in NAFLD as a risk factor for hyperoxaluria. *Cell Rep* (2021) 36(8):109526. doi: 10.1016/j.celrep.2021.109526

36. Khan SR. Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. *Transl Androl Urol* (2014) 3(3):256–76. doi: 10.3978/j.issn.2223-4683.2014.06.04

37. Yu J, Marsh S, Hu J, Feng W, Wu C. The pathogenesis of nonalcoholic fatty liver disease: interplay between diet, gut microbiota, and genetic background. *Gastroenterol Res Pract* (2016) 2016:2862173. doi: 10.1155/2016/2862173

38. Besiroglu H, Otunctemur A, Ozbek E. The metabolic syndrome and urolithiasis: a systematic review and meta-analysis. *Ren Fail* (2015) 37(1):1–6. doi: 10.3109/0886022X.2014.976133

39. Qin S, Wang J, Zhou C, Zhang Y, Xu Y, Wang X, et al. The association between a non-invasive hepatic fibrosis score and urolithiasis among non-alcoholic fatty liver disease (NAFLD) patients in China: a cross-sectional study. *BMJ Open* (2019) 9(8):e027702. doi: 10.1136/bmjopen-2018-027702

40. Qin S, Wang J, Zhou C, Zhang Y, Xu Y, Wang X, et al. The severity of NAFLD is associated with the risk of urolithiasis. *Br J BioMed Sci* (2019) 76(2):53–8. doi: 10.1080/09674845.2018.1548743

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EDITED BY
Luigi Di Filippo,
San Raffaele Hospital (IRCCS), Italy

REVIEWED BY
Sara Menotti,
Vita-Salute San Raffaele University, Italy
Rizaldy Taslim Pinzon,
Duta Wacana Christian University, Indonesia

*CORRESPONDENCE
Mei Yang
✉ yangm1047449506@sina.com

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Trends of serum 25(OH) vitamin D and association with cardiovascular disease and all-cause mortality: from NHANES survey cycles 2001–2018

Changlun Hu and Mei Yang*

Department of Internal Medicine, Chongqing Nanan District Traditional Chinese and Western Medicine Hospital, Chongqing, China

Background: The focus of this survey is on survey data for adults aged 20 and above, covering nine survey cycles from 2001 to 2018. Additionally, the present study explored the correlation between vitamin D concentrations and both cardiovascular disease (CVD) and all-cause mortality.

Objective: The objectives of this study were to evaluate the trend of changes in the serum 25(OH)D concentration changes in US adults during the survey period, the prevalence of vitamin D deficiency, and the current status of vitamin D dietary intake and supplementation.

Methods: In-home health interviews were performed using meticulously designed questionnaires that gathered information on demographic details, socioeconomic conditions, dietary patterns, and overall health status. Health assessments were conducted in specially designed mobile centers.[1]

Results: Survey data from 2001 to 2018 revealed a rise in serum 25(OH)D levels, from a weighted mean (95% CI) of 65.6 (63.8–67.4) nmol/L during 2001–2002 to 73.5 (70.4–76.5) nmol/L during 2017–2018, among US adults, while overall vitamin D deficiency rates remained stable ($p = 0.152$). Notably, in adults aged 20–39, 25(OH)D levels decreased ($p = 0.002$ for trend), and 25(OH)D deficiency increased ($p = 0.003$ for trend), especially among those with low incomes (deficiency >30%). Upon multivariable adjustment, an L-shaped relationship was found between serum 25(OH)D concentrations and both CVD and all-cause mortality ($p < 0.001$ for nonlinearity), as corroborated by sensitivity analyses.

Conclusion: From 2001 to 2018, US adults experienced a significant increase in their serum 25(OH) D concentration. However, subgroups of individuals, including young adults and individuals with lower socioeconomic status, exhibited a heightened risk of 25(OH)D deficiency. Furthermore, an L-shaped relationship was found between 25(OH)D concentration and both all-cause and CVD mortality among US adults.

KEYWORDS

serum vitamin D concentration, all-cause mortality, CVD mortality, NHANES, trends

1 https://wwwn.cdc.gov/nchs/nhanes/tutorials/default.aspx

1 Introduction

Vitamin D, a lipophilic nutrient, is primarily obtained from dietary intake and is synthesized in skin tissue. This nutrient plays a crucial role in several vital physiological processes, including calcium and phosphate homeostasis, bone metabolism, immune modulation, and diverse cellular activities (1, 2). Deficits in the serum 25(OH)D concentration have been associated with osteoporosis, metabolic syndrome, cardiovascular disease, chronic kidney disease, asthma, and respiratory tract infections (3–5). Hence, exploring the impacts of serum 25(OH)D concentration, vitamin D ingestion, and supplementation on public health is a significant scientific endeavor.

An investigation from 1988 to 2010 demonstrated an increasing trend in the serum 25(OH)D concentration over time, with disparities among races, sexes, and age groups (6). Another study investigated the trends in vitamin D deficiency from 2001 to 2018 using NHANES data. However, this study did not explore the trends in vitamin D concentrations or deficiency rates over time across different age groups, educational levels, or income strata. Understanding these trends is essential for obtaining comprehensive insight into the public's nutritional status (7). In addition, a large number of randomized controlled trials (RCTs) and meta-analyses have reported the impact of vitamin D supplementation on disease progression and specific mortality (8–11). However, the existing evidence from RCTs and meta-analyses only supports the survival benefits of vitamin D supplementation in targeted populations, such as elderly COVID-19 patients or cancer sufferers (12, 13), but fails to reveal benefits for unscreened populations in clinical studies (11). In fact, due to ethical considerations, existing clinical evidence cannot be used to determine whether vitamin D supplementation can benefit patients with vitamin D deficiency. Therefore, utilizing cycle-based nutritional survey data to investigate the relationship between the serum vitamin D concentration and mortality can serve as an important complement to RCT research.

The National Health and Nutrition Examination Survey (NHANES) is a key resource for public health research and has provided health and nutritional data for the US population since 1960 (14). This database provides valuable research data, such as serum 25(OH)D concentration, dietary and supplementary vitamin D intake, and related mortality data. Our study focused mainly on the trend of changes in the serum 25(OH)D concentration (25(OH)D2 + 25(OH) D3) in the US population, the prevalence of 25 (OHD) deficiency throughout the NHANES survey cycle, and the trend of vitamin D intake through diet and supplementation. Additionally, we also investigated the associations between serum 25(OH)D levels and cardiovascular disease (CVD) and all-cause mortality. Its purpose is to provide guidance for population health management.

2 Methods

2.1 Study design and population

The NHANES study protocol was approved by the Research Ethics Review Board of the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. In-home health interviews were performed using meticulously designed questionnaires that gathered information on demographic details, socioeconomic conditions, dietary patterns, and overall health status. Health assessments were conducted in specially designed mobile centers. These evaluations included comprehensive evaluations of medical, dental, and physiological parameters and were, supplemented by laboratory tests performed by a professional medical team (14). We used open access data from the NHANES database. There was no financial incentive or reward for participation in the NHANES project. Informed consent form was signed by all participants at the time of their recruitment. In alignment with National Institutes of Health (NIH) guidelines, our study did not involve direct interaction with the participants and hence was not categorized as involving human subjects. The focus of this survey is on survey data for adults aged 20 and above, covering nine survey cycles from 2001 to 2018. The objectives of this study included evaluating the trend of changes in the serum 25(OH)D concentration changes in US adults during the survey period, the prevalence of vitamin D deficiency, and the current status of vitamin D dietary intake and supplementation. Additionally, present the study examined the correlation between vitamin D concentrations and both CVD and all-cause mortality.

2.2 Evaluation of serum 25(OH)D levels and definition of 25(OH)D deficiency

The methods used to determine the serum 25 (OH) D concentrations and regression used equations are detailed in https://wwwn.cdc.gov/nchs/nhanes/vitamind/analyticalnote.aspx. Based on previous reports, this study defined vitamin D deficiency as a serum 25 (OH) D level less than 50 nmol/L (15).

2.3 Assessment of vitamin D intake

To gather dietary information, participants engaged in 24-h dietary recall interviews. The initial interview was performed at the Mobile Examination Center (MEC), followed by a telephone interview 3 to 10 days later. This process provides a reliable means to survey the dietary habits of the study population. Dietary and supplementary intake data on vitamin D from individuals were collected on the first day of the interviews.

2.4 Extraction of CVD and all-cause mortality

We combined NHANES data with data from the US National Death Index up to 2019 to determine the trend of mortality rates over time. All-cause mortality included all potential causes of death but was not limited to the analysis of specific causes. The definition of CVD mortality was defined according to the tenth revision of the International Classification of Diseases and Related Health Problems (encapsulating codes I00 to I09, I11, I13, I20 to I51, and I60 to I69) (16).

2.5 Assessment of covariates

Standard questionnaires were used to obtain covariates such as age, sex, race/ethnicity, education attainment, poverty income

ratio (PIR), smoking status, physical activity, and health status. Anthropometric data such as body weight and height, as well as alcohol consumption data, were obtained from mobile centers (17).

To evaluate alcohol consumption, individuals were divided into two groups: the nonalcoholic group (drinking <12 alcohol drinks for 1 year) and the alcoholic group. By answering "Have you smoked at least 100 cigarettes in your lifetime?" and "Do you smoke now?," the study population was divided into nonsmokers, former smokers, or current smokers. The incidence rate of diseases, including malignant tumors, diabetes, hypertension and hypercholesterolemia. By integrating these parameters, the scope and accuracy of epidemiological investigations have been clarified.

2.6 Statistical analysis

This study complies with the Preferred Reporting Items for Complex Sample Survey Analysis (PRICSSA) guidelines. We used sample weighting, stratification and cluster analysis to ensure the representativeness of the study population and analyzed the trend of the serum 25(OH)D concentration as a continuous variable over a two-year period. The weight variable design, PSU/stratum and survey's response rate are detailed in the NHANES.[2] The serum 25(OH)D concentration and deficiency were measured with "WTMEC2YR" as the weighting variable, vitamin D intake from food and supplements was measured with "WTDRD1," and mortality was measured with "WTMEC2YR" as the weighting variable. The unweighted sample size was reported. We utilized weighted linear regression analyses to determine the trends in the serum 25(OH)D concentration, as well as the trends in the intake of vitamin D from food and supplements. Weighted logistic regression analysis was used to estimate the trend of occurrence of serum 25(OH)D deficiency in each cycle. We employed Taylor series linearization for standard error calculations, and weighted averages and 95% confidence intervals were estimated for each follow-up cycle. We calculated the difference in estimated mean values between the first and last cycles and evaluated the trend of changes in the serum vitamin D concentration according to age, sex, PIR, education level, and ethnicity. We removed samples with missing values and reported the removal ratio. We deleted the group that retained only one primary sampling unit and reported the sample proportion of the deleted group. A weighted Cox multivariable regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) between serum 25(OH)D levels and CVD and all-cause mortality. The proportional hazards assumption was verified using Schoenfeld residual analysis. The correlation between serum 25(OH)D levels and all-cause mortality was explored using restricted cubic spline analysis, with likelihood ratio tests employed for nonlinear investigations. In this study, we developed three models: Model 1, a correlation between the serum 25(OH)D concentration and all-cause mortality without adjusting for covariates; Model 2, an adaptation of Model 1 with the inclusion of significant demographic and socioeconomic covariates; and Model 3, an extension of Model 2 with the addition of pertinent medical and lifestyle covariates. In the mortality sensitivity analysis, age -related structural variances were assessed using the standard set by the 2000 US Census population, and participants with less than 2 years of follow-up were omitted to mitigate reverse causality. For mortality correlation analysis, we stratified the serum vitamin D concentrations into four quartiles. The data were analyzed using R software (Version: 4.2.0), for which a p-value of <0.05 indicated statistical significance. We stratified the data by sex and separately investigated the relationship between serum 25(OH)D levels and mortality rates in male and female.

2 https://wwwn.cdc.gov/nchs/nhanes/tutorials/Weighting.aspx

3 Results

3.1 Population characteristics

During the period from 2001 to 2002 to 2017 to 2018, serum 25(OH)D concentrations were recorded for 92.7% (44,461 out of 47,954) of the adult participants aged 20 and above. The selection process for the data can be found in Supplementary material S1. The proportion of individuals aged 60 and above in this study increased from 21.6 to 31.4%. In terms of education, the number of participants with education below high school declined from 19.5 to 11.3%, while the number of participants with education at university or above rose from 55.2 to 61.5%. In terms of ethnic group, the proportion of non-Hispanic white participants decreased from 71.4 to 62.2% (Table 1).

3.2 Trends in the serum 25(OH)D concentration

In the current study population, we found a significant increase in the serum 25(OH)D concentration, from 65.6 nmol/L (95% CI, 63.8–67.4 nmol/L) in the 2001–2002 cycle to 73.5 nmol/L (95% CI, 70.4–76.5 nmol/L) in the 2017–2018 cycle ($p < 0.001$ for trend; Table 2). Although the serum 25(OH)D concentration showed an increasing trend, the incidence rate of 25(OH)D deficiency remained relatively stable [22.7% (95% CI, 19.7–25.8%) for 2001–2002 and 21.7% (95% CI, 18.1–25.4%) for 2017–2018] ($p = 0.152$ for trend; Table 3). According to the subgroup analysis of sex and age, the serum 25(OH)D concentration in the ≥60-year-old ($p < 0.001$ for trend) and female ($p < 0.001$ for trend) populations also showed an upward trend. Conversely, in the cohort aged 20–39 years, the exhibited a decreasing serum 25(OH)D concentration decreased trajectory from 66.2 nmol/L (95% CI, 64.3–68.1 nmol/L) in 2001–2002 to 61.9 nmol/L (95% CI, 59.1–64.8 nmol/L) in 2017–2018 ($p < 0.002$ for trend; Table 2). During the survey period, there was no significant change in the trend of serum 25(OH)D concentration in individuals with a PIR ≤ 1 ($p = 0.325$ for trend; Table 2), while the incidence of 25(OH)D deficiency remained above 30% (Table 3). A significant increase trend in the 25(OH)D concentration was noted among individuals with PIRs 1–3.9 and ≥ 4 ($p = 0.002$ and < 0.001 for trend, respectively, Table 2). The PIR ≥4 subgroup exhibited a decrease in the occurrence of serum 25(OH)D deficiency, from 15.8% (95%

TABLE 1 Sociodemographic characteristics of US adults, 2001 to 2018.

Characteristics	Percentage (%) of adults by year (95% CI)[a,b]								
	2001–2002 (*n* = 5,027)	2003–2004 (*n* = 4,742)	2005–2006 (*n* = 4,773)	2007–2008 (*n* = 5,707)	2009–2010 (*n* = 6,059)	2011–2012 (*n* = 5,319)	2013–2014 (*n* = 5,588)	2015–2016 (*n* = 5,475)	2017–2018 (*n* = 5,265)
Age group, y									
20–39	38.6 (35.6–41.6)	36.7 (33.8–39.5)	36.3 (33.7–38.9)	35.4 (32.9–37.9)	34.6 (32.2–36.9)	34.5 (30.0–39.1)	34.5 (32.2–36.9)	35.0 (32.4–37.6)	34.4 (31.9–36.9)
40–59	39.8 (37.8–41.8)	39.5 (37.2–41.8)	39.2 (36.2–42.1)	39.5 (37.4–41.6)	39.1 (37.9–40.3)	37.5 (35.1–39.9)	36.5 (34.5–38.5)	35.1 (33.0–37.3)	34.2 (31.4–36.9)
≥60	21.6 (19.7–23.6)	23.9 (21.8–26.0)	24.6 (20.7–28.4)	25.1 (23.1–27.2)	26.3 (24.1–28.6)	28.0 (25.1–30.9)	28.9 (27.1–30.8)	29.9 (27.0–32.8)	31.4 (28.2–34.7)
Sex									
Male	47.8 (46.9–48.7)	47.9 (46.6–49.3)	48.1 (47.0–49.2)	48.2 (47.1–49.3)	48.2 (47.3–49.2)	48.0 (46.4–49.6)	48.1 (46.7–49.4)	48.1 (46.9–49.2)	48.1 (46.5–49.8)
Female	52.2 (51.3–53.1)	52.1 (50.7–53.4)	51.9 (50.8–53.0)	51.8 (50.7–52.9)	51.8 (50.8–52.7)	52.0 (50.4–53.6)	51.9 (50.6–53.3)	51.9 (50.8–53.1)	51.9 (50.2–53.5)
Income-to-poverty ratio[c]									
≤1	14.3 (12.5–16.0)	13.1 (10.6–15.6)	11.2 (9.7–12.7)	14.2 (12.0–16.5)	14.8 (13.0–16.7)	18.0 (14.6–21.4)	16.2 (12.8–19.6)	14.5 (11.8–17.2)	12.9 (10.8–15.1)
1–3.9	47.2 (44.4–50.0)	50.5 (47.4–53.5)	51.6 (48.5–54.6)	47.1 (43.9–50.3)	48.4 (45.5–51.4)	45.6 (41.0–50.3)	48.5 (45.5–51.5)	48.0 (44.0–52.0)	47.8 (43.6–52.0)
≥4	38.6 (34.8–42.3)	36.4 (32.3–40.6)	37.2 (33.4–41.1)	38.7 (34.2–43.2)	36.7 (34.4–39.1)	36.4 (30.6–42.1)	35.3 (30.2–40.3)	37.5 (31.6–43.4)	39.3 (35.1–43.4)
Education level[d]									
Less than high school	19.5 (17.5–21.5)	18.5 (16.3–20.7)	17.8 (15.0–20.5)	20.5 (17.6–20.4)	19.0 (17.1–21)	16.7 (13.4–20.1)	15.3 (12.2–18.4)	14.6 (11.2–17.9)	11.3 (9.7–12.9)
High school or equivalent	25.2 (23.5–26.9)	27.1 (25.1–29.0)	25.0 (23.5–26.6)	25.5 (23.0–28.0)	22.8 (20.6–25.1)	20.2 (17.6–22.8)	21.8 (19.4–24.2)	20.7 (18.5–22.8)	27.2 (24.1–30.2)
College or more	55.2 (52.1–58.3)	54.5 (51.9–57.0)	57.2 (53.5–60.9)	54.0 (49.3–58.8)	58.1 (55.3–61.0)	63.1 (58.1–68.1)	62.9 (58.8–67.0)	64.8 (60.2–69.3)	61.5 (57.6–65.4)
Race									
Mexican American	7.2 (5.5–8.9)	7.8 (4.0–11.6)	8.0 (6.0–9.9)	8.4 (5.5–11.2)	8.6 (4.3–12.9)	7.7 (4.4–11.1)	9.2 (5.7–12.6)	8.9 (4.7–13.0)	8.8 (5.6–12.0)
Non-Hispanic White	71.4 (66.5–76.3)	72.1 (65.4–78.7)	71.8 (66.4–77.3)	69.4 (62.3–76.6)	67.9 (61.4–74.5)	66.4 (58.8–74.0)	65.8 (59.4–72.2)	63.8 (56.3–71.4)	62.2 (57.2–67.3)
Non-Hispanic Black	10.9 (7.5–14.3)	11.2 (7.6–14.8)	11.5 (7.7–15.3)	11.3 (7.6–15.0)	11.4 (9.7–13.1)	11.5 (7.1–15.9)	11.4 (8.3–14.6)	11.4 (7.1–15.6)	11.4 (8.3–14.6)
Other	10.5 (6.1–14.8)	9.0 (6.9–11.0)	8.7 (6.5–10.9)	10.9 (7.5–14.4)	12.1 (8.6–15.6)	14.3 (11.3–17.4)	13.6 (11.2–16.0)	15.9 (12.5–19.3)	17.5 (14.3–20.7)

[a]Percentages were adjusted for NHANES survey weights.
[b]The proportional sum may not be equal to 1 in some cycles due to rounding.
[c]4,115 (8.6%) samples reported income-to-poverty ratio (PIR) missing value.
[d]68 (0.1%) samples reported education level missing value.

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TABLE 2 Trends of serum vitamin D concentration (nmol/L) among adults, 2001 to 2018.

	Trends of serum vitamin D concentration (nmol/L), weighted mean (95%CI)[a]									Difference, 2017–2018 vs. 2001–2002 (95% CI)[b]	*P*-value for trend
	2001–2002 (*n* = 5,027)	2003–2004 (*n* = 4,742)	2005–2006 (*n* = 4,773)	2007–2008 (*n* = 5,707)	2009–2010 (*n* = 6,059)	2011–2012 (*n* = 5,319)	2013–2014 (*n* = 5,588)	2015–2016 (*n* = 5,475)	2017–2018 (*n* = 5,265)		
Overall	65.6 (63.8–67.4)	63.2 (60.0–66.4)	67.4 (65.4–69.5)	67.1 (65.2–69.1)	67.7 (65.0–70.4)	70.8 (67.7–73.9)	69.6 (67.0–72.1)	72.0 (68.8–75.3)	73.5 (70.4–76.5)	7.88 (4.19–11.6)	<0.001
Age											
20–39	66.2 (64.3–68.1)	63.6 (60.0–67.1)	69.0 (66.3–71.8)	66.0 (62.7–69.3)	63.4 (62.3–66.5)	62.9 (59.8–66.0)	61.6 (58.6–64.6)	62.6 (59.3–66.0)	61.9 (59.1–64.8)	−4.24 (−7.78–-0.70)	0.002
40–59	65.8 (63.7–68.0)	62.8 (59.0–66.7)	66.7 (64.5–68.9)	67.0 (64.2–69.6)	68.7 (66.1–71.3)	70.9 (66.6–75.2)	68.9 (66.0–71.7)	71.7 (68.2–75.2)	72.8 (69.6–76.0)	6.99 (3.00–11.0)	<0.001
≥60	64.0 (61.8–66.1)	63.2 (61.0–65.4)	66.0 (63.9–68.2)	69.0 (66.9–71.1)	72.6 (69.5–75.7)	81.7 (78.3–85.1)	81.4 (78.7–84.1)	84.3 (80.9–87.8)	88.3 (85.2–91.5)	24.4 (20.4–28.3)	<0.001
Sex											
Male	66.3 (64.5–68.2)	63.6 (60.2–67.0)	67.3 (65.5–69.1)	65.5 (63.1–67.9)	65.6 (63.0–68.2)	67.7 (64.5–70.9)	65.8 (63.6–68.1)	68.2 (65.5–70.9)	70.2 (67.4–73.0)	3.86 (0.40–7.32)	0.09
Female	64.9 (62.8–67.0)	62.9 (59.8–65.9)	67.6 (65.0–70.1)	68.7 (66.8–70.6)	69.6 (66.6–72.7)	73.7 (70.2–77.2)	73.1 (69.8–76.3)	75.6 (71.4–79.8)	76.5 (73.0–80.0)	11.6 (7.31–15.9)	<0.001
Income-to-poverty ratio											
≤1	60.5 (57.6–63.4)	57.0 (52.2–61.8)	61.6 (57.9–65.3)	61.2 (57.4–65.1)	60.0 (57.4–62.5)	62.2 (60.0–64.5)	59.8 (56.6–63.1)	60.9 (56.6–65.2)	64.2 (61.1–67.4)	3.8 (−0.69–8.20)	0.325
1–3.9	64.3 (62.6–66.0)	62.2 (59.2–65.3)	66.5 (64.4–68.7)	66.5 (62.9–68.1)	65.9 (62.3–69.5)	68.9 (65.3–72.5)	67.7 (65.2–70.2)	69.2 (66.3–72.2)	70.8 (66.7–74.8)	6.48 (1.87–11.09)	0.002
≥4	69.9 (67.4–72.4)	67.1 (63.4–70.9)	70.5 (68.1–72.9)	71.5 (69.9–73.2)	73.8 (71.4–76.2)	77.3 (74.0–80.6)	76.7 (73.3–80.0)	79.4 (75.3–83.5)	80.7 (77.0–84.4)	10.8 (6.2–15.4)	<0.001
Education level											
Less than high school	60.9 (58.6–63.1)	57.3 (52.9–61.7)	61.9 (58.9–64.9)	62.1 (57.6–66.5)	61.9 (59.0–64.8)	66.1 (60.1–72.0)	64.3 (61.2–67.3)	63.9 (60.0–67.8)	65.3 (60.8–69.9)	4.45 (−0.85–9.76)	0.147
High school or equivalent	65.9 (64.1–67.7)	64.6 (61.2–68.1)	66.5 (64.1–68.9)	67.8 (65.2–70.4)	67.3 (64.4–70.1)	68.2 (64.8–71.7)	68.2 (64.4–72.0)	69.4 (65.4–73.3)	71.2 (67.2–75.1)	5.29 (0.74–9.84)	0.241
College or more	67.1 (64.9–69.3)	64.5 (61.1–67.8)	69.5 (67.5–71.5)	68.7 (66.6–70.9)	69.8 (67.1–72.6)	72.8 (69.7–75.9)	71.3 (69.0–73.7)	74.6 (71.4–77.9)	76.0 (73.1–78.8)	8.90 (5.13–12.7)	<0.001
Race[c]											
Mexican American[d]	56.9 (55.0–58.7)	54.5 (51.2–57.9)	58.3 (54.6–61.9)	54.1 (50.4–57.8)	54.2 (52.8–55.7)	54.4 (50.2–58.5)	55.4 (51.2–59.6)	55.2 (52.8–57.6)	57.3 (54.5–60.1)	0.42 (−3.15–4.00)	0.276
Non-Hispanic White[e]	70.1 (68.1–72.1)	68.5 (65.3–71.7)	72.1 (70.2–73.9)	73.9 (72.1–72.6)	74.8 (72.4–77.2)	78.0 (75.3–80.6)	76.0 (73.4–78.6)	79.9 (77.5–82.4)	81.0 (77.6–84.4)	10.9 (6.79–15.0)	<0.001
Non-Hispanic Black[f]	43.6 (42.5–44.7)	41.6 (38.8–44.3)	49.0 (46.9–51.0)	42.0 (39.2–44.9)	46.5 (42.0–51.0)	50.8 (48.4–53.1)	50.4 (47.5–53.2)	51.6 (49.3–53.8)	54.7 (51.7–57.8)	11.1 (7.74–14.6)	<0.001
Other[g]	58.6 (55.4–61.9)	53.4 (50.7–56.2)	60.3 (57.2–63.4)	56.8 (53.9–59.8)	55.6 (52.8–58.5)	61.1 (58.4–63.8)	63.1 (61.4–64.8)	63.4 (60.2–66.6)	66.6 (63.7–69.5)	7.93 (3.39–12.5)	<0.001

[a]Data were adjusted for NHANES survey weights to be nationally representative.

[b]Values may not equal the difference between the beginning and ending estimates because of rounding.

[c]Stratification by ethnic characteristics leaded to stratum with a single PSU. Samples in the stratum with the singleton PSU were removed.

[d]261 (3.3%) samples removed.

[e]38 (0.2%) samples removed.

[f]147 (1.4%) samples removed.

[g]68 (0.8%) samples removed.

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TABLE 3 Trends in the weighted percentage of individuals with vitamin D deficiency among adults, 2001 to 2018.

	Weighted percentage (%) of individuals with vitamin D deficiency, % (95% CI)[a]									*P*-value for trend
	2001–2002 (*n* = 5,027)	2003–2004 (*n* = 4,742)	2005–2006 (*n* = 4,773)	2007–2008 (*n* = 5,707)	2009–2010 (*n* = 6,059)	2011–2012 (*n* = 5,319)	2013–2014 (*n* = 5,588)	2015–2016 (*n* = 5,475)	2017–2018 (*n* = 5,265)	
Overall	22.7 (19.7–25.8)	30.5 (24.5–36.5)	21.5 (17.8–25.1)	26.1 (22.4–29.7)	25.7 (21.9–29.5)	24.5 (19.5–29.6)	24.5 (20.8–28.2)	23.1 (18.8–27.4)	21.7 (18.1–25.4)	0.152
Age										
20–39	22.4 (18.8–26.1)	32.6 (25.7–39.5)	20.6 (16.1–25.2)	29.4 (23.6–35.2)	30.8 (25.6–36.0)	32.3 (25.9–38.7)	32.1 (26.9–37.2)	31.7 (25.7–37.7)	31.6 (27.0–36.2)	0.003
40–59	22.4 (18.8–25.9)	29.6 (22.6–36.6)	22.6 (17.8–27.5)	25.0 (21.0–29.0)	24.1 (19.9–28.2)	23.7 (17.8–29.6)	24.3 (19.7–28.8)	21.6 (17.0–26.2)	19.8 (15.7–23.9)	0.076
≥60	24.1 (20.2–27.9)	28.5 (24.7–32.3)	20.8 (17.3–24.3)	22.9 (19.2–26.6)	20.7 (18.0–23.4)	14.8 (11.2–18.5)	14.7 (12.3–17.0)	14.0 (11.3–16.7)	12.0 (9.1–14.9)	<0.001
Sex										
Male	19.6 (17.1–22.2)	26.9 (20.5–33.3)	19.6 (16.2–23.0)	24.3 (20.1–28.6)	24.3 (20.1–28.5)	24.7 (18.8–30.7)	25.8 (21.5–30.1)	23.5 (19.3–27.8)	23.1 (18.6–27.6)	0.364
Female	25.7 (21.7–29.6)	33.9 (27.9–39.8)	23.2 (19.0–27.5)	27.7 (24.1–31.3)	27.0 (23.1–31.0)	24.3 (19.6–29.0)	23.3 (19.5–27.1)	22.6 (17.7–27.5)	20.5 (17.2–23.8)	<0.001
Income-to-poverty ratio										
≤1	33.6 (26.9–40.3)	41.6 (31.0–52.2)	31.8 (25.3–38.4)	37.7 (29.8–45.6)	36.7 (31.5–41.9)	33.9 (28.0–39.8)	38.3 (32.4–44.1)	37.1 (30.0–44.1)	33.9 (28.7–39.0)	0.896
1–3.9	24.6 (21.5–27.7)	32.9 (26.4–39.4)	22.7 (19.0–26.4)	27.9 (23.7–32.1)	28.5 (23.5–33.5)	27.1 (20.8–33.5)	26.5 (22.5–30.4)	25.5 (20.4–30.6)	23.6 (18.5–28.6)	0.2667
≥4	15.8 (12.1–19.6)	22.6 (16.5–28.7)	15.6 (11.4–19.7)	19.0 (15.9–22.1)	16.4 (12.6–20.2)	16.5 (12.6–20.4)	15.3 (11.6–19.0)	14.6 (10.4–18.8)	13.4 (10.0–16.8)	0.024
Education level										
Less than high school	32.2 (28.2–36.1)	40.5 (30.5–50.6)	31.7 (26.0–37.4)	33.9 (26.4–41.3)	33.4 (28.8–38.0)	29.8 (20.9–38.8)	32.1 (26.3–37.9)	31.8 (25.6–37.9)	30.7 (25.5–35.9)	0.144
High school or equivalent	23.4 (19.6–27.1)	27.4 (21.1–33.7)	23.3 (18.7–28.0)	25.2 (20.7–29.7)	25.9 (22.3–29.6)	29.6 (22.5–36.6)	26.2 (21.4–31.1)	24.9 (19.1–30.8)	23.2 (19.2–27.3)	0.953
College or more	19.2 (15.7–22.7)	28.7 (22.7–34.8)	17.5 (13.9–21.2)	23.6 (19.7–27.5)	23.0 (18.8–27.2)	21.6 (17.2–25.9)	22.0 (18.6–25.5)	20.6 (16.7–24.5)	19.4 (15.7–23.2)	0.279
Race[b]										
Mexican American[c]	33.8 (28.9–38.7)	43.5 (35.9–51.2)	34.6 (26.2–42.9)	43.7 (34.3–53.0)	43.1 (38.8–47.4)	44.0 (32.6–55.4)	41.1 (31.4–50.8)	45.2 (39.2–51.3)	40.2 (34.5–46.0)	0.174
Non-Hispanic White[d]	14.0 (11.5–16.4)	21.1 (16.1–26.1)	12.7 (9.7–15.7)	15.9 (13.8–18.0)	14.7 (11.9–17.4)	14.3 (10.6–18.0)	15.5 (12.0–18.9)	12.4 (9.7–15.1)	12.2 (8.7–15.7)	0.027
Non-Hispanic Black[e]	72.4 (69.5–75.3)	71.5 (64.5–78.5)	60.8 (54.1–67.5)	70.7 (64.8–76.6)	64.4 (55.3–73.4)	58.3 (54.2–62.3)	58.3 (53.2–63.5)	56.3 (51.6–61.1)	53.1 (46.7–59.5)	<0.001
Other[f]	32.6 (25.3–40.0)	46.7 (39.1–54.4)	32.7 (26.1–39.2)	38.0 (29.6–46.4)	42.0 (35.3–48.5)	36.5 (30.4–42.6)	30.4 (25.9–35.0)	31.1 (26.6–35.6)	26.9 (23.2–30.6)	<0.001

[a]Data were adjusted for NHANES survey weights to be nationally representative.
[b]Stratification by ethnic characteristics leaded to stratum with a single PSU. Samples in the stratum with the singleton PSU were removed.
[c]261 (3.3%) samples removed.
[d]38 (0.2%) samples removed.
[e]147 (1.4%) samples removed.
[f]68 (0.8%) samples removed.

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CI, 12.1–19.6%) in 2001–2002 to 13.4% (95% CI, 10.0–16.8%) in 2017–2018 (Table 3), suggesting at a notable association between the incidence of 25(OH)D deficiency and poverty level. According to the stratified analysis of population education level, there was no significant change in the trend of serum 25(OH)D concentration or the incidence of vitamin D deficiency among individuals with lower levels of university education.

3.3 Trends in dietary supplements and vitamin D

During the survey period from 2007 to 2018, the intake of vitamin D in dietary supplements increased (Supplementary material S2). Regarding the survey of food vitamin D intake, we found that during the 2017–2018 period, the total study population had lower vitamin D intake (difference: −0.13 nmol/L; 95% CI, −0.52 to 0.26 nmol/L). However, vitamin D intake was greater in the other survey cycles than in the compared to 2007–2008 cycle (Supplementary material S3).

3.4 Associations between the serum 25(OH)D concentration and mortality

After stratification by serum 25(OH)D deficiency status, the baseline characteristics of the study population are shown in Supplementary material S4. Among the 426,438 individuals, 6,870 had all-cause mortality. There was a V-shaped nonlinear correlation between the serum 25(OH)D concentration and all-cause mortality in Model 1 ($p < 0.001$ for nonlinearity; Figure 1A) and an L-shaped nonlinear association in Models 2 and 3 ($p < 0.001$ for nonlinearity; Figures 1B,C). Moreover, we studied the relationship between serum 25(OH)D levels and CVD mortality and found 2,120 CVD deaths. According to the three models established, it was found that the serum vitamin D concentration was associated with CVD mortality in a nonlinear L-shaped fashion ($p < 0.001$ for nonlinear; Figures 1D–F).

3.5 Sensitivity analysis

The sensitivity analysis was partitioned into five parts: (1) we used the 2000 US Census data for age standardization and observed no significant changes in the serum vitamin D concentration, the incidence rate of vitamin D deficiency, or the trend of dietary vitamin D intake (Supplementary materials S5–S8); (2) after excluding participants with a follow-up time of 2 years, the results showed an L-shaped relationship between the serum 25(OH) D concentration and CVD mortality as well as all-cause mortality ($p < 0.001$ for nonlinearity; Supplementary materials S9, S10). (3) We divided the concentration of serum 25(OH)D into four concentration gradients: (1) < 25 nmol/L, (2) 25.0–49.9 mmoL/L, (3) 50–74.9 nmol/L, and (4) ≥ 75 nmol/L. We adjusted for multiple variables using 25.0–49.9 nmol/L subgroup as the reference standard for all-cause mortality. The results showed that the hazard ratio (HR) and 95% confidence interval (CI) were 1.48 (1.18–1.86) for the <25 nmol/L group, 0.74 (0.68–0.81) for the 50–74.9 nmol/L group and 0.66 (0.60–0.73) for the ≥75 nmol/L group (Supplementary material S11). (5) CVD mortality was greater when the concentration was lower than 25 nmol/L. The other subgroups of HR and 95% CI subgroups were as follows: 1.76 (1.22–2.53) for the <25 nmol/L group; 0.68 (0.58–0.80) for the 50–74.9 nmol/L group; and 0.67 (0.56–0.79) for the ≥75 nmol/L group (Supplementary material S12). We investigated the impact of serum vitamin D levels on all-cause and cardiovascular mortality in both the male and female groups. We observed that, in each sex subgroup, there was an L-shaped nonlinear relationship between 25(OH)D concentration and mortality risk. These findings are largely consistent with previous conclusions (Supplementary material S13).

4 Discussion

Our analysis revealed an upwards trend in the serum 25(OH)D concentrations among American adults from 2001 to 2018. Furthermore, we identified an L-shaped correlation between serum 25(OH)D levels and both CVD and all-cause mortality, which became more pronounced when the serum 25(OH)D concentration decreased to less than 50 nmol/L. By eliminating reverse causality, converting the concentration of 25-hydroxyvitamin D [25(OH)D] into categorical variables, and conducting sensitivity analysis using methods such as stratification by gender, we have confirmed the reliability of these results. Our conclusions were corroborated through an exhaustive stratification and sensitivity analysis.

Cui et al. reported on the trends in vitamin D levels during the 2001–2018 NHANES survey cycle, focusing on the changes in the proportions of patients with different vitamin D concentrations. Their overall conclusion aligns with our study, which showed a general decrease in the proportion of the population with a serum vitamin D concentrations < 50 nmol/L (7). This consistent trend may be attributed to increased public health awareness about the role of vitamin D, improvements in living standers, and a growing understanding of the negative health consequences associated with vitamin D deficiency. Such awareness likely spurred interventions, including greater emphasis on dietary vitamin D intake and more widespread use of supplements, Supporting this, studies have shown the beneficial effects of sun exposure in ameliorating vitamin D deficiency (18, 19). Nevertheless, the need for additional vitamin D supplementation remains critical, especially for those at high risk of deficiency (20). However, our study, using finer stratification, draws additional conclusions on temporal trends. Our study highlights an escalating trend in the serum 25(OH)D concentration among American adults, particularly in elderly people (≥ 60 years old) and female, which parallels reports from the NHANES study for the period 1988–2010 (6). Previous studies overlooked income and education-based stratification, factors that can significantly affect serum 25(OH)D levels. While no significant vitamin D deficiency fluctuations were observed in our study population, disaggregation by age, sex, or income revealed stark disparities. Serum 25(OH)D levels among 20–39-year-olds remained stable during the 1988–2010 survey phase (6). However, this age bracket exhibited the highest vitamin D deficiency rates in a 2001–2018 study, despite the absence of an age-stratified trend analysis (7). Our study reveals a decline in serum vitamin D and a rise in deficiency among this demographic,

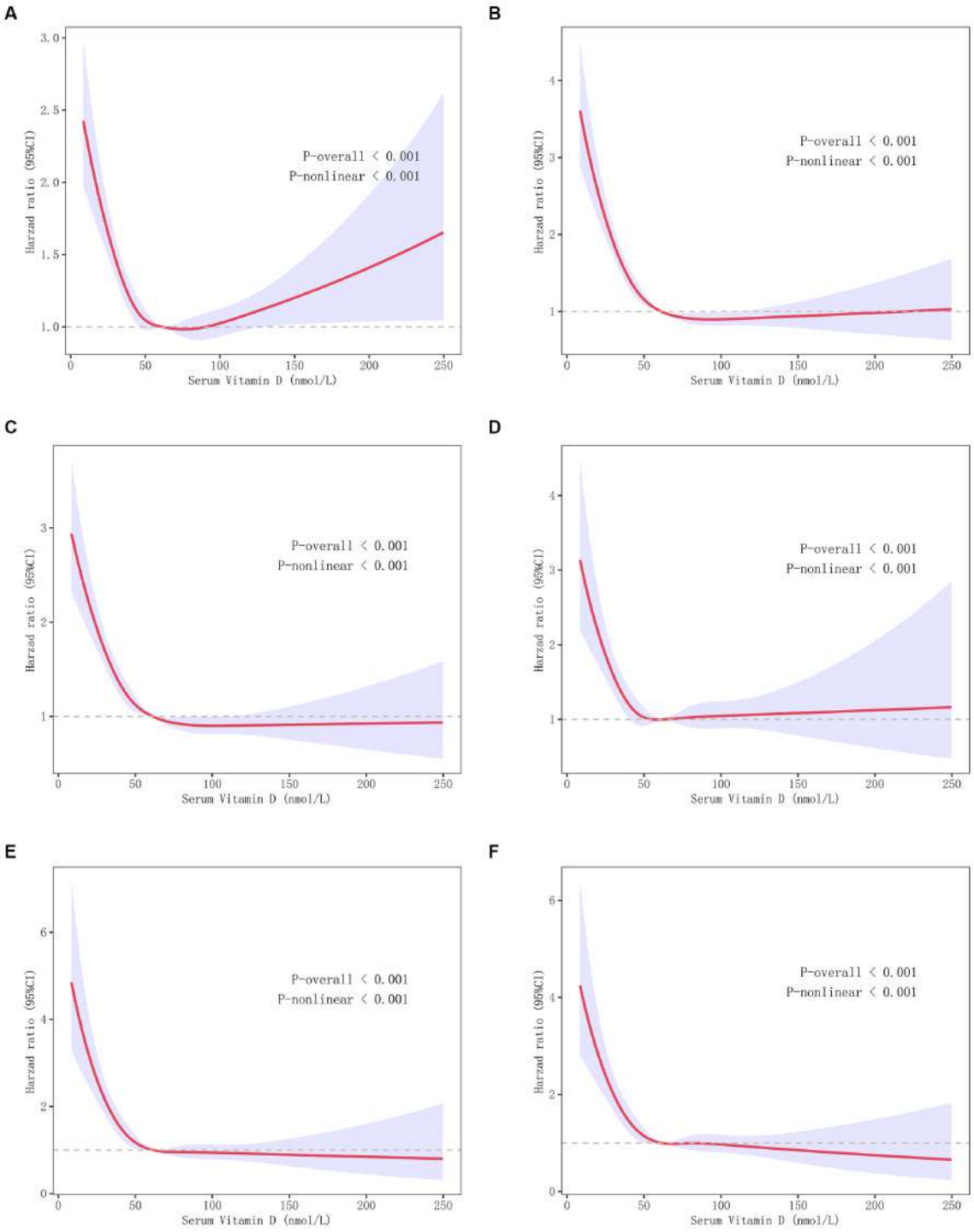

FIGURE 1
Correlations between the serum 25 (OH) D concentration and CVD and all-cause mortality. **(A)** V-shaped nonlinear correlation between serum 25(OH) D concentration and all-cause mortality in Model 1. **(B)** The L-relationship between serum 25 (OH) D concentration and all-cause mortality in Model 2. **(C)** The L-shaped relationship between the serum 25 (OH) D concentration and all-cause mortality in Model 3. **(D–F)** The L-shaped relationship between the serum 25 (OH) D concentration and CVD mortality in models 1 **(D)**, 2 **(E)**, and 3 **(F)**. Model 1, without adjusting covariates; Model 2, adjusting for covariates such as age, education level, PIR, sex, and race; Model 3, adjusted for include Model 2 covariates and disease history, as well as covariates such as smoking and alcohol consumption.

contrasting with decreased deficiency in individuals aged 40 and older. Changes in lifestyle patterns may explain this discrepancy, as indicated by increases in indoor occupations, decreases in outdoor activities, and shifts in dietary intake in younger cohorts (21–23). Therefore, a decrease in dietary intake or vitamin D supplementation alone cannot fully explain the observed serum 25(OH)D concentration and the simultaneous increase in vitamin D deficiency in the 20–39 age cohort. Obesity and lack of exercise were noted as primary vitamin

D causes of deficiency causes in young populations in an Australian study (21).

In addition, we discerned a significant increase in 25(OH)D levels in more educated and higher-income (PIR ≥ 4) individuals. Individuals lacking college education revealed no significant increase in the serum vitamin D concentration, with deficiency rates consistently exceeding 30%. Consequently, we posit that these specific populations warrant special attention. Interestingly, a downwards trend in vitamin D deficiency incidence prevalence was detected in high-income groups (PIR ≥ 4), suggesting a relationship between 25(OH)D levels and economic income. In the low-income groups (PIR ≤ 1), no significant shifts in serum 25(OH)D concentration were observed across the nine survey cycles. However, a persistently high prevalence of vitamin D deficiency, approximately 30%, was observed in low-income populations, aligning with findings from other demographic studies. For instance, studies involving UK children have indicated that low household income as a significant risk factor for vitamin D deficiency (23). Similarly, a separate study of Chinese women of reproductive age identified a clear correlation between economic income level and vitamin D deficiency incidence (24). A systematic review echoed these findings, analyzing populations in 29 low- and middle-income countries (25). A density-equalizing mapping analysis revealed that, globally, epidemiological surveys of vitamin D are extremely limited, especially in Asian, African, and South American countries (26). The prevalence rates of vitamin D deficiency that are affected by poverty, worldwide, and the extent to which comprehensive epidemiological studies are conducted is influenced by poverty. This indicates that it is very necessary to conduct similar surveys in less developed countries and regions.

To further elucidate the connection between vitamin D and overall mortality, we adjusted for an array of covariates, such as sociodemographic factors and health conditions. Following this adjustment, an L-shaped inverse association was observed between serum 25(OH)D levels and CVD mortality and between 25(OH)D levels and all-cause mortality. Below a certain inflection point of vitamin D concentration, no additional reduction in CVD or all-cause mortality was observed. Specifically, a serum 25(OH)D concentrations less than 50 nmol/L was significantly negatively correlated with all-cause mortality. With 25(OH)D levels between 50 and 75 nmol/L, all-cause mortality seemed to plateau. Echoing the findings of previous, we found a nonlinear association between 25(OH)D levels and both CVD and all-cause mortality. Specifically, when serum vitamin D concentrations dipped are less than 25 ng/L, the risk of CVD and all-cause mortality significantly increased (27–30).

Based on our results and previous studies, boosting vitamin D intake could benefit public health and potentially lower CVD and all-cause mortality. Given the L-shaped relationship between the serum vitamin D concentration and all-cause mortality, vitamin D supplementation may primarily benefit deficient individuals, emphasizing the need for wide-scale screening and intervention within these groups. A recent meta-analysis and a Cochrane review indicated that while vitamin D and calcium supplementation may not reduce all-cause mortality, they do decrease the risk of cancer death (8). This is further supported by the RCTs, which revealed a significant reduction in cancer mortality (9, 13). Consistent with our hypothesis, in a real-world study based on the UK Biobank, the prescription of vitamin D supplements did indeed reduce cancer mortality, all-cause mortality, or the risk of respiratory infections (2).

5 Strengths and limitations

The current research has the following strengths. First, it offers a comprehensive long-term analysis reflecting serum vitamin D trends and their association with CVD and all-cause mortality across a large population. Second, by employing stratification based on age and socioeconomic factors, this study effectively elucidated vitamin D variations and their impact on deficiency prevalence. Third, after we adjusted for several demographic factors, a detailed exploration of the relationship between the serum 25(OH)D concentration and mortality was performed to further guarantee the accuracy of the study. However, our study has several limitations: (1) the trend-based nature of the study restricts causal interpretation of the observed outcomes; (2) potential biases may emerge from a single measurement of serum vitamin D concentrations; (3) vitamin D deficiency may be influenced by various uncontrollable factors, such as sunlight exposure and genetic variations; (4) recall bias due to self-reported dietary data and disease status; and (5) measurement errors from different serum 25(OH)D assessment methods in the NHANES study could also increase potential error; (6) In the estimation of the association between 25(OH)D concentrations and mortality, the interval with concentrations >125 nmol/L exhibited a wide 95% CI for the HR, indicating a lack of precision in this value interval.

6 Conclusion

From 2001 to 2018, serum vitamin D levels in U.S. adults increased, but deficiency rates remained unchanged. We observed an L-shaped correlation between vitamin D levels and all-cause mortality, showing a significant inverse relationship when vitamin D levels are below 50 nmol/L. This highlights the importance of a nationwide survey on serum vitamin D levels to inform targeted public health strategies, particularly for high-risk populations.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: all data for this study can be obtained from NHANES (https://www.cdc.gov/nchs/nhanes/index.htm).

Ethics statement

The NHANES study protocol was approved by the Research Ethics Review Board of the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/ next of kin.

Author contributions

CH: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft. MY: Conceptualization, Methodology, Supervision, Writing – review & editing.

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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/fnut.2024.1328136/full#supplementary-material

References

1. Force USPST, Krist AH, Davidson KW, Mangione CM, Cabana M, Caughey AB, et al. Screening for vitamin D deficiency in adults: US preventive services task Force recommendation statement. *JAMA*. (2021) 325:1436–42. doi: 10.1001/jama.2021.3069

2. Sha S, Nguyen TMN, Kuznia S, Niedermaier T, Zhu A, Brenner H, et al. Real-world evidence for the effectiveness of vitamin D supplementation in reduction of total and cause-specific mortality. *J Intern Med*. (2023) 293:384–97. doi: 10.1111/joim.13578

3. Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmun Rev*. (2010) 9:709–15. doi: 10.1016/j.autrev.2010.06.009

4. Pilz S, Iodice S, Zittermann A, Grant WB, Gandini S. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. *Am J Kidney Dis*. (2011) 58:374–82. doi: 10.1053/j.ajkd.2011.03.020

5. Aspray TJ, Bowring C, Fraser W, Gittoes N, Javaid MK, Macdonald H, et al. National osteoporosis society vitamin D guideline summary. *Age Ageing*. (2014) 43:592–5. doi: 10.1093/ageing/afu093

6. Schleicher RL, Sternberg MR, Lacher DA, Sempos CT, Looker AC, Durazo-Arvizu RA, et al. The vitamin D status of the US population from 1988 to 2010 using standardized serum concentrations of 25-hydroxyvitamin D shows recent modest increases. *Am J Clin Nutr*. (2016) 104:454–61. doi: 10.3945/ajcn.115.127985

7. Cui A, Xiao P, Ma Y, Fan Z, Zhou F, Zheng J, et al. Prevalence, trend, and predictor analyses of vitamin D deficiency in the US population, 2001-2018. *Front Nutr*. (2022) 9:965376. doi: 10.3389/fnut.2022.965376

8. Zhang Y, Fang F, Tang J, Jia L, Feng Y, Xu P, et al. Association between vitamin D supplementation and mortality: systematic review and meta-analysis. *BMJ*. (2019) 366:l4673. doi: 10.1136/bmj.l4673

9. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D supplements and prevention of Cancer and cardiovascular disease. *N Engl J Med*. (2019) 380:33–44. doi: 10.1056/NEJMoa1809944

10. Barbarawi M, Kheiri B, Zayed Y, Barbarawi O, Dhillon H, Swaid B, et al. Vitamin D supplementation and cardiovascular disease risks in more than 83 000 individuals in 21 randomized clinical trials: a meta-analysis. *JAMA Cardiol*. (2019) 4:765–76. doi: 10.1001/jamacardio.2019.1870

11. Neale RE, Baxter C, Romero BD, McLeod DSA, English DR, Armstrong BK, et al. The D-health trial: a randomised controlled trial of the effect of vitamin D on mortality. *Lancet Diabetes Endocrinol*. (2022) 10:120–8. doi: 10.1016/S2213-8587(21)00345-4

12. Annweiler C, Beaudenon M, Gautier J, Gonsard J, Boucher S, Chapelet G, et al. High-dose versus standard-dose vitamin D supplementation in older adults with COVID-19 (COVIT-TRIAL): a multicenter, open-label, randomized controlled superiority trial. *PLoS Med*. (2022) 19:e1003999. doi: 10.1371/journal.pmed.1003999

13. Keum N, Lee DH, Greenwood DC, Manson JE, Giovannucci E. Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials. *Ann Oncol*. (2019) 30:733–43. doi: 10.1093/annonc/mdz059

14. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on NHANES dietary data: focus on collection, release, analytical considerations, and uses to inform public policy. *Adv Nutr*. (2016) 7:121–34. doi: 10.3945/an.115.009258

15. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*. (2011) 96:53–8. doi: 10.1210/jc.2010-2704

16. Liu Y, Geng T, Wan Z, Lu Q, Zhang X, Qiu Z, et al. Associations of serum folate and vitamin B12 levels with cardiovascular disease mortality among patients with type 2 diabetes. *JAMA Netw Open*. (2022) 5:e2146124. doi: 10.1001/jamanetworkopen.2021.46124

17. Wang JS, Xia PF, Ma MN, Li Y, Geng TT, Zhang YB, et al. Trends in the prevalence of metabolically healthy obesity among US adults, 1999-2018. *JAMA Netw Open*. (2023) 6:e232145. doi: 10.1001/jamanetworkopen.2023.2145

18. Weber B, Bachmann CC, Braun R, Abraham AG, Serra AL, Hofbauer GFL. 25-Hydroxyvitamin-D3 serum modulation after use of sunbeds compliant with European Union standards: a randomized open observational controlled trial. *J Am Acad Dermatol*. (2017) 77:48–54. doi: 10.1016/j.jaad.2017.02.029

19. Vahavihu K, Ylianttila L, Kautiainen H, Viljakainen H, Lamberg-Allardt C, Hasan T, et al. Narrowband ultraviolet B course improves vitamin D balance in women in winter. *Br J Dermatol*. (2010) 162:848–53. doi: 10.1111/j.1365-2133.2010.09629.x

20. Nowson CA, McGrath JJ, Ebeling PR, Haikerwal A, Daly RM, Sanders KM, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust*. (2012) 196:686–7. doi: 10.5694/mja11.10301

21. Horton-French K, Dunlop E, Lucas RM, Pereira G, Black LJ. Prevalence and predictors of vitamin D deficiency in a nationally representative sample of Australian adolescents and young adults. *Eur J Clin Nutr*. (2021) 75:1627–36. doi: 10.1038/s41430-021-00880-y

22. Levy MA, McKinnon T, Barker T, Dern A, Helland T, Robertson J, et al. Predictors of vitamin D status in subjects that consume a vitamin D supplement. *Eur J Clin Nutr*. (2015) 69:84–9. doi: 10.1038/ejcn.2014.133

23. Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One*. (2011) 6:e22179. doi: 10.1371/journal.pone.0022179

24. Lin S, Jiang L, Zhang Y, Chai J, Li J, Song X, et al. Socioeconomic status and vitamin D deficiency among women of childbearing age: a population-based, case-control study in rural northern China. *BMJ Open*. (2021) 11:e042227. doi: 10.1136/bmjopen-2020-042227

25. Cashman KD, Sheehy T, O'Neill CM. Is vitamin D deficiency a public health concern for low middle income countries? A systematic literature review. *Eur J Nutr*. (2019) 58:433–53. doi: 10.1007/s00394-018-1607-3

26. Bruggmann D, Alafi A, Jaque J, Klingelhofer D, Bendels MH, Ohlendorf D, et al. World-wide research architecture of vitamin D research: density-equalizing mapping studies and socio-economic analysis. *Nutr J*. (2018) 17:3. doi: 10.1186/s12937-018-0313-6

27. Chen X, Zhou M, Yan H, Chen J, Wang Y, Mo X. Association of serum total 25-hydroxy-vitamin D concentration and risk of all-cause, cardiovascular and malignancies-specific mortality in patients with hyperlipidemia in the United States. *Front Nutr*. (2022) 9:971720. doi: 10.3389/fnut.2022.971720

28. Xiao Q, Cai B, Yin A, Huo H, Lan K, Zhou G, et al. L-shaped association of serum 25-hydroxyvitamin D concentrations with cardiovascular and all-cause mortality in individuals with osteoarthritis: results from the NHANES database prospective cohort study. *BMC Med*. (2022) 20:308. doi: 10.1186/s12916-022-02510-1

29. Wan Z, Guo J, Pan A, Chen C, Liu L, Liu G. Association of Serum 25-Hydroxyvitamin D concentrations with all-cause and cause-specific mortality among individuals with diabetes. *Diabetes Care*. (2021) 44:350–7. doi: 10.2337/dc20-1485

30. Emerging Risk Factors Collaboration E-CVDVDSC. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol*. (2021) 9:837–46. doi: 10.1016/S2213-8587(23)00364-9

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Jie Lu,
The Affiliated Hospital of Qingdao University, China
Federica Piani,
University of Bologna, Italy
Shaoyong Xu,
Xiangyang Central Hospital, China

*CORRESPONDENCE
Hongguang Yang
yanghg3@mail2.sysu.edu.cn
Shuhan Zhou
shuhan_zhou@hbtcm.edu.cn

†These authors have contributed equally to this work

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Sex-specific differences in the associations between adiposity indices and incident hyperuricemia among middle-aged and older adults: a nationwide longitudinal study

Zhiyi Liu[1†], Qinwu Zhou[2†], Yuqiong Tang[3], Jiyong Li[4], Qiutong Chen[5], Hongguang Yang[6*] and Shuhan Zhou[1,7*]

[1]College of Traditional Chinese Medicine, Hubei University of Chinese Medicine, Wuhan, China, [2]Department of Dermatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3]The First Clinical Medical School, Hubei University of Chinese Medicine, Wuhan, China, [4]Huangpi Hospital of Traditional Chinese Medicine, Wuhan, China, [5]College of Language Intelligence, Sichuan International Studies University, Chongqing, China, [6]Department of Clinical Nutrition, Union Shenzhen Hospital of Huazhong University of Science and Technology, Shenzhen, Guangdong, China, [7]Hubei Shizhen Laboratory, Hubei University of Chinese Medicine, Wuhan, China

Objective: Although obesity is a known risk for hyperuricemia (HUA), the associations between adiposity indices and incident HUA and whether sex-specific differences exist is still unknown. We aimed to investigate the associations between adiposity indices and incident HUA in a longitudinal study.

Methods: Data from the China Health and Retirement Longitudinal Study (CHARLS) in 2011–2012 and 2015–2016 were used to conduct a cohort study. Participants aged ≥45 years without HUA at baseline were included in this study. Adiposity indices, including body mass index (BMI), waist circumference (WC), waist-to-height ratio body roundness index (BRI), conicity index (CI), lipid accumulation product (LAP) index, waist-to-height ratio (WHtR), visceral adiposity index (VAI), and Chinese visceral adiposity index (CVAI), were calculated. Logistic analysis was used to analyze the association between adiposity indices and incident HUA risk stratified by gender. Receiver operating characteristic curve analysis was performed to evaluate the power of predictions for incident HUA.

Results: Of 5,873 participants aged 59.0 ± 8.7 years enrolled in this study, 578 (9.8%) participants developed HUA during the 4-year follow-up period. After adjusting for confounding variables, LAP, VAI, and CVAI showed significant association with incident HUA. BMI, WC, WHtR, BRI, and CI were significantly associated with incident HUA in women but not in men. LAP had the highest area under the curve (AUC) (0.612) followed by CVAI (0.596) in men, while CVAI had the highest AUC (0.707) followed by LAP (0.691) in women. All indices showed better predictive ability in women than in men.

Conclusion: Our findings indicated that adiposity indices were effective predictors of incident HUA and showed better predictive power in women than men. In clinical practice, adiposity indices could be used to assess and prevent incident HUA among Chinese middle-aged and older adults.

KEYWORDS

obesity, adiposity indices, hyperuricemia, longitudinal study, CHARLS

Introduction

Uric acid is the end enzymatic product of purine metabolism (1). Hyperuricemia (HUA) is the elevation of serum uric acid (SUA) concentrations, usually resulting from excessive production or reduced urinary excretion of uric acid (1, 2). In recent years, HUA has become a critical public health issue worldwide. It was reported that 14.0% of Chinese adults had HUA during 2018–2019 according to a nationwide study (3), and approximately 20% of American adults had HUA according to the National Health and Nutrition Examination Survey (4). HUA is a known risk factor for gout, diabetes, chronic kidney disease, hypertension, cardiovascular diseases, and all-cause mortality (5–7). Given the increasing number of people with HUA, it is important to understand the modifiable risk factors and well-predictive indicators for HUA in clinical practice.

Although the impact of uric acid on metabolic syndrome has been thoroughly studied (8), only a few studies to date have linked obesity to serum uric acid and incident HUA (9–12). Accumulating evidence demonstrates obesity, especially visceral obesity, is positively associated with SUA levels and HUA (9). Obesity is highly related to metabolic disorders, which is a major risk of HUA. Ryu et al. reported that obesity was a primary determinant risk factor of HUA in middle-aged South Korean men (13). Moreover, obese individuals, especially those with abdominal obesity, displayed a significantly increased risk of HUA (9–11). In recent years, many adiposity indices have been developed to evaluate obesity status, body fat distribution, and visceral adiposity proportions (14). The associations between these adiposity indices and HUA have been explored in cross-sectional studies (11, 15, 16). However, these studies yielded inconsistent results. Furthermore, some traditional adiposity indices without lipid parameters such as body mass index (BMI), waist circumference (WC), waist-to-height ratio (WHtR), and body roundness index (BRI) reported inconsistent associations in different studies, especially in different genders (11, 15, 16). However, several novel adiposity indices containing lipid parameters such as lipid accumulation product (LAP) index and visceral adiposity index (VAI), which have shown stronger correlations with metabolic abnormality, consistently show a positive association with HUA (9, 11). In addition, previous studies reported the effect of cumulative burden of abnormal VAI, and its components were more pronounced in women (17), and several adiposity indices showed higher predictive power for HUA in women (15, 16), which suggested that there might be gender differences in relationships between adiposity indices and HUA.

Although several cross-sectional studies have explored the associations between adiposity indices and HUA, the results were still mixed, and longitudinal evidence was lacking (9, 11, 12, 18). Therefore, we aimed to investigate the relationship between adiposity indices and incident HUA in a cohort of Chinese middle-aged and older adults, which may provide the basis for individuals to prevent the incidence of HUA.

Methods

Study population

Data used in this study were from the China Health and Retirement Longitudinal Study (CHARLS), an ongoing national representative survey conducted in China. Details of the study could be found elsewhere (19). Briefly, this survey recruited participants from 450 urban communities and rural areas in 28 provinces of China from 2011 to 2012 (Wave 1). They were followed up every 2 years subsequently. In the present study, data collected at Wave 1 and Wave 3 (2015 to 2016) were used because data on laboratory blood samples were only available in the two waves.

Of 17,708 participants, we excluded 6,053 participants with missing data on SUA, 243 participants aged <45 years, 2,031 participants in whom all the eight adiposity indices were unable to be calculated, 101 participants with missing data on other blood tests such as blood urea nitrogen (BUN) and plasma creatinine, and 528 participants with HUA at baseline in Wave 1. We further excluded 2,879 participants lost to follow-up in Wave 3. Finally, we included a total of 5,873 participants in the present study. Details regarding the study population selection are provided in Figure 1.

Anthropometric and laboratory measurements

Face-to-face interviews were performed to collect data on demographic information. Participants reported their age, gender,

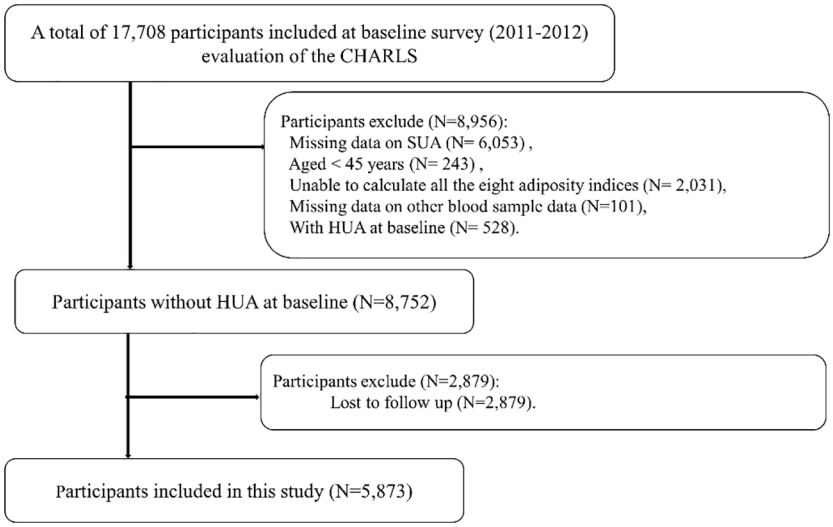

FIGURE 1
Flowchart of study population.

educational levels (primary school or below, middle school, and high school or above), places of residence (rural and urban), marital status, and history of smoking and alcohol drinking. Their height, weight, and WC were measured by trained investigators using standard methods.

Fasting blood samples were collected to measure the blood biochemical indices, including SUA, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL), low-density lipoprotein-cholesterol (LDL), C-reactive protein (CRP), blood urea nitrogen, and serum creatinine. SUA was measured using the UA plus method. FPG, TG, TC, HDL, and LDL were measured using an enzymatic colorimetric test method. The HbA1c assay was performed using the boronate-affinity high-performance liquid chromatography (HPLC) method. BUN was measured using the enzymatic ultraviolet method with urease. CRP was measured using an immunoturbidimetric assay. Serum creatinine was measured by rate-blanking and compensated Jaffe creatinine method.

Definitions of HUA and adiposity indices

According to a previous study, HUA was defined as SUA ≥7.0 mg/dL in men and ≥6.0 mg/dL in women (20, 21).

Adiposity indices were calculated using formulas in Table 1 and were then categorized into quantiles (14, 22, 23).

Hypertension was defined as self-reported hypertension, systolic blood pressure (SBP) ≥140 mmHg, and/or diastolic blood pressure (DBP) ≥90 mmHg.

Statistical analysis

Continuous data are presented as means ± SD, and categorical data are presented as frequency (percentage). The differences between continuous variables and categorical variables were compared using t-test and chi-squared test, respectively. Logistic regression analyses were performed to examine the association between adiposity indices and incident HUA using three models. Model 1 was unadjusted. Model 2 was adjusted for age, educational levels, places of residence, drinking status, smoking status, marital status, and SBP. Model 3 was further adjusted for BMI, WC, BRI, WHtR, and conicity index (CI) and for LAP, VAI, and Chinese visceral adiposity index (CVAI), without TG. In addition, a sensitivity analysis was performed under the definition that HUA was defined as SUA ≥ 7.0 mg/dL in men and women.

The area under the curve (AUC) was calculated by receiver operating characteristic (ROC) curve analysis to compare the predictive ability of different adiposity indices for incident HUA. The cutoff value of adiposity indices calculated by ROC curve analysis was determined using the maximized Youden index value (24). Finally, chi-squared tests were used to determine differences in the incidence of HUA based on the cutoff values obtained by ROC analysis, and then Cramer's V was applied to determine the interpreted effect size (23, 25). All data analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA) and R software (version 4.1.0, http://www.r-project.org). A two-sided *p*-value < 0.05 was considered statistically significant.

Results

Of 5,873 participants (mean age, 59.0 ± 8.7 years) without HUA at baseline enrolled in this study, 2,669 (45.4%) were men. During the 4-year follow-up period, 578 (9.8%) participants developed HUA, with 296 (11.1%) in male participants and 282 (8.8%) in female participants.

Baseline characteristics

The baseline characteristics of participants are summarized in Table 2. Compared to those without incident HUA, male

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TABLE 1 Formulas of adiposity indices.

Adiposity index	Formula
BMI	$weight_{(kg)}/height^2_{(m^2)}$
WHtR	$WC_{(m)}/height_{(m)}$
CI	$(WC_{(m)}) \times 0.109^{-1} \times (weight_{(kg)}/height_{(m)})^{-1/2}$
BRI	$364.2 - 365.5 \times [1 - [(WC/2\pi)/(0.5 \times height)]^2]^{1/2}$
LAP	$TG_{(mmol/L)} \times (WC_{[cm]} - 65)$ (men)
	$TG_{(mmol/L)} \times (WC_{[cm]} - 58)$ (women)
VAI	$[(WC_{(cm)}/[39.68 + (1.88 \times BMI)]] \times (TG_{(mmol/L)}/1.03) \times (1.31/HDL_{(mmol/L)})$ (men)
	$[(WC_{(cm)}/[36.58 + (1.889 \times BMI)]] \times (TG_{(mmol/L)}/0.81) \times (1.52/HDL_{(mmol/L)})$ (women)
CVAI	$-267.93 + 0.68 \times age + 0.03 \times BMI + 4 \times WC + 22 \times logTG_{(mmol/L)} - 16.32 \times HDL_{(mmol/L)}$ (men)
	$-187.32 + 1.71 \times age + 4.23 \times BMI + 1.12 \times WC + 39.76 \times logTG_{(mmol/L)} - 11.66 \times HDL_{(mmol/L)}$ (women)

BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; BRI, body roundness index; CI, conicity index; LAP, lipid accumulation product index; VAI, visceral adiposity index; CVAI, Chinese visceral adiposity index.

participants with incident HUA had a higher prevalence of hypertension; higher SBP, DBP, SUA, serum creatinine, TC, and TG; and lower HDL. Female participants with incident HUA had higher age; higher prevalence of hypertension; higher SBP, DBP, SUA, CRP, serum creatinine, TC, TG, FPG, HbA1c, and BUN; and lower HDL. Regarding the adiposity indices, participants with incident HUA had higher levels of all eight adiposity indices than those without incident HUA in both male and female participants.

Associations between adiposity indices with incident HUA stratified by gender

The associations between adiposity indices with incident HUA in men are shown in Table 3. After adjusting for potential covariates, LAP, VAI, and CVAI were significantly associated with incident HUA. Compared with the first quartile, the hazard ratio (HR) of incident HUA in the highest quartile of the LAP was 1.821 (95%CIs, 1.18 to 2.852); the HR in the highest quartile of the VAI was 1.735 (95%CIs, 1.162 to 2.617); the HR in the highest quartile of the CVAI was 1.547 (95%CIs, 1.023 to 2.360).

The associations between adiposity indices with incident HUA in women are shown in Table 4. After adjusting for potential covariates, all adiposity indices were significantly associated with incident HUA. Compared with the first quartile, the HRs for HUA in the highest quartile of the adiposity indices were 3.550 (95%CIs, 2.173 to 5.989) for CVAI, 2.099 (95%CIs, 1.396 to 3.204) for VAI, 3.616 (95%CIs, 2.258 to 5.996) for LAP, 3.370 (95%CIs, 2.136 to 5.485) for BMI, 2.596 (95%CIs, 1.668 to 4.136) for WC, 3.206 (95% CIs, 1.976 to 5.391) for WHtR, 3.206 (95%CIs, 1.976 to 5.391) for BRI, and 1.673 (95%CIs, 1.089 to 2.602) for CI. The sensitivity analyses showed a similar association with the primary analyses in that all adiposity indices except CI were significantly associated with incident HUA (Supplementary Table 1).

Predictive ability of the adiposity indices to identify incident HUA stratified by gender

Results from the ROC analysis and AUCs for the eight indices are shown in Figure 2. Results of the ROC analysis of the adiposity indices to identify incident HUA in male and female participants are shown in Tables 5, 6, respectively. In male participants, LAP had the highest AUC (0.612), followed by CVAI (0.596), VAI (0.593), BRI (0.592), WHtR (0.592), BMI (0.586), WC (0.579), and CI (0.563). In female participants, CVAI had the highest AUC (0.707), followed by LAP (0.691), BRI (0.669), WHtR (0.669), VAI (0.664), WC (0.664), BMI (0.644), and CI (0.616) (all $p < 0.001$). The sensitivity analyses showed similar AUCs to the primary analyses (Supplementary Table 2).

Incidence of HUA according to adiposity indices

Compared with their counterparts, participants with high levels of adiposity indices had a significantly higher risk of HUA in both men and women. All eight adiposity indices had higher Cramer's V to identify HUA in women than in men (Figures 3, 4).

Discussion

In this nationally prospective study, we investigated the relationships of adiposity indices with HUA risk in 5,873 Chinese middle-aged and older adults during 4 years of follow-up. The present study demonstrated that high visceral adiposity levels were positively associated with a higher risk of HUA among Chinese adults. All adiposity indices showed significant association with incident HUA in female participants, while only LAP, VAI, and CVAI, which contained lipid parameters, were significantly associated with incident HUA in male participants. Furthermore, all the adiposity indices showed better predictive power for incident HUA in women than men. CVAI had the highest AUC for the prediction of incident HUA, followed by LAP and VAI in the female participants, while LAP had the highest AUC for the prediction of incident HUA, followed by VAI in male participants. The results suggested that adiposity indices containing lipid parameters had stronger predictive power for incident HUA than other indices in both genders. In this study, the cumulative incidence of HUA was 9.8% in total, with 11.1% in men and 8.8% in women. Different HUA incidences have been reported in previous studies. For example, Zhang et al. showed that the cumulative incidence of hyperuricemia was 20.2% in total participants, with 21.3% in men and 16.6% in women, in an 8-

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TABLE 2 Baseline characteristics of the study participants were classified by the presence of different genders and incident HUA.

	Male (N = 2,669)			Female (N = 3,204)		
	Not incident HUA (N = 2,373)	Incident HUA (N = 296)	*p*-Value	Not incident HUA (N = 2,922)	Incident HUA (N = 282)	*p*-Value
Age, year	60.0 ± 8.7	60.5 ± 9.0	0.341	57.9 ± 8.6	60.3 ± 9.4	<0.001
Smoking, n (%)	1,801 (75.9)	217 (73.3)	0.329	205 (7.0)	27 (9.6)	0.113
Drinking, n (%)	1,332 (56.1)	176 (59.5)	0.276	342 (11.7)	36 (12.8)	0.598
Marriage status, n (%)			0.410			0.064
Married	2,154 (90.8)	273 (92.2)		2,558 (87.5)	236 (83.7)	
Not married	219 (9.2)	23 (7.8)		364 (12.5)	46 (16.3)	
Places of residence, n (%)			0.143			0.183
Rural	2,032 (85.6)	244 (82.4)		2,433 (83.3)	226 (80.1)	
Urban	341 (14.4)	52 (17.6)		489 (16.7)	56 (19.9)	
Educational level, n (%)			0.526			0.988
Primary school and lower	1,449 (61.0)	190 (64.1)		2,321 (79.4)	225 (79.8)	
Middle school	630 (26.6)	75 (25.4)		424 (14.5)	40 (14.2)	
High school and above	294 (12.4)	31 (10.5)		177 (6.1)	17 (6.0)	
Hypertension, n (%)	880 (37.1)	156 (52.7)	<0.001	1,150 (39.4)	162 (57.4)	<0.001
SBP	129.0 ± 19.7	134.7 ± 22.5	<0.001	129.4 ± 22.5	136.0 ± 22.1	<0.001
DBP	75.6 ± 12.2	78.2 ± 12.9	<0.001	75.1 ± 11.8	78.4 ± 12.3	<0.001
HDL (mmol/L)	1.32 ± 0.42	1.25 ± 0.41	<0.001	1.35 ± 0.37	1.20 ± 0.36	<0.001
LDL (mmol/L)	2.9 ± 0.9	3.0 ± 0.9	0.125	3.1 ± 0.9	3.1 ± 1.1	0.548
TG (mmol/L)	1.4 ± 1.0	1.7 ± 1.3	<0.001	1.5 ± 1.0	2.0 ± 1.2	<0.001
TC (mmol/L)	4.8 ± 0.9	5.0 ± 0.9	<0.001	5.1 ± 1.0	5.2 ± 1.1	0.017
FPG (mmol/L)	6.1 ± 2.0	6.2 ± 2.0	0.556	6.1 ± 1.9	6.3 ± 2.1	0.033
HbA1c, %	5.2 ± 0.7	5.3 ± 0.8	0.672	5.3 ± 0.8	5.4 ± 1.0	0.007
Creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.2	<0.001	0.7 ± 0.1	0.7 ± 0.1	<0.001
CRP	2.7 ± 8.1	2.7 ± 5.5	0.911	2.2 ± 5.8	3.2 ± 7.5	0.007
BUN (mg/dL)	16.4 ± 4.4	16.4 ± 4.0	0.798	14.8 ± 4.2	15.2 ± 3.9	0.014
SUA (mg/dL)	4.6 ± 1.0	5.7 ± 0.9	<0.001	3.8 ± 0.8	4.8 ± 0.7	<0.001
BMI	23.1 ± 11.2	23.8 ± 3.3	0.277	23.9 ± 4.0	25.8 ± 4.0	<0.001
WC	84.6 ± 9.4	87.4 ± 9.9	<0.001	85.3 ± 10.0	91.1 ± 9.6	<0.001
CI	1.27 ± 0.08	1.28 ± 0.08	<0.001	1.30 ± 0.09	1.33 ± 0.09	<0.001
WHtR	0.52 ± 0.07	0.53 ± 0.06	<0.001	0.56 ± 0.07	0.60 ± 0.06	<0.001
BRI	3.8 ± 1.8	4.1 ± 1.2	<0.001	4.6 ± 1.4	5.4 ± 1.3	<0.001
LAP	29.3 ± 35.2	40.3 ± 41.8	<0.001	42.9 ± 37.3	66.3 ± 47.2	<0.001
VAI	1.6 ± 2.3	2.3 ± 3.6	<0.001	2.7 ± 3.1	4.0 ± 4.4	<0.001
CVAI	91.6 ± 42.8	105.9 ± 44.9	<0.001	97.1 ± 34.3	122.2 ± 33.1	<0.001

HUA, hyperuricemia; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL, high-density lipoprotein; TC, total cholesterol; LDL, low-density lipoprotein; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; CRP, C-reactive protein; BUN, blood urea nitrogen; SUA, serum uric acid; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; BRI, body roundness index; CI, conicity index; LAP, lipid accumulation product index; VAI, visceral adiposity index; CVAI, Chinese visceral adiposity index.

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TABLE 3 Association between adiposity indices with incident hyperuricemia multivariate logistic regression analysis in male participants.

		Model 1		Model 2		Model 3	
		HR (95%CIs)	*p*-Value	HR (95%CIs)	*p*-Value	HR (95%CIs)	*p*-Value
BMI	Q1 (<20.525)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (20.525–22.540)	1.501 (1.013, 2.240)	0.044	1.419 (0.959, 2.115)	0.082	1.378 (0.902, 2.117)	0.140
	Q3 (22.540–24.972)	2.029 (1.390, 2.994)	<0.001	1.843 (1.261, 2.721)	0.002	1.481 (0.979, 2.258)	0.065
	Q4 (>24.972)	2.487 (1.707, 3.672)	<0.001	2.099 (1.429, 3.119)	<0.001	1.421 (0.924, 2.204)	0.113
WC	Q1 (<78.00)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (78.00–84.00)	1.184 (0.804, 1.750)	0.393	1.130 (0.767, 1.668)	0.537	1.267 (0.835, 1.929)	0.267
	Q3 (84.00–91.20)	1.513 (1.046, 2.205)	0.029	1.376 (0.950, 2.008)	0.094	1.122 (0.747, 1.696)	0.581
	Q4 (>91.20)	2.123 (1.484, 3.067)	<0.001	1.818 (1.263, 2.641)	0.001	1.326 (0.877, 2.018)	0.184
WHtR	Q1 (<0.477)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (0.477–0.513)	1.293 (0.869, 1.933)	0.207	1.214 (0.817, 1.815)	0.339	1.140 (0.744, 1.753)	0.548
	Q3 (0.513–0.557)	1.845 (1.269, 2.709)	0.002	1.670 (1.147, 2.454)	0.008	1.295 (0.861, 1.962)	0.217
	Q4 (>0.557)	2.468 (1.718, 3.593)	<0.001	2.109 (1.457, 3.089)	<0.001	1.449 (0.959, 2.207)	0.081
BRI	Q1 (<2.945)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (2.945–3.615)	1.293 (0.869, 1.933)	0.207	1.214 (0.817, 1.815)	0.339	1.140 (0.744, 1.753)	0.548
	Q3 (3.615–4.482)	1.845 (1.269, 2.709)	0.002	1.670 (1.147, 2.454)	0.008	1.295 (0.861, 1.962)	0.217
	Q4 (>4.482)	2.468 (1.718, 3.593)	<0.001	2.109 (1.457, 3.089)	<0.001	1.449 (0.959, 2.207)	0.081
CI	Q1 (<1.222)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (1.222–1.271)	1.314 (0.906, 1.915)	0.152	1.292 (0.890, 1.885)	0.180	1.110 (0.741, 1.669)	0.614
	Q3 (1.271–1.321)	1.485 (1.032, 2.151)	0.034	1.413 (0.981, 2.049)	0.065	1.180 (0.793, 1.765)	0.416
	Q4 (>1.321)	1.783 (1.247, 2.572)	0.002	1.626 (1.132, 2.353)	0.009	1.280 (0.858, 1.919)	0.229
LAP	Q1 (<11.211)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (11.211–20.227)	1.828 (1.206, 2.807)	0.005	1.715 (1.134, 2.627)	0.012	1.65 (1.059, 2.599)	0.028
	Q3 (20.227–37.687)	2.715 (1.829, 4.105)	<0.001	2.462 (1.659, 3.718)	<0.001	1.957 (1.274, 3.051)	0.003
	Q4 (>37.687)	3.337 (2.259, 5.029)	<0.001	2.919 (1.970, 4.407)	<0.001	1.821 (1.180, 2.852)	0.008
VAI	Q1 (<0.690)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (0.690–1.132)	1.352 (0.912, 2.015)	0.135	1.398 (0.945, 2.081)	0.095	1.254 (0.821, 1.925)	0.297
	Q3 (1.132–1.949)	1.949 (1.349, 2.849)	<0.001	1.601 (1.091, 2.369)	0.017	1.286 (0.847, 1.965)	0.240
	Q4 (>1.949)	2.354 (1.645, 3.414)	<0.001	2.575 (1.795, 3.743)	<0.001	1.735 (1.162, 2.617)	0.008
CVAI	Q1 (<61.339)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (61.339–88.559)	1.396 (0.939, 2.089)	0.101	1.345 (0.906, 2.009)	0.144	1.425 (0.933, 2.191)	0.103
	Q3 (88.559–122.368)	2.013 (1.384, 2.962)	<0.001	1.827 (1.255, 2.689)	0.002	1.637 (1.091, 2.481)	0.019
	Q4 (>122.368)	2.466 (1.705, 3.612)	<0.001	2.137 (1.471, 3.143)	<0.001	1.547 (1.023, 2.36)	0.040

Abbreviations are the same as in Table 1. HR, hazard ratio; CIs, confidence intervals.
Model 1 was unadjusted. Model 2 was adjusted for age, educational levels, places of residence, drink history, smoke history, marital status, and SBP. Model 3 was further adjusted for history of hypertension, LDL, CRP, creatinine, BUN, FPG, TG, and SUA for BMI, WC, BRI, WHtR, and CI and for LAP, VAI, and CVAI, without TG.

year follow-up study (20), and 20.3% in total participants, with 27.7% in men and 13.2% in women in a prospective study performed in Tianjin, China, from 2013 to 2019 (26). The main reason for the lower incidence of HUA in our study compared with other studies was the shorter follow-up time. Consistent with previous studies, our study also demonstrated that men had a greater risk of developing HUA than women. This observation may be partially attributed to the fact that men typically exhibit higher baseline levels of uric acid compared to women (27). Moreover, men were more likely to drink alcohol, which had

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TABLE 4 Association between adiposity indices with incident hyperuricemia multivariate logistic regression analysis in female participants.

		Model 1		Model 2		Model 3	
		HR (95%CIs)	*p*-Value	HR (95%CIs)	*p*-Value	HR (95%CIs)	*p*-Value
BMI	Q1 (<21.436)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (21.436–23.766)	2.390 (1.542, 3.793)	<0.001	2.664 (1.707, 4.254)	<0.001	2.196 (1.368, 3.604)	0.001
	Q3 (23.766–26.440)	2.549 (1.652, 4.032)	<0.001	2.846 (1.827, 4.541)	<0.001	1.948 (1.211, 3.203)	0.007
	Q4 (>26.440)	4.554 (3.036, 7.046)	<0.001	5.162 (3.387, 8.107)	<0.001	3.089 (1.949, 5.026)	<0.001
WC	Q1 (<78.50)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (78.50–86.00)	1.788 (1.150, 2.830)	0.011	1.807 (1.160, 2.867)	0.010	1.539 (9.580, 2.513)	0.079
	Q3 (86.00–92.60)	2.571 (1.679, 4.022)	<0.001	2.471 (1.608, 3.881)	<0.001	1.821 (1.145, 2.951)	0.013
	Q4 (>92.60)	4.581 (3.090, 6.991)	<0.001	4.232 (2.834, 6.502)	<0.001	2.596 (1.668, 4.136)	<0.001
WHtR	Q1 (<0.515)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (0.515–0.560)	2.348 (1.441, 3.944)	<0.001	2.333 (1.430, 3.924)	<0.001	2.015 (1.201, 3.475)	0.009
	Q3 (0.560–0.606)	4.228 (2.691, 6.913)	<0.001	3.994 (2.534, 6.547)	<0.001	3.091 (1.902, 5.205)	<0.001
	Q4 (>0.606)	5.844 (3.767, 9.462)	<0.001	5.207 (3.327, 8.494)	<0.001	3.206 (1.976, 5.391)	<0.001
BRI	Q1 (<3.636)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (3.636–4.544)	2.348 (1.441, 3.944)	<0.001	2.333 (1.430, 3.924)	<0.001	2.015 (1.201, 3.475)	0.009
	Q3 (4.544–5.565)	4.228 (2.691, 6.913)	<0.001	3.994 (2.534, 6.547)	<0.001	3.091 (1.902, 5.205)	<0.001
	Q4 (>5.565)	5.844 (3.767, 9.462)	<0.001	5.207 (3.327, 8.494)	<0.001	3.206 (1.976, 5.391)	<0.001
CI	Q1 (<1.240)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (1.240–1.300)	1.375 (0.905, 2.107)	0.138	1.356 (0.890, 2.083)	0.159	1.137 (0.724, 1.797)	0.578
	Q3 (1.300–1.357)	2.170 (1.477, 3.238)	<0.001	2.027 (1.372, 3.040)	<0.001	1.548 (1.015, 2.392)	0.045
	Q4 (>1.357)	2.902 (2.005, 4.278)	<0.001	2.420 (1.636, 3.642)	<0.001	1.673 (1.089, 2.602)	0.020
LAP	Q1 (<20.866)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (20.866–33.892)	1.649 (1.008, 2.749)	0.049	1.592 (0.971, 2.658)	0.069	1.42 (0.845, 2.427)	0.191
	Q3 (33.892–56.491)	3.034 (1.945, 4.881)	<0.001	2.884 (1.842, 4.654)	<0.001	2.150 (1.336, 3.556)	0.002
	Q4 (>56.491)	6.313 (4.173, 9.920)	<0.001	5.918 (3.881, 9.363)	<0.001	3.37 (2.136, 5.485)	<0.001
VAI	Q1 (<1.168)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (1.168–1.895)	1.369 (0.928, 2.031)	0.115	0.829 (0.515, 1.326)	0.435	0.66 (0.399, 1.085)	0.102
	Q3 (1.895–3.133)	1.585 (1.086, 2.332)	0.018	2.009 (1.359, 3.013)	<0.001	1.560 (1.021, 2.415)	0.042
	Q4 (>3.133)	2.570 (1.809, 3.702)	<0.001	3.424 (2.371, 5.042)	<0.001	2.099 (1.396, 3.204)	<0.001
CVAI	Q1 (<74.526)	1 (Ref)		1 (Ref)		1 (Ref)	
	Q2 (74.526–96.724)	1.702 (1.024, 2.888)	0.043	1.653 (0.991, 2.812)	0.058	1.420 (0.832, 2.469)	0.204
	Q3 (96.724–122.642)	3.246 (2.055, 5.307)	<0.001	3.081 (1.935, 5.074)	<0.001	2.115 (1.294, 3.561)	0.004
	Q4 (>122.642)	7.156 (4.678, 11.419)	<0.001	6.587 (4.191, 10.758)	<0.001	3.550 (2.173, 5.989)	<0.001

Abbreviations are the same as in Table 1. HR, hazard ratio; CIs, confidence intervals.
Model 1 was unadjusted. Model 2 was adjusted for age, educational levels, places of residence, drink history, smoke history, marital status, and SBP. Model 3 was further adjusted for history of hypertension, LDL, CRP, creatinine, BUN, FPG, TG, and SUA for BMI, WC, BRI, WHtR, and CI and for LAP, VAI, and CVAI, without TG.

been significantly associated with HUA (28). Thus, more interventions should be taken to change the risk factors for HUA, especially in men.

The present study showed that several adiposity indices containing lipid parameters, such as CVAI, VAI, and LAP, were positively associated with incident HUA in both genders. Our results were consistent with several previous studies (11, 22, 23). Liu et al. reported that LAP was positively associated with HUA in China's Yangtze River Delta region population (11), and Kahaer et al. found that LAP and VAI were positively associated with HUA in the Chinese Xinjiang population (16). Huang et al. reported that evaluated VAI increased the risk of hyperuricemia, independently

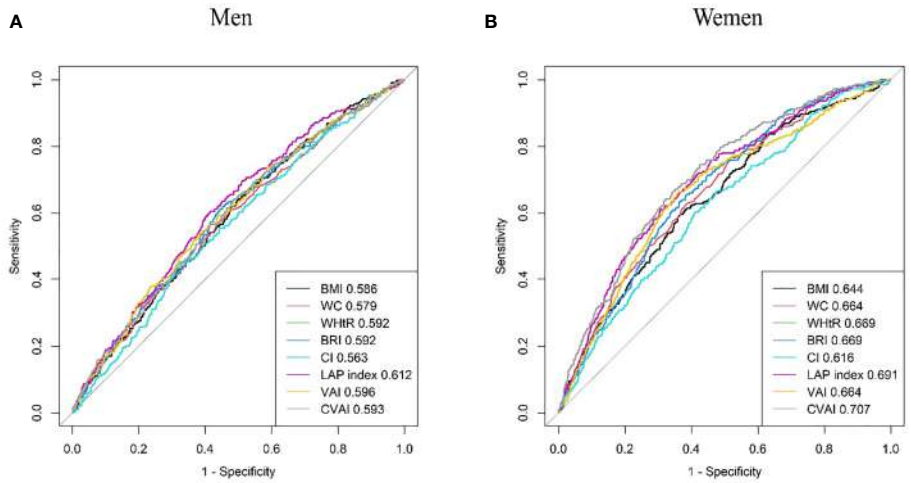

FIGURE 2
Comparison of the predictive value of eight adiposity indices for diagnosis of incident HUA in **(A)** men and **(B)** women. HUA, hyperuricemia.

of BMI and waist circumference, among middle-aged and elderly Chinese adults (21). Previous studies demonstrated that adiposity indices containing lipid parameters showed a better ability to identify obesity and the distribution of adipose tissue, especially visceral adiposity tissue (VAT), than those that did not contain lipid parameters (11, 14, 22). It was indicated that the distribution of adipose tissue instead of the amount had major effects on metabolism (29). Previous studies suggested that uric acid and metabolic syndrome were closely related and that such an association might be bidirectional (8). Some possible mechanisms

TABLE 5 Area under the curve (AUC), cutoff value, sensitivity, specificity, and Youden index of eight adiposity indices in male participants.

Adiposity indices	AUC (95%CIs)	*p*-Value	Cutoff value	Sensitivity	Specificity	Youden index
BMI	0.586 (0.552, 0.619)	<0.001	22.443	0.639	0.504	0.143
WC	0.579 (0.545, 0.614)	<0.001	84.050	0.605	0.530	0.135
WHtR	0.592 (0.558, 0.626)	<0.001	0.519	0.611	0.555	0.166
BRI	0.592 (0.558, 0.626)	<0.001	3.728	0.611	0.555	0.166
CI	0.563 (0.529, 0.597)	<0.001	1.303	0.453	0.676	0.129
LAP	0.612 (0.579, 0.645)	<0.001	23.553	0.605	0.584	0.189
VAI	0.596 (0.562, 0.630)	<0.001	1.372	0.537	0.617	0.154
CVAI	0.593 (0.559, 0.627)	<0.001	88.454	0.642	0.515	0.157

Abbreviations are the same as in Table 1. CIs, confidence intervals.

TABLE 6 Area under the curve (AUC), cutoff value, sensitivity, specificity, and Youden index of eight adiposity indices in female participants.

Adiposity indices	AUC (95%CIs)	*p*-Value	Cutoff value	Sensitivity	Specificity	Youden index
BMI	0.644 (0.611, 0.678)	<0.001	24.881	0.592	0.638	0.230
WC	0.664 (0.632, 0.696)	<0.001	84.850	0.766	0.483	0.249
WHtR	0.669 (0.638, 0.700)	<0.001	0.576	0.652	0.617	0.269
BRI	0.669 (0.638, 0.700)	<0.001	4.890	0.652	0.617	0.269
CI	0.616 (0.583, 0.650)	<0.001	1.316	0.603	0.588	0.191
LAP	0.691 (0.660, 0.723)	<0.001	46.747	0.610	0.694	0.304
VAI	0.664 (0.630, 0.697)	<0.001	2.331	0.663	0.639	0.302
CVAI	0.707 (0.676, 0.737)	<0.001	109.471	0.663	0.657	0.320

Abbreviations are the same as in Table 1. CIs, confidence intervals.

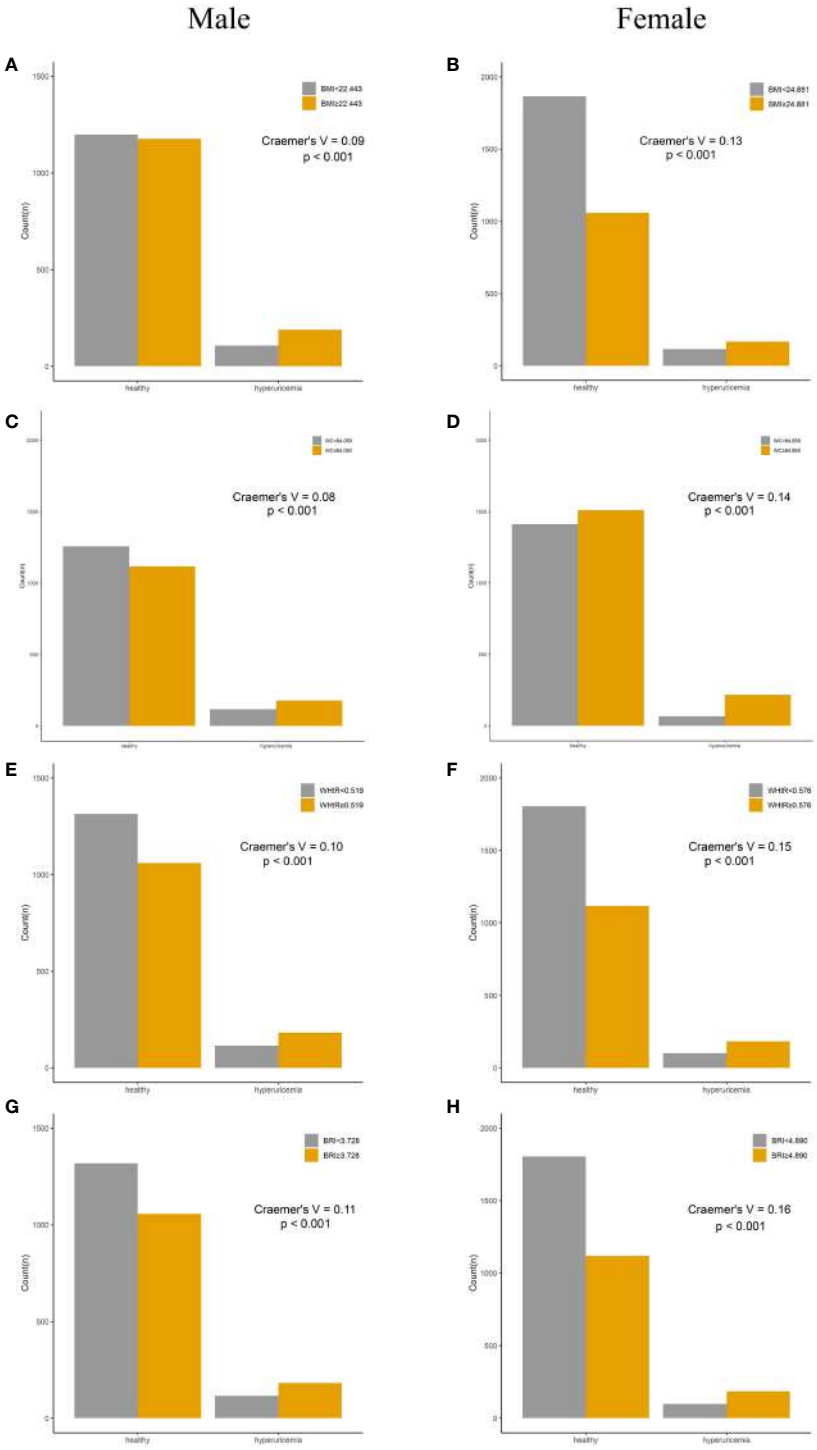

FIGURE 3
Incidence of HUA according to adiposity index cutoff values in men and women. **(A, B)** BMI. **(C, D)** WC. **(E, F)** WHtR. **(G, H)** BRI. HUA, hyperuricemia; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; BRI, body roundness index.

might explain the effect of metabolic disorders on HUA. First, increased VAT accumulation caused the free flow of free fatty acids to the liver and the overproduction of very-low-density lipoprotein (VLDL) and TG. The increase in lipid synthesis increased the need for NADPH and then accelerated the pentose phosphate pathway, which led to the *de novo* purine synthesis, thus increasing the production of UA (20, 30). Second, the elevation of TG and/or TC lipid metabolism disorders may impair kidney function and result in decreased renal blood flow and reduction of the excretion and reabsorption of uric acid (31, 32). Third, previous studies demonstrated that VAT-induced adiponectin and leptin were significantly associated with insulin resistance, which could affect kidney functions, increase reabsorption of uric acid, decrease excretion of uric acid, and lead to hyperuricemia (33, 34).

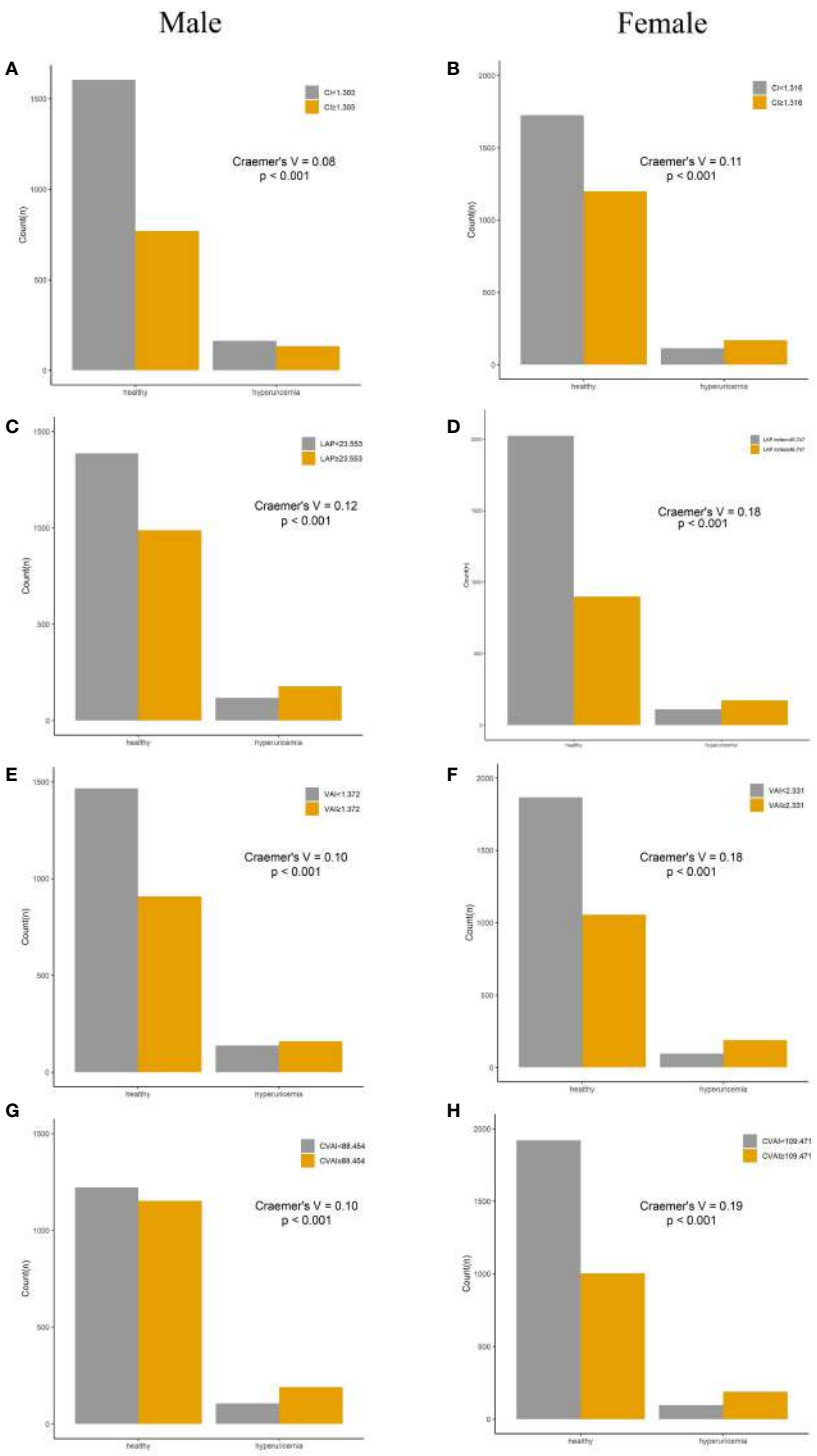

FIGURE 4
Incidence of HUA according to adiposity index cutoff values in men and women. **(A, B)** CI. **(C, D)** LAP. **(E, F)** VAI. **(G, H)** CVAI. HUA, hyperuricemia; CI, conicity index; LAP, lipid accumulation product; VAI, visceral adiposity index; CVAI, Chinese visceral adiposity index.

Our results showed that several adiposity indices, including BMI, WC, WHtR, BRI, and CI, displayed gender-specific associations with incident HUA between men and women. The significant associations between these incidences and incident HUA were observed exclusively in women. Furthermore, all adiposity indices in the present study showed a higher predictive ability of HUA in women than in men. Previous studies reported inconsistent results on the association between these indices and HUA. Kahaer et al. reported that BMI, WC, WHtR, and BRI exhibited no significant association with HUA in both men and women (16), while Zhang et al. reported that BRI, WHtR, BMI, and WC were associated with HUA in both genders (15). Several previous studies also demonstrated that the adiposity indices had a greater predictive ability of HUA in women than men in longitudinal and cross-

sectional studies (15, 16). The gender variation regarding the association between adiposity indices with HUA and the predictive ability may be explained by biological differences between men and women. Middle-aged and older women showed age-related increases in serum uric acid levels, while such a trend was not found in men (35). In the present study, the female participants were generally in perimenopause and menopause. Their estrogen levels decreased with aging (36, 37), thus reducing the renal clearance of urate and resulting in increased SUA levels (36). Furthermore, a clinical study reported that sex hormone replacement therapy can reduce SUA concentrations and decrease the risk of gout flare in postmenopausal women. This suggested that the decreased estrogen levels in postmenopausal women could increase the risk of HUA (38). Taken together, these physiological changes may cause women to be more sensitive to metabolic changes and thus more likely to develop HUA. In addition, the associations were explored in 4-year folloe-up study.

The present study has several strengths. The data in this study came from a population-based nationwide prospective survey, which provides a representative sample of the population. Then, a longitudinal study was conducted, whose evidence power was stronger than that of previous cross-sectional studies. Furthermore, the predictive ability of a total of eight adiposity indices with HUA was investigated, and for the first time, the associations between several adiposity indices (such as CVAI, VAI, and LAP) and incident HUA were reported in a longitudinal survey. In addition, we also proposed optimal cutoff values for these indices by ROC curve analysis and further verified the effectiveness in the diagnosis of HUA. Nevertheless, there are several limitations in this study. First, although we have adjusted for several potential confounding variables, we did not include data on variables that could potentially influence uric acid levels, such as dietary factors (consumption of dairy, meat, and micronutrients). Second, the use of uric acid-lowering drugs, such as allopurinol, was not available in the study, which may cause diagnostic bias. Third, the participants were Chinese adults aged ≥45 years, which may limit the generalizability of our results to the younger population and other ethnic groups.

In conclusion, our study provided strong evidence that the adiposity indices were effective predictors of incident HUA in general middle-aged and older Chinese adults. Furthermore, some indices, including BMI, WC, WHtR, BRI, and CI, displayed gender-specific associations with incident HUA, with a significant association observed exclusively in women. All adiposity indices showed better predictive ability for incident HUA in women than men. For the preventive prospect and clinical practice, adiposity indices, especially those containing lipid parameters, were obtainable and cost-effective and could be used in HUA prevention among Chinese middle-aged and older adults.

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 below: http://charls.pku.edu.cn/.

Author contributions

ZL: Conceptualization, Writing – original draft. QZ: Conceptualization, Methodology, Writing – original draft. YT: Writing – original draft. JL: Writing – original draft. QC: Funding acquisition, Methodology, Writing – original draft. HY: Conceptualization, Formal analysis, Methodology, Software, Writing – original draft. SZ: Formal analysis, Funding acquisition, Writing – original draft.

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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.2024.1336471/full#supplementary-material

References

1. Cheang C, Law S, Ren J, Chan W, Wang C, Dong Z. Prevalence of hyperuricemia in patients with severe obesity and the relationship between serum uric acid and severe obesity: A decade retrospective cross-section study in Chinese adults. *Front Public Health* (2022) 10:986954. doi: 10.3389/fpubh.2022.986954

2. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. *Int J Cardiol* (2016) 213:8–14. doi: 10.1016/j.ijcard.2015.08.109

3. Zhang M, Zhu X, Wu J, Huang Z, Zhao Z, Zhang X, et al. Prevalence of hyperuricemia among chinese adults: findings from two nationally representative cross-sectional surveys in 2015-16 and 2018-19. *Front Immunol* (2021) 12:791983. doi: 10.3389/fimmu.2021.791983

4. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. *Arthritis Rheum* (2011) 63:3136–41. doi: 10.1002/art.30520

5. Choi BG, Kim DJ, Baek MJ, Ryu YG, Kim SW, Lee MW, et al. Hyperuricaemia and development of type 2 diabetes mellitus in Asian population. *Clin Exp Pharmacol Physiol* (2018) 45:499–506. doi: 10.1111/1440-1681.12911

6. Eren M. The association between serum uric acid level and heart failure and mortality in the early period of STEMI. *Turk Kardiyol Dern Ars* (2014) 42:509–10. doi: 10.5543/tkda.2014.15729

7. Chang JB, Chen YL, Hung YJ, Hsieh CH, Lee CH, Pei D, et al. The role of uric acid for predicting future metabolic syndrome and type 2 diabetes in older people. *J Nutr Health Aging* (2017) 21:329–35. doi: 10.1007/s12603-016-0749-3

8. Borghi C, Fogacci F, Piani F. Not all the eggs and the chickens are the same: The case of uric acid and metabolic syndrome. *Eur J Intern Med* (2022) 103:36–7. doi: 10.1016/j.ejim.2022.07.006

9. Dong H, Xu Y, Zhang X, Tian S. Visceral adiposity index is strongly associated with hyperuricemia independently of metabolic health and obesity phenotypes. *Sci Rep* (2017) 7:8822. doi: 10.1038/s41598-017-09455-z

10. Rospleszcz S, Dermyshi D, Muller-Peltzer K, Strauch K, Bamberg F, Peters A. Association of serum uric acid with visceral, subcutaneous and hepatic fat quantified by magnetic resonance imaging. *Sci Rep* (2020) 10:442. doi: 10.1038/s41598-020-57459-z

11. Liu XZ, Li HH, Huang S, Zhao DB. Association between hyperuricemia and nontraditional adiposity indices. *Clin Rheumatol* (2019) 38:1055–62. doi: 10.1007/s10067-018-4374-x

12. Zhou Z, Li K, Li X, Luan R, Zhou R. Independent and joint associations of body mass index, waist circumference, waist-height ratio and their changes with risks of hyperuricemia in middle-aged and older Chinese individuals: a population-based nationwide cohort study. *Nutr Metab (Lond)* (2021) 18:62. doi: 10.1186/s12986-021-00590-z

13. Ryu S, Chang Y, Zhang Y, Kim SG, Cho J, Son HJ, et al. A cohort study of hyperuricemia in middle-aged South Korean men. *Am J Epidemiol* (2012) 175:133–43. doi: 10.1093/aje/kwr291

14. Lee WC, Wu PY, Huang JC, Tsai YC, Chiu YW, Chen SC, et al. Sex difference in the associations among obesity-related indices with incident hypertension in a large Taiwanese population follow-up study. *J Pers Med* (2022) 12. doi: 10.3390/jpm12060972

15. Zhang N, Chang Y, Guo X, Chen Y, Ye N, Sun Y. A Body Shape Index and Body Roundness Index: Two new body indices for detecting association between obesity and hyperuricemia in rural area of China. *Eur J Intern Med* (2016) 29:32–6. doi: 10.1016/j.ejim.2016.01.019

16. Kahaer M, Zhang B, Chen W, Liang M, He Y, Chen M, et al. Triglyceride glucose index is more closely related to hyperuricemia than obesity indices in the medical checkup population in Xinjiang, China. *Front Endocrinol (Lausanne)* (2022) 13:861760. doi: 10.3389/fendo.2022.861760

17. Tian X, Chen S, Xu Q, Zhang Y, Wu S, Wang A, et al. Cumulative burden of abnormal visceral adiposity index and its components on the risk of hyperuricemia. *Nutr Metab Cardiovasc Dis* (2023) 33:340–49. doi: 10.1016/j.numecd.2022.11.016

18. Su SY, Lin TH, Liu YH, Wu PY, Huang JC, Su HM, et al. Sex difference in the associations among obesity-related indices with hyperuricemia in a large Taiwanese population study. *Nutrients* (2023) 15. doi: 10.3390/nu15153419

19. Zhao Y, Hu Y, Smith JP, Strauss J, Yang G. Cohort profile: the China health and retirement longitudinal study (CHARLS). *Int J Epidemiol* (2014) 43:61–8. doi: 10.1093/ije/dys203

20. Zhang Y, Zhang M, Yu X, Wei F, Chen C, Zhang K, et al. Association of hypertension and hypertriglyceridemia on incident hyperuricemia: an 8-year prospective cohort study. *J Transl Med* (2020) 18:409. doi: 10.1186/s12967-020-02590-8

21. Huang X, Jiang X, Wang L, Chen L, Wu Y, Gao P, et al. Visceral adipose accumulation increased the risk of hyperuricemia among middle-aged and elderly adults: a population-based study. *J Transl Med* (2019) 17:341. doi: 10.1186/s12967-019-2074-1

22. Wu J, Gong L, Li Q, Hu J, Zhang S, Wang Y, et al. A Novel Visceral Adiposity Index for Prediction of Type 2 Diabetes and Pre-diabetes in Chinese adults: A 5-year prospective study. *Sci Rep* (2017) 7:13784. doi: 10.1038/s41598-017-14251-w

23. Ramirez-Velez R, Perez-Sousa MA, Gonzalez-Ruiz K, Cano-Gutierrez CA, Schmidt-RioValle J, Correa-Rodriguez M, et al. Obesity- and lipid-related parameters in the identification of older adults with a high risk of prediabetes according to the american diabetes association: an analysis of the 2015 health, well-being, and aging study. *Nutrients* (2019) 11. doi: 10.3390/nu11112654

24. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J* (2005) 47:458–72. doi: 10.1002/bimj.200410135

25. McHugh ML. The chi-square test of independence. *Biochem Med (Zagreb)* (2013) 23:143–49. doi: 10.11613/bm.2013.018

26. Zhang T, Gan S, Ye M, Meng G, Zhang Q, Liu L, et al. Association between consumption of ultra-processed foods and hyperuricemia: TCLSIH prospective cohort study. *Nutr Metab Cardiovasc Dis* (2021) 31:1993–2003. doi: 10.1016/j.numecd.2021.04.001

27. Lin YK, Lin YP, Lee JT, Lin CS, Wu TJ, Tsai KZ, et al. Sex-specific association of hyperuricemia with cardiometabolic abnormalities in a military cohort: The CHIEF study. *Med (Baltimore)* (2020) 99:e19535. doi: 10.1097/MD.0000000000019535

28. Hong F, Zheng A, Xu P, Wang J, Xue T, Dai S, et al. High-protein diet induces hyperuricemia in a new animal model for studying human gout. *Int J Mol Sci* (2020) 21. doi: 10.3390/ijms21062147

29. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* (2008) 359:2105–20. doi: 10.1056/NEJMoa0801891

30. Tanaka K, Ogata S, Tanaka H, Omura K, Honda C, Hayakawa K. The relationship between body mass index and uric acid: a study on Japanese adult twins. *Environ Health Prev Med* (2015) 20:347–53. doi: 10.1007/s12199-015-0473-3

31. Seyed-Sadjadi N, Berg J, Bilgin AA, Grant R. Visceral fat mass: is it the link between uric acid and diabetes risk? *Lipids Health Dis* (2017) 16:142. doi: 10.1186/s12944-017-0532-4

32. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *Jama* (1991) 266:3008–11. doi: 10.1001/jama.1991.03470210076036

33. Adnan E, Rahman IA, Faridin HP. Relationship between insulin resistance, metabolic syndrome components and serum uric acid. *Diabetes Metab Syndr* (2019) 13:2158–62. doi: 10.1016/j.dsx.2019.04.001

34. McCormick N, O'Connor MJ, Yokose C, Merriman TR, Mount DB, Leong A, et al. Assessing the causal relationships between insulin resistance and hyperuricemia and gout using bidirectional mendelian randomization. *Arthritis Rheumatol* (2021) 73:2096–104. doi: 10.1002/art.41779

35. Akizuki S. Serum uric acid levels among thirty-four thousand people in Japan. *Ann Rheum Dis* (1982) 41:272–74. doi: 10.1136/ard.41.3.272

36. Anton FM, Garcia PJ, Ramos T, Gonzalez P, Ordas J. Sex differences in uric acid metabolism in adults: evidence for a lack of influence of estradiol-17 beta (E2) on the renal handling of urate. *Metabolism* (1986) 35:343–48. doi: 10.1016/0026-0495(86)90152-6

37. Guan S, Tang Z, Fang X, Wu X, Liu H, Wang C, et al. Prevalence of hyperuricemia among Beijing post-menopausal women in 10 years. *Arch Gerontol Geriatr* (2016) 64:162–66. doi: 10.1016/j.archger.2016.02.002

38. Hak AE, Choi HK. Menopause, postmenopausal hormone use and serum uric acid levels in US women–the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* (2008) 10:R116. doi: 10.1186/ar2519

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Rifka C. Schulman-Rosenbaum,
Northwell Health, United States
Ivana Rabbone,
University of Eastern Piedmont, Italy

*CORRESPONDENCE
Jing-Wen Duan
duanjingwen314@163.com
Hong-Li Liu
lhly123@sina.com

†These authors have contributed equally to this work

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Association between vitamin D status and cardiometabolic risk factors in adults with type 2 diabetes in Shenzhen, China

Yan-Jing Liu[1†], Jing-Wen Duan[2,3*†], Dong-Hui Lu[1], Fan Zhang[1] and Hong-Li Liu[1*]

[1]Department of Endocrinology and Metabolism, Peking University Shenzhen Hospital, Peking University Shenzhen Hospital, Shenzhen, Guangdong, China, [2]Department of Cardiology, The Third Xiangya Hospital, Central South University, Changsha, China, [3]Clinical Research Center, The Third Xiangya Hospital, Central South University, Changsha, China

Background: Evidence of vitamin D status and cardiometabolic health in adults with type 2 diabetes mellitus (T2DM) is still limited. This study aimed to investigate the association between vitamin D status and cardiometabolic risk factors among adults with T2DM in Shenzhen, China.

Methods: This cross-sectional study included 164 adults (aged ≥18 years) with T2DM who were hospitalized at Peking University Shenzhen Hospital from March 1, 2023, to May 31, 2023. Serum 25-hydroxyvitamin D [25(OH)D] concentration, the active marker of vitamin D, and three major cardiometabolic risk factors including blood pressure (BP), glucose metabolism-related indicators, and blood lipid profiles were collected. Vitamin D deficiency (VDD) was defined as 25(OH)D < 20 ng/mL. Correlation, Regression, and Logistic analysis were applied to verify the association among serum 25(OH)D concentration, VDD, and 11 cardiometabolic risk factors.

Results: Median 25(OH)D concentration was 21.78 [interquartile range (IQR) =17.51-28.05] ng/mL. The prevalence of VDD was 40.24%. Serum 25(OH)D concentration was significantly negatively correlated with diastolic BP (DBP) and glycated hemoglobin A1c (HbA1c) rather than systolic BP, plasma glucose, plasma C-peptide, and blood lipid profiles among adults with T2DM in both correlation and linear regression analysis. Furthermore, the adjusted odd ratio for poor DBP control (≥90 mmHg) of T2DM patients with VDD was 3.164 (95% confidence interval=1.303, 7.683; P=0.011) compared to those without VDD.

Conclusion: In China, VDD was highly prevalent among adults with T2DM and associated with greater cardiovascular risk factors, especially with increased chances of uncontrolled DBP. These findings suggest that vitamin D levels should be monitored in T2DM patients, especially those with high DBP.

KEYWORDS

vitamin D, blood pressure, HbA1c, C-peptide, lipid profiles

1 Introduction

Diabetes mellitus (DM), especially type 2 DM (T2DM), is known as one of the most prevalent chronic, life-threatening diseases worldwide. According to the latest estimates from the International Diabetes Federation, there are 536.6 million adult (20-79 years old) people with DM in 2021, which represents more than 10% of the global adult population (1). Compared to people without T2DM, in addition, those with T2DM are at higher risk of cardiovascular disease (CVD), which is a well-known cause of morbidity and mortality (2). Although advanced progress has been made in preventing future CVD risk among patients with T2DM, mainly due to early screening and treatment of its risk factors like blood pressure (BP), blood lipid, and glucose metabolism, CVD remains the main cause of death among patients with T2DM (3). Thus, the identification of other modifiable risk factors is of great significance in postponing the development of CVD among patients with T2DM.

Over the last decade, along with the discovery of the expression of vitamin D receptors in non-skeletal organs and tissues, including adipose, cardiomyocytes, pancreatic β-cells, and others, vitamin D status has been described to be linked with the onset of T2DM and the development of complications among patients with T2DM, including CVD (4–8). Also, increasing studies have linked vitamin D status with the common and important cardiometabolic risk factors including BP, blood lipids profile, and glucose metabolism-related indicators in the non-diabetes population. However, the association between vitamin D and these cardiometabolic risk factors remains controversial and limited, especially for patients with T2DM. To date, only two studies have investigated the association between vitamin D status and BP and hypertension, respectively, and one of these studies simultaneously looked at its association with dyslipidemia among patients with T2DM according to the recent systematic review by Md et al. (9–11) In terms of glucose metabolism, most results of related studies are consistent, suggesting that lower concentrations of serum vitamin D are closely related to poor glycemic control, however, these studies were limited to the general population, the older, pregnant women, and other nondiabetic populations, and few studies focused on patients with T2DM, who are more prone to vitamin D deficiency (VDD) (12–18).

Above all, the association between vitamin D status and BP, blood lipids, and glucose metabolism remains further explored. Thus, this study aims to comprehensively explore the association of serum 25(OH) level, the most common active marker of vitamin D, and VDD with BP [including systolic BP (SBP) and diastolic BP (DBP)], glucose metabolism-related indicators [including oral glucose tolerance test (OGTT) that includes both fasting plasma glucose (FPG) and 2h post-load plasma glucose (2hPG), fasting plasma C-peptide, glycated hemoglobin A1c (HbA1c), and insulin resistance (IR)], and blood lipids profile [including total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), and triglyceride (TG)] among adult T2DM patients in China, to provide more theoretical clues for reducing risk of CVD in this population.

2 Materials and methods

2.1 Study population and design

In this cross-sectional study, we initially included 488 adults (aged ≥18 years) with DM who were hospitalized in the endocrinology units of the Peking University Shenzhen Hospital from March 1, 2023, to May 31, 2023. First of all, those well-diagnosed with DM were included according to the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2020 edition) as follows (19): 1) FPG ≥7.0 mmol/L, or 2h post glucose load BG ≥ 11.1 mmol/L, or HbA1c ≥6.5%; 2) self-reported diagnosis of DM in a secondary or tertiary hospital, or 3) currently receiving hypoglycemic therapy, including oral hypoglycemic agents and the use of insulin. Then, we excluded those with a diagnosis of unclassified DM, type 1 DM, gestational DM, and other special types of DM. Next, we further excluded participants with conditions that could affect the production and secretion of 25(OH) D, which included hyperparathyroidism, hypoparathyroidism, and hepatic insufficiency.

In addition, participants with pregnancy, infection, and cancer or missing data were also excluded. Finally, 164 patients with diagnosed T2DM were included in the analysis. The flowchart of the study population is shown in Figure 1. This study followed the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Peking University Shenzhen Hospital. All the participants signed an informed consent form at admission and agreed to share their health information for medical research.

2.2 Data collection

The basic information, clinical assessment, and biochemical indicators (serum) of all participants were collected from the Hospital Information System (HIS), including age, sex (female/male), weight, height, SBP, DBP, 25(OH)D, TC, TG, LDL-c, HDL-c, HbA1c, FPG, 2hPG, fasting plasma C-peptide, calcium (Ca), calcitonin, parathyroid hormone (PTH), osteocalcin (OC), β-C-terminal cross-linked telopeptide of type I collagen (β-CTX), and procollagen-1 N-terminal peptide (P1NP), creatinine (Cr), calcitonin (CT), uric acid (UA), urinary Albumin-to-Creatinine Ratio (uACR), 24-hour urinary protein quantity (UP), alanine aminotransferase (ALT), thyrotropin (TRH), thyroid stimulating hormone (TSH), and free thyroxine (FT4).

2.3 Clinical assessment

Each participant's weight, height, and BP were measured by well-trained nurses at the time of admission. Specifically, weight and height were measured with the participants wearing light clothing and no shoes to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of height in meters (m). SBP and DBP of the right upper arm were measured using a validated

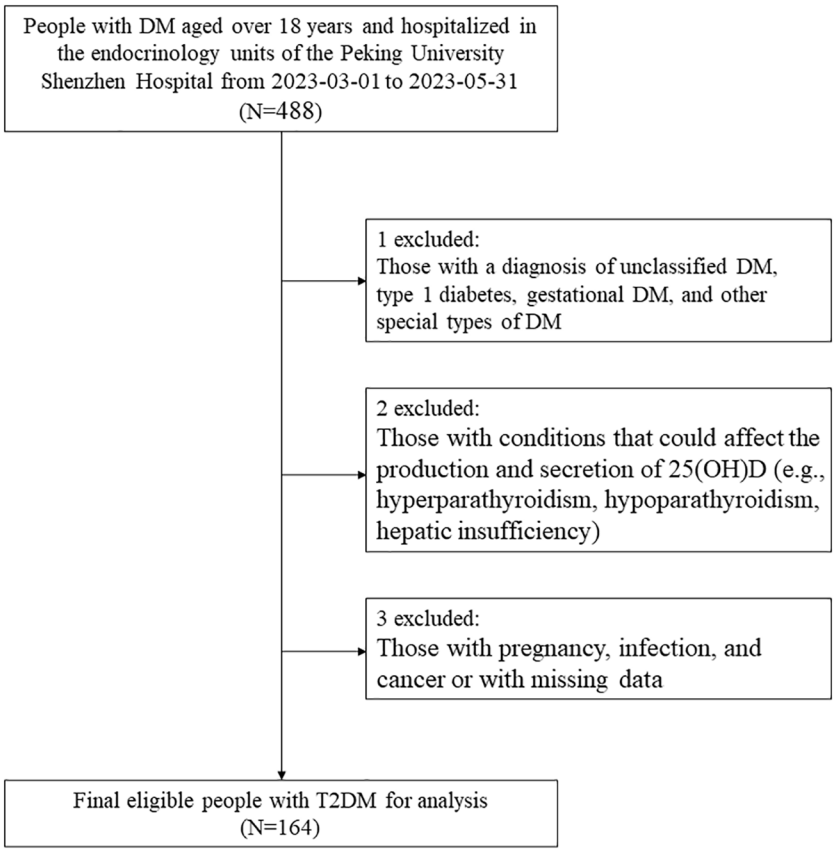

FIGURE 1
Study flowchart. DM, diabetes; 25(OH)D, 25-hydroxyvitamin D; T2DM, type 2 diabetes mellitus.

digital automatic analyzer (Omron, Japan) in a seated position after at least 5 minutes of rest and an additional recording after a 2-minute break. SBP and DBP were each measured twice and the mean of the two readings was used for analyses. If the two readings differed by >5 mmHg, a third measurement was performed, and the average of all three readings was applied.

2.4 Biochemical measurements

Blood samples of each participant who was included in the final analysis were drawn in the morning after overnight fasting (≥8 hours) during hospitalization. The main outcome parameters of the laboratory included blood lipids (TC, TG, LDL-c, HDL-c) and glucose metabolism-related indicators (HbA1c, FPG, 2hPG, and fasting plasma C-peptide). Participants underwent a 75 g OGTT with plasma glucose measured using a routine hexokinase method and C-peptide measured by Chemiluminescence immunoassay before (fasting) and 2 h post glucose load (i.e., FPG, 2hPG, and fasting plasma C-peptide). HbA1c was measured using capillary electrophoresis. TG, TC, and LDL-c/HDL-c were measured using enzymatic assays (GK-GPO-POD colorimetric method), cholesterol oxidase methods, and homogeneous methods, respectively. Levels of all the above parameters were measured by an automatic biochemical analyzer (AU5800 Series Chemistry Analyzers, Beckman Coulter) in the clinical laboratory of Peking University Shenzhen Hospital.

2.5 Measurement of serum 25(OH)D and classification of vitamin D status

Serum 25(OH)D concentration was measured by electrochemiluminescence immunoassay (ECLIA) using the same automatic biochemical analyzer in the clinical laboratory of Peking University Shenzhen Hospital. VDD was defined as 25(OH)D <20 ng/mL, and vitamin D sufficiency (VDS) was defined as 25(OH)D ≥20 ng/mL according to the Endocrine Society clinical practice guidelines and methods provided by serval published research (20).

2.6 Definition of outcomes

The cardiometabolic risk factors of interest in this study include BP (including SBP and DBP), glucose metabolism-related indicators (including FPG, 2hPG, fasting plasma C-peptide, HbA1c, and IR), and blood lipid profile (including TC, TG, LDL-c, and HDL-c). Usually, IR is expressed by HOMA, which can calculated from fasting blood insulin, blood glucose, and C-peptide values. Since most patients with T2DM may use insulin to control

blood glucose levels, the measurement of fasting insulin may be affected by exogenous insulin; therefore, C-peptide values were used to calculate HOMA in this study [i.e., HOMA2 based on C-peptide (HOMA2-Cpep)]. According to the method provided by Ferrannini G et al. (21), HOMA2-Cpep was calculated at https://www.dtu.ox.ac.uk/homacalculator/, with high HOMA-IR values indicating insulin resistance.

Poor BP control included the following two situations: SBP ≥140 mmHg or DBP ≥90 mmHg. Poor blood lipid control was defined as the following four situations: hypertriglyceridemia (TG ≥2.26 mmol/L), hypercholesterolemia (TC ≥6.2 mmol/L), high levels of LDL-c (LDL-c ≥4.14 mmol/L), or low levels of HDL-c (HDL-c ≤1.04 mmol/L). Poor glycemic control was defined as the occurrence of any one of the following three situations: HbA1c ≥7%, FPG ≥ 7.0 mmol/L, or 2h post glucose load BG ≥ 11.1 mmol/L.

2.7 Statistical analysis

Baseline characteristics of the participants classified by vitamin D status were presented as means ± standard deviation (SD) or median [interquartile range (IQR)] for continuous variables with normal or skewed distribution, respectively; and percentages (%) for categorical variables. To compare the differences between VDD and VDS groups, the student T-test or Mann-Whitney U test was performed for continuous variables with normal or skewed distribution, respectively; and the Chi-square test was performed for categorical variables. Pearson correlation analysis or Spearman correlation analysis for continuous variables with normal or skewed distribution were used to analyze whether 25(OH)D concentrations linked to SBP, DBP, HbA1c, FPG, 2hPG, fasting plasma C-peptide, HOMA2-Cpep, TC, TG, LDL-$_C$, and HDL-$_C$, respectively.

To determine whether 25(OH)D levels independently affected these indicators, multivariable linear regression analysis was further performed by three models (model 1: unadjusted; model 2: adjusted for age and sex; and model 3: adjusted for age, sex, BMI, ALT, CR, TSH, and FT4). Furthermore, multivariable logistic analysis was used to clarify the association between VDD and these risk factors that were significant in the correlation analysis and linear regression analysis based on the same models. All statistical analysis was performed by Stata statistical software version 17.0 (Stata Corp LP, College Station, TX, United States). The value of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Characteristics of the study population

A total of 164 adults with T2DM with a mean age of 58.2 ± 11.0 years met the criteria and were finally enrolled in this study. The characteristics of these participants are shown in Table 1. The median 25(OH)D concentration in the present study was 21.78 (IQR=17.51-28.05) ng/mL. Overall, 66 (40.24%) participants were confirmed to have VDD. In addition, adults with T2DM in the VDD group exhibited significantly elevated DBP (80.91 ± 11.04 vs 74.76 ± 10.67; *P*=0.001), β-CTX [0.45 (0.31-0.58) vs 0.36 (0.23-0.51); 0.048], HbA1c (8.69 ± 1.70 vs 8.03 ± 1.69; 0.016), and uACR [21.66 (7.40-67.60) vs 10.89 (5.68-27.61); 0.038], whereas those in the VDS group exhibited significantly higher age (55.7 ± 11.6 vs 59.9 ± 10.3; 0.014).

3.2 Serum 25(OH)D concentration and cardiometabolic health

Table 2 and Figure 2 show the results of the correlation analysis of 25(OH)D and cardiometabolic risk factors. For both measures of BP, the correlation analysis showed that serum 25(OH)D concentration was only significantly negatively correlated with DBP (r=-0.219, *P*=0.005), but SBP (r=-0.008, *P*=0.922) among adult T2DM patients in China. For different indicators of lipid profile (TC, TG, LDL-$_C$, HDL-$_C$) and glucose metabolism (HbA1c, FPG, 2hPG, fasting plasma C-peptide, HOMA2-Cpep), serum 25(OH)D concentration was only significantly negatively correlated with TG (r=-0.247, *P*=0.001), FPG (r=-0.152, *P*=0.053), and HbA1c (r=-0.200, *P*=0.010) among this population.

The results of multiple linear regression analysis of serum 25(OH)D concentrations and cardiometabolic risk factors are shown in Table 3. Among adults with T2DM, serum 25(OH)D concentration were significantly negatively correlated with HbA1c [β (95% CI): -0.037 (-0.070, -0.005), *P*=0.026], TG [-0.028 (-0.054, -0.001), 0.040], and DBP [-0.303 (-0.513, -0.093), 0.005], and they were significantly positively correlated with HDL [0.006 (0.001, 0.012), 0.027]. Similar results were obtained when some or all potential confounders were adjusted, that is, serum 25(OH)D concentration was significantly negatively correlated with HbA1c [-0.040 (-0.075, -0.005), 0.024] and DBP [-0.252 (-0.470, -0.034), 0.024] and significantly positively correlated with HDL [0.007 (0.001, 0.012), 0.016], but there was no significant correlation with TG [-0.015 (-0.0413, 0.012),0.280].

3.3 Vitamin D deficiency and cardiometabolic health

Compared to adults with T2DM in the VDS group, the logistic analysis showed that the unadjusted odds ratios (ORs) for poor DBP control (≥90 mmHg), low levels of HDL (≤1.04 mmol/L), and poor glycemic control (HbA1c ≥7%) among those in the VDD group were 3.164 (95%CI=1.303, 7.683; P=0.011), 1.163 (0.616, 2.196; 0.640), and 1.800 (0.839, 3.863; 0.131) and adjusted ORs were 3.168 (95%CI=1.303, 7.683; P=0.019), 1.258 (0.611, 2.592; 0.534), and 2.177 (0.942, 5.030; 0.069), respectively (shown in Figure 3).

4 Discussion

This study demonstrated that there was a significant correlation between serum 25(OH)D concentration and cardiometabolic risk factors of DBP, HbA1c, TG, and HDL-c, especially DBP, in Chinese adults with T2DM. Specifically, lower concentrations of serum 25(OH)D significantly correlated with higher levels of DBP, HbA1c,

TABLE 1 Baseline characteristics of the study participants.

Characteristics	All participants	Vitamin D deficiency (<20 ng/mL)		Vitamin D sufficiency (≥20 ng/mL)		*P*-value
		n	Mean ± SD or Median (IQR) *	n	Mean ± SD or Median (IQR)	
Female/male	70/94	28/38	/	42/56	/	0.956
Age, year	58.2 ± 11.0	66	55.7 ± 11.6	98	59.9 ± 10.3	**0.014**
Clinical assessment						
BMI, kg/m^2	24.06 (22.13-25.90)	66	24.24 (26.30-22.49)	98	23.94 (21.87-25.85)	0.474
SBP, mmHg	122 (112.5-136)	66	122 (113-136)	98	119.5 (108-136)	0.508
DBP, mmHg	77.23 ± 11.20	66	80.91 ± 11.04	98	74.76 ± 10.67	**0.001**
Laboratory assessment (serum)						
Osteocalcin, ng/mL	13.2 (10.45-16.8)	66	14.00 (10.60-17.70)	98	12.5 (10.4-15.9)	0.148
β-CTX, ng/mL	0.395 (0.26-0.54)	66	0.45 (0.31-0.58)	98	0.36 (0.23-0.51)	**0.048**
P1NP, g/mL	35.6 (27.5-48.2)	66	39.35 (29.5-50.5)	98	34 (26.3-46.3)	0.066
25(OH)D, g/mL	21.78 (17.51-28.05)	66	16.73 (14.57-18.43)	98	27.02 (22.98-32.96)	**<0.001**
Ca, mmol/L	2.26 (2.20-2.31)	66	2.27 (2.20-2.31)	98	2.25 (2.19-2.31)	0.365
Cr, μmol/L	67.00 (53.50-78.50)	66	64.00 (51.00-80.00)	98	67.00 (54.00-77.00)	0.735
uACR, mg/g	14.11 (5.75-39.61)	66	21.66 (7.40-67.60)	98	10.89 (5.68-27.61)	**0.038**
24hUP, g/24h	0.11 (0.08-0.16)	66	0.10 (0.06-0.16)	98	0.11 (0.08-0.15)	0.385
PTH, pmol/L	3.4 (2.7-4.5)	34	3.5 (2.9-4.9)	61	3.4 (2.6-4.2)	0.125
Calcitonin, pg/ml	0.58 (0.50-2.02)	32	0.50 (0.50-1.34)	54	0.77 (0.50-2.14)	0.211
UA, μmol/L	330.18 ± 85.50	66	336.36 ± 84.95	98	326.01 ± 86.05	0.449
ALT, U/L	19.34 ± 8.55	66	20.55 ± 9.10	98	18.52 ± 8.09	0.291
TSH, mIU/L	1.71 (1.09-2.38)	64	1.77 (1.14-2.60)	98	1.60 (0.97-2.14)	0.231
FT4, pmol/L	11.18 (9.95-12.74)	66	11.25 (10.01-13.00)	98	11.12 (9.75-12.61)	0.837
Glucometabolic status						
FPG, mmol/L	6.66 (5.68-8.50)	66	7.41 (6.02-9.04)	98	6.46 (5.45-8.09)	0.057
2hPG, mmol/L	16.63 ± 4.84	66	17.01 ± 5.02	98	16.38 ± 4.73	0.418
HbA1c (%)	8.29 ± 1.72	66	8.69 ± 1.70	98	8.03 ± 1.69	**0.016**
Fasting plasma C-peptide, nmol/L	0.44 (0.25-0.67)	66	0.40 (0.27-0.67)	98	0.46 (0.24-0.66)	0.960
HOMA2-Cpep #	1.23 (0.89-1.73)	58	1.17 (0.82-1.79)	80	1.28 (1.00-1.72)	0.449
Lipid profile						
TG, mmol/L	1.28 (0.94-2.06)	66	1.35 (1.00-2.30)	98	1.27 (0.86-1.78)	0.078
TC, mmol/L	4.32 (3.43-5.23)	66	4.34 (3.44-5.24)	98	4.31 (3.42-5.14)	0.667
HDL-c, mmol/L	1.08 (0.93-1.28)	66	1.06 (0.91-1.25)	98	1.09 (0.93-1.34)	0.282
LDL-c, mmol/L	2.73 (2.05-3.38)	66	2.73 (2.16-3.41)	98	2.73 (2.01-3.31)	0.473

*Data present as Mean ± SD or Median (IQR) for continuous variables and percentages (%) for categorical variables.
#HOMA2-Cpep was calculated at https://www.dtu.ox.ac.uk/homacalculator/.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; β-CTX, β-C-terminal cross-linked telopeptide of type I collagen; P1NP, procollagen-1 N-terminal peptide; 25(OH)D, 25-hydroxyvitamin D; Ca, calcium; Cr, creatinine; HbA1c, glycated hemoglobin A1c; uACR, urinary Albumin-to-Creatinine Ratio; UP, 24-hour urinary protein quantity; PTH, parathyroid hormone; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; TSH, thyroid stimulating hormone; FT4, free thyroxine; FBG, fasting plasma glucose; 2hPG, 2h post-load plasma glucose; HOMA2-Cpep, HOMA2 based on C-peptide.
The bold values mean their value less than 0.05, which is statistically significant (i.e., P<0.05).

TABLE 2 Correlation between serum 25(OH)D concentrations and cardiometabolic risk factors in adult T2DM patients.

Cardiometabolic risk factors	Serum 25(OH) D concentrations	
	r *	*P*-value
Blood pressure		
SBP, mmHg	-0.008	0.922
DBP, mmHg	-0.242	**0.002**
Glucometabolic status		
HbA1c, %	-0.200	**0.010**
FPG, mmol/L	-0.152	0.053
2hPG, mmol/L	-0.026	0.740
Fasting plasma C-peptide, nmol/L	-0.041	0.604
HOMA2-Cpep	-0.018	0.834
Lipid profile		
TG, mmol/L	-0.247	**0.001**
TC, mmol/L	-0.078	0.324
HDL-c, mmol/L	0.139	0.077
LDL-c, mmol/L	-0.101	0.197

*Data are Spearman Correlation Coefficient. Spearman rank correlation analysis was used due to the skewed distribution of serum 25(OH)D in this study.
25(OH)D, 25-hydroxyvitamin D; T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; HbA1c, glycated hemoglobin A1c; FBG, fasting plasma glucose; 2hPG, 2h post-load plasma glucose; HOMA2-Cpep, HOMA2 based on C-peptide.
The bold values mean their value less than 0.05, which is statistically significant (i.e., P<0.05).

and TG, but with lower levels of HDL-$_C$. These associations were robust after adjusting multiple variables including age, sex, BMI, ALT, Scr, TSH, and FT4. Further analysis revealed that the prevalence of VDD reached 40.24% of the studied population, and adults with T2DM in the VDD group increased the risk of poor DBP control compared to those in the VDS group in China. These findings suggest that adults with T2DM who have poor BP control, especially DBP, should monitor their vitamin D levels and consider taking vitamin D supplementation, if necessary, to reduce future CVD risk.

At present, CVD remains the main cause of death among adults with T2DM (22). For example, a contemporary multi-ethnic population-based observational study reported that the adjusted hazard ratio of CVD events for patients with T2DM was 1.28 (1.09-1.51) in individuals of South Asian ethnicity compared to those without T2DM (23). In addition, poor glycemic control is a major risk factor for the microvascular and macrovascular complications of patients with T2DM, such as retinopathy, diabetic nephropathy, and CVD. In this context, good glycemic control is a priority of T2DM management, but no more than 50% of patients are satisfied with glycemic control, especially in China. Therefore, the identification of risk factors other than the well-known ones such as poor glycemic control, increased BP or hypertension, and obesity is essential to improve glycemic control rates. Over the last decade, the pleiotropic metabolic roles of vitamin D have attracted widespread concern, especially in glucose metabolism and cardiovascular health, along with the discovery of the expression of vitamin D receptors in non-skeletal organs and tissues such as adipose, vascular smooth muscles, cardiomyocytes, and pancreatic β-cells (11). Most of the epidemiologic and meta-analysis studies have reported consistent inverse associations between serum 25(OH)D level and the risk of incident diabetes in the general population and that of poor glycemic control among adults with T2DM. For example, Khan et al. (24) found that vitamin D at baseline is inversely associated with future risks of T2DM in apparently healthy adults. Song et al. (25) combined data from 21 longitudinal cohorts with a total of 76,220 participants and showed that the estimated risk reduction for incident diabetes in the highest versus the lowest category of 25(OH)D was 38%.

In terms of vitamin D and glycemic control of patients with T2DM, several previous research studies have shown that low vitamin D levels have been associated with poor control of FPG and HbA1c. Consistent with these studies, our study found that 25(OH)D levels were significantly inversely associated with HbA1c, but not FBG, in both correlation and linear regression analyses, which provided more evidence that vitamin D may play an important role in the long-term glycemic control of patients with T2DM. The underlying mechanism by which VDD affects glycemic control is not fully understood. In recent years, some studies have suggested that VDD might indirectly decrease the level of intracellular calcium, thereby reducing the level of insulin secretion and beta-cellular dysfunction and, or directly reducing intracellular calcium level, as a result, impaired glucose tolerance (26–28). In addition, vitamin D may reduce systemic inflammation via the vitamin D receptor on pancreatic beta cells and in muscles and the liver to improve peripheral IR. However, our study found no significant association between vitamin D and HOMA2-Cpep, a marker of IR, suggesting that the inverse association of vitamin D with long-term poor glycemic control may not be acting through insulin function and accurate underlying mechanisms need to be further explored in the future.

In addition to glycemic control, poor control of BP and blood lipid are the two main factors contributing to the elevated risk of future CKD in patients with T2DM. For the association between vitamin D and BP, early in the 1980s, Sowers et al. (29) found a significant inverse association between the estimated dietary intake of vitamin D and SBP in younger women. Since then, an increasing number of studies have explored the association between 25(OH)D levels and BP and lipid profile in the general population, overweight/obese population, childhood, and pregnant (30–32). However, few relevant studies have focused on patients with T2DM, although this group has a higher risk of VDD (33). Of them, the case-control study included 80 overweight/obese subjects with T2DM and 77 healthy subjects matched by sex, age, and BMI from Tabriz, identifying that there was a significant negative association between serum 25(OH)D concentrations and DBP among adults with T2DM compared with healthy controls (9). However, another study by Ahmed et al. (10) suggested the association between vitamin D and BP was influenced by vitamin D type, and higher levels of vitamin D2, but not vitamin D3, are associated with hypertension in patients with diabetes. Consistent with the case-control study, this study observed a significant inverse association between 25(OH)D concentrations and DBP, which provides a new possible treatment strategy for T2DM patients with high DBP.

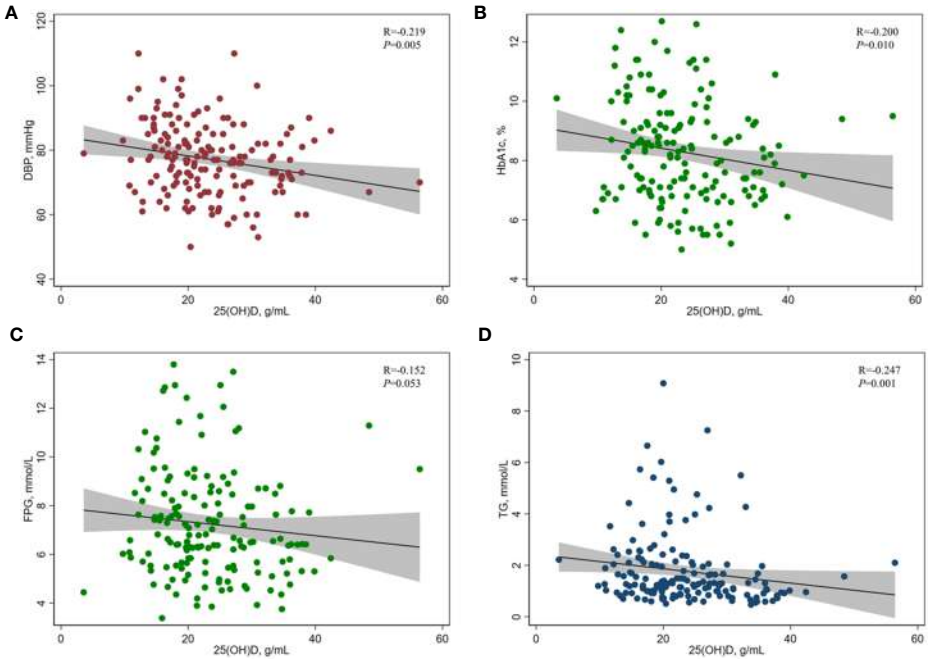

FIGURE 2
Correlation between serum 25(OH)D concentration and **(A)** DBP, **(B)** HbA1c, **(C)** FPG, and **(D)** TG in adults with T2DM. Spearman rank correlation analysis was used due to the skewed distribution of serum 25(OH)D in this study. 25(OH)D, 25-hydroxyvitamin D; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; TG, triglyceride; DBP, diastolic blood pressure.

TABLE 3 Regression analyses between serum 25(OH)D concentrations and cardiometabolic risk factors in adult T2DM patients.

Outcomes	Model 1 *		Model 2		Model 3	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value
No. participants	164		164		162	
Blood pressure						
SBP, mmHg	0.008 (-0.351, 0.366)	0.967	-0.097 (-0.460, 0.267)	0.600	-0.067 (-0.439, 0.306)	0.724
DBP, mmHg	-0.303 (-0.513, -0.093)	**0.005**	-0.244 (-0.453, -0.034)	**0.023**	-0.252 (-0.470, -0.034)	**0.024**
Glucometabolic status						
HbA1c, %	-0.037 (-0.070, -0.005)	**0.026**	-0.035 (-0.069, -0.002)	**0.039**	-0.040 (-0.075, -0.005)	**0.024**
FPG, mmol/L	-0.029 (-0.071, 0.013)	0.177	-0.027 (-0.070, 0.016)	0.222	-.0295 (-0.073, 0.014)	0.185
2hPG, mmol/L	0.008 (-0.085, 0.101)	0.869	-0.015 (-0.109, 0.080)	0.760	-.0235 (-0.120, 0.073)	0.632
Fasting plasma C-peptide, nmol/L	-3.257 (-10.398, 3.885)	0.369	-0.747 (-7.919, 6.425)	0.837	1.913 (-5.118, 8.944)	0.592
HOMA2-Cpep	-0.008 (-0.026, 0.011)	0.415	-0.003 (-0.022, 0.015)	0.726	0.001 (-0.017, 0.019)	0.898
Lipid profile						
TG,	-0.028 (-0.054, -0.001)	**0.040**	-0.023(-0.050, 0.004)	0.096	-0.015 (-0.0413, 0.012)	0.280
TC	-0.013 (-0.039, 0.012)	0.305	-0.009 (-0.035, 0.017)	0.502	-0.003 (-0.029, 0.023)	0.832
HDL-c	0.006 (0.001, 0.012)	**0.027**	0.005 (-0.000, 0.011)	0.067	0.007 (0.001, 0.012)	**0.016**
LDL-c	-0.011 (-0.031, 0.009)	0.264	-0.008 (-0.028, 0.013)	0.466	-0.004 (-0.024, 0.017)	0.726

*Model 1: unadjusted; Model 2: adjusted for age and sex; and Model 3: adjusted for age, sex, BMI, ALT, CR, TSH, and FT4.
CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; HbA1c, glycated hemoglobin A1c; FBG, fasting plasma glucose; 2hPG, 2h post-load plasma glucose; HOMA2-Cpep, HOMA2 based on C-peptide; BMI, body mass index; ALT, alanine aminotransferase; Cr, creatinine; TSH, thyroid stimulating hormone; FT4, free thyroxine.
The bold values mean their value less than 0.05, which is statistically significant (i.e., P<0.05).

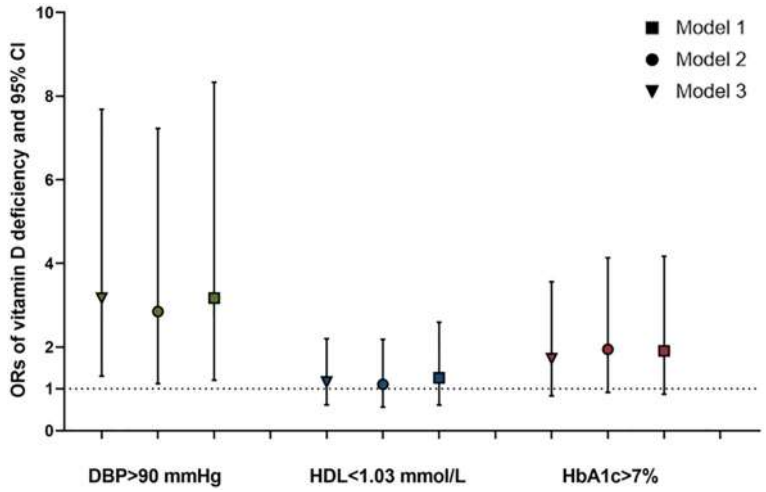

FIGURE 3
ORs and 95%CI of the vitamin D deficiency for poor control of DBP, HDL, and HbA1c in adults with T2DM. *Model 1: unadjusted; Model 2: adjusted for age and sex; and Model 3: adjusted for age, sex, BMI, ALT, CR, TSH, and FT4. ORs, odds ratios; CI, confidence interval; HbA1c, glycated hemoglobin A1c; DBP, diastolic blood pressure; HDL, high-density lipoprotein-cholesterol; BMI, body mass index; ALT, alanine aminotransferase; Cr, creatinine; TSH, thyroid stimulating hormone; FT4, and free thyroxine.

In addition, our study also found that lower 25(OH)D concentrations were associated with poorer lipid management including TG and HDL-$_C$, especially lower HDL-c levels, among adults with T2DM. This trend is consistent with some previous studies focusing on the non-diabetes population. The underlying mechanisms by which vitamin D influences the BP and lipid profile have not been fully elucidated. First, it has been recognized that vitamin D might lower BP by suppressing renin synthesis to downregulate the activity of the renin-angiotensin-aldosterone system (RASS) (34). Second, reduced active vitamin D can lead to increased iPTH production in the parathyroid gland and stimulate the expression of PTH2 receptors in vascular smooth muscle cells, thereby up-regulating the expression of the receptor for advanced glycation end products (RAGE) and the production of monocyte-macrophage cytokines and IL-6, as a result, promoting calcium deposition in the arterial wall, leading to collagen deposition, and increased vascular stiffness (35). These pathways can also affect lipid metabolism, for example, high iPTH levels may also accelerate calcium influx into adipocytes, thereby increasing lipase expression, and then increasing various lipid factors (36). Furthermore, vitamin D can inhibit the synthesis and excretion of TG by promoting the intestinal absorption of calcium. Furthermore, vitamin D may participate in the reverse cholesterol transport process by regulating the efflux of cholesterol from cholesterol-carrying macrophages, which removes excess cholesterol from the liver, thus causing an increase in HDL levels (37). But for now, why VDD is only significantly associated with DBP in BP and with HDL in lipid profile needs to be further verified and explored by studies with larger sample sizes.

4.1 Strengths and limitations

To the best of our knowledge, this is the first population-based study from China to simultaneously compare the association between vitamin D status and multiple cardiovascular risk factors in the same population with T2DM. Meanwhile, it should be noted that our study also has some limitations. First, this study is a cross-sectional study, the results of which cannot determine the causal association. Second, several vital potential indicators were not included in the final analysis, such as the duration of T2DM and whether use of VD supplements. Moreover, the sample size of this study was relatively small.

5 Conclusions

In conclusion, this population-based study showed serum 25 (OH)D concentration was significantly associated with multiple cardiometabolic risk factors including DBP, HbA1c, TG, and HDL-c among adults with T2DM, especially with DBP, which suggests that vitamin D may contribute to increased CVD risk through poor management of DBP in patients with T2DM. The role of vitamin D in the management of BP, especially DBP, and this role in the increased risk of CVD among adults with T2DM deserves further investigation.

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/s.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of Peking University Shenzhen Hospital. 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

Y-JL: Methodology, Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation. J-WD: Project administration, Writing – review & editing, Conceptualization, Methodology, Validation, Visualization, Writing – original draft. D-HL: Investigation, Writing – review & editing. FZ: Investigation, Writing – review & editing. H-LL: Investigation, Writing – review & editing, Data curation, Project administration.

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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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References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843.

2. Pearson-Stuttard J, Bennett J, Cheng YJ, Vamos EP, Cross AJ, Ezzati M, et al. Trends in predominant causes of death in individuals with and without diabetes in England from 2001 to 2018: an epidemiological analysis of linked primary care records. *Lancet Diabetes Endocrinol* (2021) 9:165–73. doi: 10.1016/S2213-8587(20)30431-9.

3. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. *Diabetologia* (2019) 62:3–16. doi: 10.1007/s00125-018-4711-2.

4. Wan Z, Geng T, Li R, Chen X, Lu Q, Lin X, et al. Vitamin D status, genetic factors, and risks of cardiovascular disease among individuals with type 2 diabetes: a prospective study. *Am J Clin Nutr* (2022) 116:1389–99. doi: 10.1093/ajcn/nqac183.

5. Jayedi A, Daneshvar M, Jibril AT, Sluyter JD, Waterhouse M, Romero BD, et al. Serum 25(OH)D concentration, vitamin D supplementation, and risk of cardiovascular disease and mortality in patients with type 2 diabetes or prediabetes: a systematic review and dose-response meta-analysis. *Am J Clin Nutr* (2023) 118:697–707. doi: 10.1016/j.ajcnut.2023.07.012.

6. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. *Diabetes Care* (2006) 29:722–4. doi: 10.2337/diacare.29.03.06.dc05-2148.

7. Pittas AG, Dawson-Hughes B, Sheehan P, Ware JH, Knowler WC, Aroda VR, et al. Vitamin D supplementation and prevention of type 2 diabetes. *N Engl J Med* (2019) 381:520–30. doi: 10.1056/NEJMc1912185.

8. Lu L, Bennett DA, Millwood IY, Parish S, McCarthy MI, Mahajan A, et al. Association of vitamin D with risk of type 2 diabetes: A Mendelian randomisation study in European and Chinese adults. *PloS Med* (2018) 15:e1002566. doi: 10.1371/journal.pmed.1002566.

9. Aghamohammadzadeh N, Dolatkhah N, Hashemian M, Shakouri SK, Hasanpour S. The relationship between serum 25-hydroxy vitamin D and blood pressure and quality of life in overweight and obese patients with type 2 diabetes mellitus compared with healthy subjects. *Caspian J Intern Med* (2020) 11:267–77.

10. Ahmed LHM, Butler AE, Dargham SR, Latif A, Robay A, Chidiac OM, et al. Association of vitamin D(2) and D(3) with type 2 diabetes complications. *BMC Endocr Disord* (2020) 20:65. doi: 10.1186/s12902-020-00549-w.

11. Md Isa Z, Amsah N, Ahmad N. The impact of vitamin D deficiency and insufficiency on the outcome of type 2 diabetes mellitus patients: A systematic review. *Nutrients* (2023) 15(10). doi: 10.3390/nu15102310.

12. Darraj H, Badedi M, Poore KR, Hummadi A, Khawaji A, Solan Y, et al. Vitamin D deficiency and glycemic control among patients with type 2 diabetes mellitus in Jazan City, Saudi Arabia. *Diabetes Metab Syndr Obes* (2019) 12:853–62. doi: 10.2147/DMSO.

13. Salih YA, Rasool MT, Ahmed IH, Mohammed AA. Impact of vitamin D level on glycemic control in diabetes mellitus type 2 in Duhok. *Ann Med Surg (Lond)* (2021) 64:102208. doi: 10.1016/j.amsu.2021.102208.

14. Karau PB, Kirna B, Amayo E, Joshi M, Ngare S, Muriira G. The prevalence of vitamin D deficiency among patients with type 2 diabetes seen at a referral hospital in Kenya. *Pan Afr Med J* (2019) 34:38. doi: 10.11604/pamj.2019.34.38.18936.

15. Saif-Elnasr M, Ibrahim IM, Alkady MM. Role of Vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus. *J Res Med Sci* (2017) 22:22. doi: 10.4103/1735-1995.200278.

16. Kajbaf F, Mentaverri R, Diouf M, Fournier A, Kamel S, Lalau JD. The association between 25-hydroxyvitamin D and hemoglobin A1c levels in patients with type 2 diabetes and stage 1-5 chronic kidney disease. *Int J Endocrinol* (2014) 2014:142468. doi: 10.1155/2014/142468.

17. Gandhe MB, Jain K, Gandhe SM. Evaluation of 25(OH) vitamin D3 with reference to magnesium status and insulin resistance in T2DM. *J Clin Diagn Res* (2013) 7(11):2438–41. doi: 10.7860/JCDR/2013/6578.3568.

18. Hurskainen AR, Virtanen JK, Tuomainen TP, Nurmi T, Voutilainen S. Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. *Diabetes Metab Res Rev* (2012) 28(5):418–23. doi: 10.1002/dmrr.2286.

19. [Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition)]. *Zhonghua Nei Ke Za Zhi* (2022) 61(1):12–50. doi: 10.3760/cma.j.cn112138-20211027-00751

20. Wang L, Lv S, Li F, Yu X, Bai E, Yang X. Vitamin D deficiency is associated with metabolic risk factors in women with polycystic ovary syndrome: A cross-sectional study in Shaanxi China. *Front Endocrinol (Lausanne)* (2020) 11:171. doi: 10.3389/fendo.2020.00171.

21. Ferrannini G, De Bacquer D, Erlund I, Gyberg V, Kotseva K, Mellbin L, et al. Measures of insulin resistance as a screening tool for dysglycemia in patients with coronary artery disease: A report from the EUROASPIRE V population. *Diabetes Care* (2022) 45(9):2111–7. doi: 10.2337/dc22-0272.

22. Wong ND, Sattar N. Cardiovascular risk in diabetes mellitus: epidemiology, assessment and prevention. *Nat Rev Cardiol* (2023) 20(10):685–95. doi: 10.1038/s41569-023-00877-z.

23. Coles B, Zaccardi F, Ling S, Davies MJ, Samani NJ, Khunti K. Cardiovascular events and mortality in people with and without type 2 diabetes: An observational study in a contemporary multi-ethnic population. *J Diabetes Investig* (2021) 12(7):1175–82. doi: 10.1111/jdi.13464.

24. Khan H, Kunutsor S, Franco OH, Chowdhury R. Vitamin D, type 2 diabetes and other metabolic outcomes: a systematic review and meta-analysis of prospective studies. *Proc Nutr Soc* (2013) 72(1):89–97. doi: 10.1017/S0029665112002765.

25. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* (2013) 36(5):1422–8. doi: 10.2337/dc12-0962.

26. Volek JS, Fernandez ML, Feinman RD, Phinney SD. Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Prog Lipid Res* (2008) 47(5):307–18. doi: 10.1016/j.plipres.2008.02.003.

27. Contreras-Bolívar V, García-Fontana B, García-Fontana C, Muñoz-Torres M. Mechanisms involved in the relationship between vitamin D and insulin resistance: impact on clinical practice. *Nutrients* (2021) 13(10). doi: 10.3390/nu13103491.

28. Gysemans CA, Cardozo AK, Callewaert H, Giulietti A, Hulshagen L, Bouillon R, et al. 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. *Endocrinology* (2005) 146(4):1956–64. doi: 10.1210/en.2004-1322.

29. Sowers MR, Wallace RB, Lemke JH. The association of intakes of vitamin D and calcium with blood pressure among women. *Am J Clin Nutr* (1985) 42(1):135–42. doi: 10.1093/ajcn/42.1.135.

30. Del Pinto R, Wright JT, Monaco A, Pietropaoli D, Ferri C. Vitamin D and blood pressure control among hypertensive adults: results from NHANES 2001-2014. *J Hypertens* (2020) 38(1):150–8. doi: 10.1097/HJH.0000000000002231.

31. Sauder KA, Stamatoiu AV, Leshchinskaya E, Ringham BM, Glueck DH, Dabelea D. Cord blood vitamin D levels and early childhood blood pressure: the healthy start study. *J Am Heart Assoc* (2019) 8(9):e011485. doi: 10.1161/JAHA.118.011485.

32. Kim D, Kim J. Age and sex differences in the relationship between serum 25-hydroxyvitamin D and hypertension in the general Korean population. *Eur J Clin Nutr* (2016) 70(3):326–32. doi: 10.1038/ejcn.2015.115.

33. Gendy HIE, Sadik NA, Helmy MY, Rashed LA. Vitamin D receptor gene polymorphisms and 25(OH) vitamin D: Lack of association to glycemic control and metabolic parameters in type 2 diabetic Egyptian patients. *J Clin Transl Endocrinol* (2019) 15:25–9. doi: 10.1016/j.jcte.2018.11.005.

34. Li YC. Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem* (2003) 88(2):327–31. doi: 10.1002/jcb.10343.

35. Zittermann A, Trummer C, Theiler-Schwetz V, Lerchbaum E, März W, Pilz S. Vitamin D and cardiovascular disease: an updated narrative review. *Int J Mol Sci* (2021) 22(6). doi: 10.3390/ijms22062896.

36. Wang JM, Ye SD, Li SM, Hu W. Correlations of 25(OH)D level with blood lipid, inflammatory factors and vascular endothelial function in diabetic patients. *Eur Rev Med Pharmacol Sci* (2018) 22(3):731–5. doi: 10.26355/eurrev_201802_14303

37. Kazlauskaite R, Powell LH, Mandapakala C, Cursio JF, Avery EF, Calvin J. Vitamin D is associated with atheroprotective high-density lipoprotein profile in postmenopausal women. *J Clin Lipidol* (2010) 4(2):113–9. doi: 10.1016/j.jacl.2010.01.006.

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Giuseppe Murdaca,
University of Genoa, Italy
Rizaldy Taslim Pinzon,
Duta Wacana Christian University, Indonesia

*CORRESPONDENCE
Yehai Li
ahyylyh@163.com

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Machine learning-based prediction of vitamin D deficiency: NHANES 2001-2018

Jiale Guo[1], Qionghan He[2] and Yehai Li[1]*

[1]Department of Orthopedics, Chaohu Hospital of Anhui Medical University, Hefei, China,
[2]Department of Infection, Chaohu Hospital of Anhui Medical University, Hefei, China

Background: Vitamin D deficiency is strongly associated with the development of several diseases. In the current context of a global pandemic of vitamin D deficiency, it is critical to identify people at high risk of vitamin D deficiency. There are no prediction tools for predicting the risk of vitamin D deficiency in the general community population, and this study aims to use machine learning to predict the risk of vitamin D deficiency using data that can be obtained through simple interviews in the community.

Methods: The National Health and Nutrition Examination Survey 2001-2018 dataset is used for the analysis which is randomly divided into training and validation sets in the ratio of 70:30. GBM, LR, NNet, RF, SVM, XGBoost methods are used to construct the models and their performance is evaluated. The best performed model was interpreted using the SHAP value and further development of the online web calculator.

Results: There were 62,919 participants enrolled in the study, and all participants included in the study were 2 years old and above, of which 20,204 (32.1%) participants had vitamin D deficiency. The models constructed by each method were evaluated using AUC as the primary evaluation statistic and ACC, PPV, NPV, SEN, SPE, F1 score, MCC, Kappa, and Brier score as secondary evaluation statistics. Finally, the XGBoost-based model has the best and near-perfect performance. The summary plot of SHAP values shows that the top three important features for this model are race, age, and BMI. An online web calculator based on this model can easily and quickly predict the risk of vitamin D deficiency.

Conclusion: In this study, the XGBoost-based prediction tool performs flawlessly and is highly accurate in predicting the risk of vitamin D deficiency in community populations.

KEYWORDS

machine learning, vitamin D deficiency, clinical decision rules, nutrition surveys, public health

1 Introduction

Vitamin D is a unique fat-soluble vitamin, and as it is produced primarily through exposure of human skin to sunlight, few foods contain natural vitamin D (1). Its main role in humans is to increase the absorption of calcium and phosphate to mineralize the bones (2). In children, vitamin D deficiency leads to growth retardation and rickets (3). In adults, vitamin D deficiency can lead to osteochondrosis and osteoporosis (3). Vitamin D deficiency and its health consequences first gained attention with the industrialization of Northern Europe. As research progressed, vitamin D deficiency was also found to be strongly associated with the development of diabetes (4), sarcopenia (5), psychiatric disorders (6), autoimmune diseases (7), cardiovascular diseases (8), and tumors (9). Because of the role of vitamin D in the antiviral immune response (10, 11), vitamin D-related studies have gained more attention since the COVID-19 pandemic. Vitamin D levels have also been shown to be associated with the prevention and prognosis of COVID-19 (12–14). Vitamin D deficiency has now been defined as a pandemic. As an important part of public health, identifying vitamin D deficiency is vital. However, a single measurement of vitamin D costs £9.86 and between 70.4% and 77.5% of tests are likely to be inappropriate (15). Testing for vitamin D in all populations does not appear to be appropriate. An Endocrine Society Clinical Practice Guideline recommends screening for vitamin D in people at risk for deficiency; they do not recommend screening for vitamin D in people who are not at risk (16). The use of prediction tools to identify patients at high risk of vitamin D deficiency is necessary. As of now, there are no prediction tools for predicting vitamin D risk in the general community population.

Machine learning is one of the fastest growing technology areas today and is widely used to enable evidence-based decision making in industries such as healthcare, manufacturing, and education (17). Machine learning is primarily based on large datasets to develop robust risk models and predict the type of person being studied (18, 19). Prediction tools developed using machine learning can be a good predictor of vitamin D deficiency risk in participants. The purpose of this study was to construct a prediction tool to predict participants' risk of vitamin D deficiency using a machine learning method based on data that can be easily collected in a general community population.

2 Materials and methods

2.1 Data sources and study population

Data for this study were obtained from the National Health and Nutritional Examination Surveys (NHANES), a population-based, cross-sectional survey study conducted in two-year cycles since 1999 to assess the health and nutritional status of adults and children in the United States. Serum 25(OH)D as a good biomarker for evaluating vitamin D status was used in this study as a laboratory test to determine vitamin D deficiency (20). The definition of vitamin deficiency used in this study was 25(OH)D < 50 nmol/L as recommended by an Endocrine Society Clinical Practice Guideline (16). Data from NHANES 2001-2018 containing 25(OH)D measurements were included in this study. In particular, serum 25(OH)D data from NHANES 2001-2006 were determined by the radioimmunoassay (RIA) method, which, due to excessive methodological bias and inaccuracy, was switched to liquid chromatography-tandem mass spectrometry (LC-MS/MS), a method that has better specificity and sensitivity, in the follow-up to NHANES 2007-2018 (21). Whereas serum 25(OH)D data from NHANES 2001-2006 have been converted to 25(OH)D measurements from equivalent LC-MS/MS methods by using regression.

For simplicity and ease of use of the model, only information that could be obtained in the community through a simple interview was included as variables for instrument development: gender, age, race, total number of people in the Household (H.Size), household income to poverty income ratio (H.PIR), body mass index (BMI), whether or not someone smokes in the household (H.Smoke), past 30-day milk product consumption (Milk), diabetes. Race is categorized as Mexican American, Non-Hispanic White, Non-Hispanic Black, Other Hispanic, or Other Race. For H.Size over 7 or more defined as 7. For H.PIR more than 5 is defined as 5. For the past 30-day milk product consumption, four frequencies were used to distinguish between never, rarely, sometimes, and often, with never meaning never drinking milk; rarely meaning less than once a week; sometimes meaning once a week or more but less than once a day; and often meaning once a day or more.

The data analyzed in this study were obtained from NHANES and did not require additional ethical review by the investigator's affiliated institution. NHANES has received approval from the National Center for Health Statistics (NCHS) Research Ethics Review Board.

2.2 Statistical analysis

Normally distributed continuous variables are expressed as mean ± standard deviation, non-normally distributed continuous variables as median (interquartile range), and categorical variables as percentages. Continuous variables were analyzed with the Independent Student's t-test or Mann-Whitney U analysis; categorical variables were analyzed with the chi-square test or Fisher's test. All statistical analyses were realized based on the "CBCgrps" package in R software.

2.3 Model construction, evaluation and validation

Data from the NHANES database for nine cycles from 2001-2018 were included for analysis. The included data were randomly divided into training and validation sets in the ratio of 70:30. We used the extracted variables as machine learning features for analysis. Six machine learning algorithms, Gradient Boosting

Machine (GBM), Logistic Regression (LR), Neural Network (NNet), Random Forest (RF), Support Vector Machine (SVM), and eXtreme Gradient Boosting (XGBoost), were used to construct the classification model. Ten 10-fold cross validation resampling was used to ensure stability and reproducibility of model performance. Receiver operating characteristic (ROC) curves were plotted to evaluate the discriminative performance of the model, and the area under the curve (AUC) of the ROC curve was calculated. The AUC value was used as the main statistical indicator to evaluate the predictive performance of the model. To evaluate the predictive performance of the model more comprehensively, this study also reports accuracy (ACC), positive predictive value (PPV), negative predictive value (NPV), sensitivity (SEN), specificity (SPE), F1 score, Matthews correlation coefficient (MCC). The closer these statistics are to 1 the better the predictive performance of the model. Kappa values are used to determine whether the model's results are consistent with actual results. The Kappa value is between -1 and +1, the closer the Kappa value is to 1, the better the consistency is, and if it is greater than 0.75, the consistency is excellent. The Brier Score combines the differentiation and calibration of the model and is used to evaluate the overall performance of the model, and the closer the Brier Score is to 0, the closer the predicted value is to the actual value (22). Decision curve analysis (DCA) is used to assess the clinical utility of models in decision making (23). The best machine learning predictive model was selected using AUC statistic value as the main statistic combined with various statistical indicators. Shapely Additive exPlanations (SHAP) values were used to interpret the best machine learning models (24). In addition, for the best machine learning models, an online web calculator is further constructed to facilitate the use of the models.

All statistical analyses, model construction and validation in this study were based on R software (version 4.1.3).

3 Results

There were 62,919 participants enrolled in the study, all the participants included in the study were 2 years old and above, of which 20,204 (32.1%) participants had vitamin D deficiency. The entire flow of the analysis is shown in the flowchart (Figure 1). The included data were randomly divided into training and validation sets in a ratio of 70:30, and the characteristics of the patients in the training set are shown in Table 1. The performance of the models constructed by each method was determined by resampling with ten ten-fold cross validation. AUC values were calculated based on the ROC curves (Figures 2A, B). The AUC values of GBM, LR, NNet, RF, SVM, and XGBoost in the training set are 0.796, 0.76, 0.778, 0.96, 0.8, and 0.995, respectively; and in the validation set are 0.786, 0.767, 0.79, 0.979, 0.837, and 1, respectively (Table 2). The model constructed by the XGBoost method has the best and near-perfect prediction performance in both the training and validation sets. To avoid the bias caused by data imbalance, this study further calculates ACC, PPV, NPV, SEN, SPE, F1 score, and MCC to evaluate the prediction performance of the model more comprehensively, as shown in Table 2. XGBoost obtained excellent results on all types of statistical metrics used to evaluate differentiation. The Kappa values of GBM, LR, NNet, RF, SVM, XGBoost in the training set are: 0.407, 0.353, 0.382, 0.745, 0.476, 0.928; and in the validation set are: 0.395, 0.36, 0.38, 0.821, 0.53, 0.997 (Table 2). The Brier score values of GBM, LR, NNet, RF, SVM, XGBoost in the training set are: 0.165, 0.178, 0.172, 0.084, 0.166, 0.042 respectively; and in the validation set are: 0.168, 0.175, 0.166, 0.068, 0.154, 0.013 respectively (Table 2). The XGBoost method also shows excellent consistency. The DCA curves show that the XGBoost-based model achieves higher net gains than the "all intervention" or "no intervention" strategies over the full range

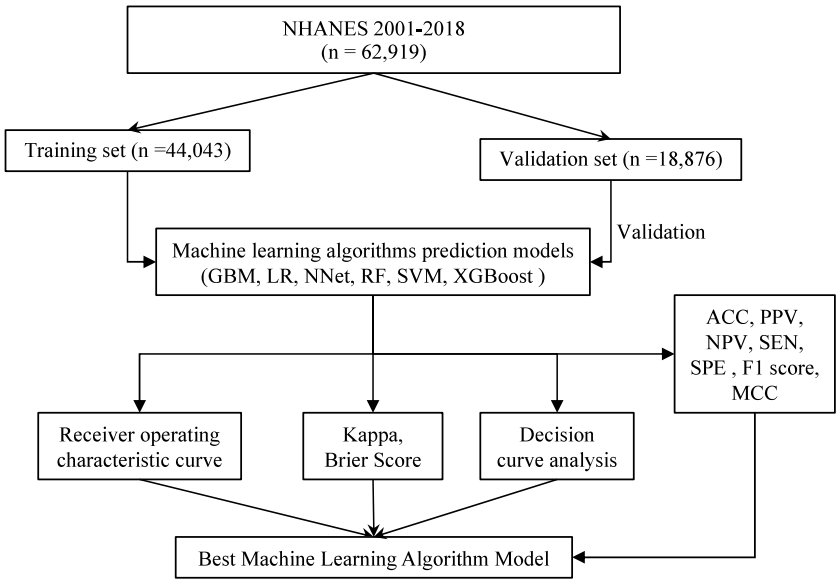

FIGURE 1
Flowchart of data screening and analysis. NHANES, National Health and Nutritional Examination Surveys; GBM, Gradient Boosting Machine; LR, Logistic Regression; NNet, Neural Network; RF, Random Forest; SVM, Support Vector Machine; XGBoost, eXtreme Gradient Boosting; ACC, accuracy; PPV, positive predictive value; NPV, negative, predictive value; SEN, sensitivity; SPE, specificity; MCC, Matthews correlation coefficient.

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TABLE 1 Characterization of participants in the training set.

Variables	Total (n = 44043)	NVDD (n = 29818)	VDD (n = 14225)	p
Gender, n (%)				< 0.001
Female	22419 (51)	14712 (49)	7707 (54)	
Male	21624 (49)	15106 (51)	6518 (46)	
Age, Median (Q1, Q3)	31 (14, 54)	31 (12, 56)	30 (17, 51)	< 0.001
Race, n (%)				< 0.001
Mexican American	8954 (20)	5636 (19)	3318 (23)	
Non-Hispanic Black	9958 (23)	4191 (14)	5767 (41)	
Non-Hispanic White	17339 (39)	14743 (49)	2596 (18)	
Other Hispanic	3538 (8)	2463 (8)	1075 (8)	
Other Race	4254 (10)	2785 (9)	1469 (10)	
H.Size, n (%)				< 0.001
1	3935 (9)	2635 (9)	1300 (9)	
2	9364 (21)	6673 (22)	2691 (19)	
3	7570 (17)	4964 (17)	2606 (18)	
4	8782 (20)	6030 (20)	2752 (19)	
5	6991 (16)	4750 (16)	2241 (16)	
6	3746 (9)	2462 (8)	1284 (9)	
7	3655 (8)	2304 (8)	1351 (9)	
H.PIR, Median (Q1, Q3)	1.87 (0.99, 3.69)	2.04 (1.05, 3.98)	1.6 (0.88, 3.1)	< 0.001
BMI, Median (Q1, Q3)	25.2 (20.45, 30.2)	24.4 (19.6, 29.2)	26.9 (22.16, 32.49)	< 0.001
H.Smoke, n (%)				< 0.001
No	34722 (79)	23934 (80)	10788 (76)	
Yes	9321 (21)	5884 (20)	3437 (24)	
Milk, n (%)				< 0.001
Never	5329 (12)	3029 (10)	2300 (16)	
Often	22374 (51)	16986 (57)	5388 (38)	
Rarely	5618 (13)	3172 (11)	2446 (17)	
Sometimes	10722 (24)	6631 (22)	4091 (29)	
Diabetes, n (%)				< 0.001
No	40652 (92)	27637 (93)	13015 (91)	
Yes	3391 (8)	2181 (7)	1210 (9)	

NVDD, non-vitamin D deficiency; VDD, vitamin D deficiency; H.Size, total number of people in the Household; H.PIR, household income to poverty income ratio; BMI, body mass index; H.Smoke, whether or not someone smokes in the household; Milk, past 30-day milk product consumption.

of thresholds, both in the training set (Figure 2C) and the validation set (Figure 2D). Combined with the various model performance evaluation statistics, the XGBoost-based model has the best and almost perfect performance.

We further plotted a summary of SHAP values (Figure 3) to interpret the XGBoost model results. For each feature, a point corresponds to a patient. The position of the point on the X-axis (i.e., the actual SHAP value) indicates the effect of the feature on the model output for that particular patient.The higher the feature on the Y-axis, the more important the feature is to the model. The results show that for this model, the features included are, in order of importance, Race, Age, BMI, H.PIR, Milk, H.Size, Gender, H.Smoke, and Diabetes. We also constructed an online web calculator based on the XGBoost method in order to facilitate the use of the model (Figure 4, https://jialeguo.shinyapps.io/vitamin_D_deficiency/).

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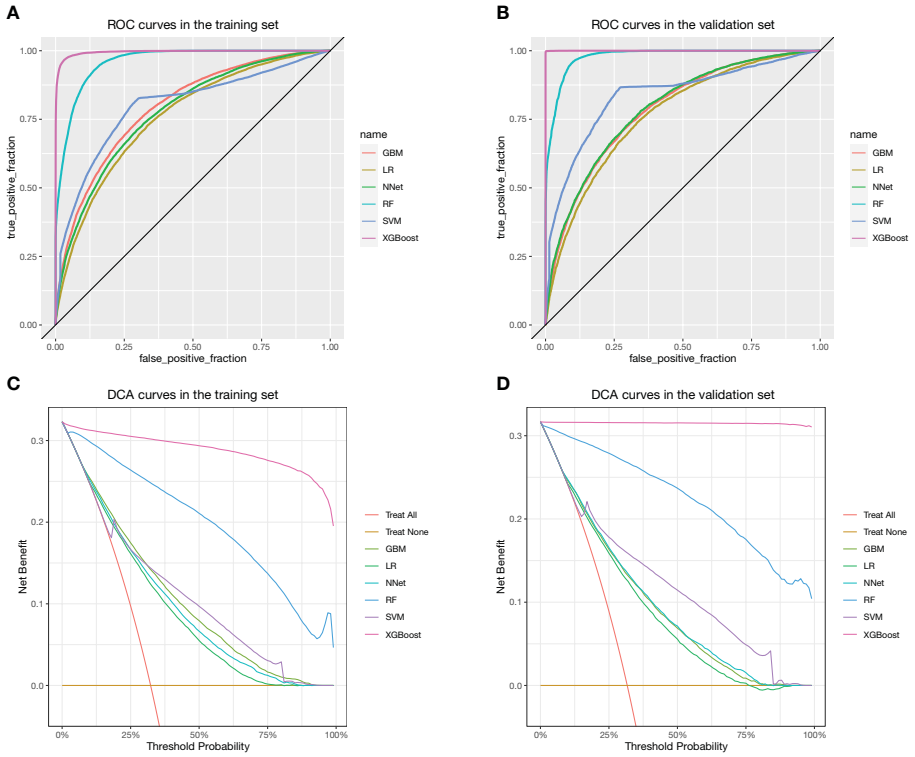

FIGURE 2
ROC and DCA curves for each method. **(A)** ROC in the training set. **(B)** ROC in the validation set. **(C)** DCA curves in the training set. **(D)** DCA curves in the validation set.

4 Discussion

This study uses data collected through interviews in a community-based population: gender, age, race, H.Size, H.PIR, BMI, H.Smoke, Milk, and diabetes. These nine variables were used as machine learning features to construct the model. Six machine learning methods (GBM, LR, NNet, RF, SVM, and XGBoost) were used to construct the model, and the model was evaluated for discrimination, fit, and clinical efficacy. Figures 2A, B show the main evaluation result of the discrimination: the ROC

TABLE 2 Evaluation metrics of the models constructed by each method.

	Method	AUC	ACC	PPV	NPV	SEN	SPE	F1 score	MCC	KAPPA	Brier score
Tra	GBM	0.796	0.717	0.546	0.849	0.736	0.708	0.627	0.419	0.407	0.165
	LR	0.76	0.685	0.509	0.837	0.728	0.664	0.599	0.368	0.353	0.178
	Nnet	0.778	0.706	0.534	0.836	0.71	0.705	0.61	0.392	0.382	0.172
	RF	0.96	0.882	0.76	0.962	0.928	0.86	0.835	0.754	0.745	0.084
	SVM	0.8	0.747	0.577	0.887	0.808	0.718	0.674	0.494	0.476	0.166
	XGBoost	0.995	0.968	0.937	0.984	0.967	0.969	0.952	0.929	0.928	0.042
Val	GBM	0.786	0.716	0.539	0.842	0.709	0.719	0.612	0.404	0.395	0.168
	LR	0.767	0.689	0.506	0.847	0.74	0.665	0.601	0.378	0.36	0.175
	Nnet	0.79	0.694	0.511	0.865	0.78	0.654	0.618	0.404	0.38	0.166
	RF	0.979	0.919	0.817	0.98	0.96	0.9	0.882	0.828	0.821	0.068
	SVM	0.837	0.772	0.597	0.921	0.866	0.728	0.706	0.554	0.53	0.154
	XGBoost	1	0.999	0.998	0.999	0.998	0.999	0.998	0.997	0.997	0.013

Tra, training set; Val, validation set; AUC, area under the curve; ACC, accuracy; PPV, positive predictive value; NPV, negative predictive value; SEN: sensitivity; SPE: specificity, MCC, Matthews correlation coefficient.

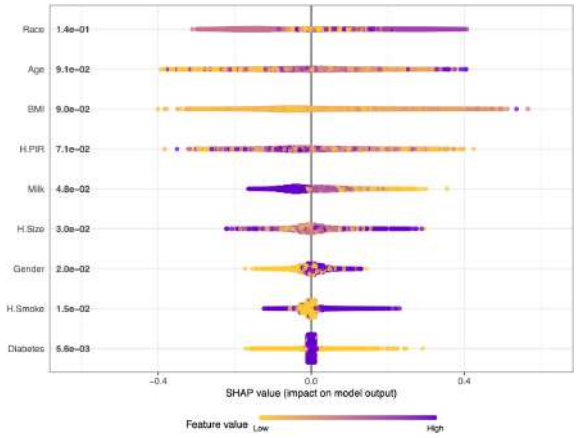

FIGURE 3
Summary plot of SHAP values for the model constructed by XGBoost algorithm. The horizontal position "SHAP value" indicates whether the impact of the value is associated with a higher or lower prediction, and the color of each SHAP value point indicates whether the observed value is higher (purple) or lower (yellow). The vertical coordinates show the importance of the features, sorted by the importance of the variables in descending order, with the upper variables being more important to the model.

curve. The higher the convexity and the more skewed towards the upper left corner of the corresponding curve for each machine learning model, the better its differentiation. The results of the ROC curves in this study show that XGBoost-based has the best discrimination performance both in the training and validation sets. This is also confirmed in other complementary evaluation metrics: ACC, PPV, NPV, SEN, SPE, F1 score, and MCC. The results of the evaluation of clinical efficacy are presented in Figures 2C, D: DCA curves. The line corresponding to "Treat All" in the DCA curves shows the net benefit of intervening on all participants, and the line corresponding to "Treat None" shows the net benefit of not intervening on all participants. Therefore, it makes sense to construct a model that has a threshold probability that the net benefit is higher than both "Treat All" and "Treat None". In this study, all the models have some clinical utility within a certain threshold. In particular, the model constructed by the XGBoost method has a higher net benefit than the "Treat All" or "Treat None" strategies within all thresholds. Ultimately, the model of the XGBoost method has the best and near perfect performance. This study further used SHAP values to interpret the model of XGBoost method, and among the variables included, race, age, and BMI were the top three important characteristics. In addition, an online web calculator was constructed based on the model of the XGBoost method for ease of use. Using this online web calculator, it is possible to screen community populations for vitamin D deficiency through a simple interview. The population in this study originated from the American community, where the prevalence of vitamin D deficiency was 32.11%. Vitamin D

FIGURE 4
Online web calculator based on XGBoost modeling. Race is categorized as Mexican American, Non-Hispanic White, Non-Hispanic Black, Other Hispanic, or Other Race. Household Size: total number of people in the Household. Household Size over 7 or more defined as 7. Household PIR: household income to poverty income ratio. Household PIR more than 5 is defined as 5. BMI, body mass index; Household smoking, whether or not someone smokes in the household; Milk consumption, past 30-day milk product consumption. For Milk consumption, four frequencies were used to distinguish between never, rarely, sometimes, and often, with never meaning never drinking milk; rarely meaning less than once a week; sometimes meaning once a week or more but less than once a day; and often meaning once a day or more.

deficiency, a global public health problem, has different prevalence rates in different regions. Defined as vitamin D deficiency with 25 (OH)D less than 50 nmol/L as recommended by an Endocrine Society Clinical Practice Guideline, the prevalence of vitamin D deficiency is 34.22% in Africa (25); 34.76% in South America (26); and 57.69% in Asia (27).

Both major forms of vitamin D forms (vitamin D2 and vitamin D3) are rarely found in food; vitamin D2 is found in plants and mushrooms; vitamin D3 is found in foods of animal origin, e.g., salmon, butter, and liver. Vitamin D in the body comes mainly from ultraviolet light exposure of the skin rather than through food intake. When human skin is exposed to ultraviolet light at wavelengths between 290 and 315 nm, it converts 7-dehydrocholesterol present in the epidermis to pre-vitamin D3 (28, 29). In turn, it is rapidly metabolized to vitamin D3 by thermal isomerization, which in turn is bound to vitamin D-binding proteins in the blood and transported to the liver. Converted to 1α,25(OH)2D3, the major biologically active metabolite form of vitamin D, sequentially by primary hydroxylation in the liver and kidney, respectively (28). This major source form of vitamin D in the body determines differences in vitamin D levels among different races and populations. The risk of vitamin D deficiency is related to race (30, 31), with darker-skinned races being less able to synthesize vitamin D from sunlight (32). In addition, latitude, season, atmospheric pollution, time spent outdoors, use of sunscreen, and habitual dress of some races, all factors that can affect the skin's exposure to ultraviolet light, contribute to differences in vitamin D levels (32). The effect of age on vitamin D deficiency presents a different role in adults and minors. The results of a multicenter cross-sectional study of adults aged 30-75 years in Saudi Arabia suggest that older age is a protective factor against vitamin D deficiency (33). This has been confirmed in studies from other regions (34–36). Instead, for minors, a higher risk of vitamin D deficiency was predicted with increasing age (37, 38). Obesity increases the risk of vitamin D deficiency in different regions and ages (39–41). The results of a meta-analysis showed a positive association between BMI and vitamin D deficiency (42). Several Mendelian randomization studies have also demonstrated this relationship at the causal level (43, 44). Low vitamin D levels in the obese population may be caused by the deposition of vitamin D in the adipose zone of the body, which reduces its bioavailability (45).

Vitamin D plays a crucial role in the maintenance of calcium and phosphate homeostasis, normal bone growth and mineralization (46). The effect of vitamin D on mineral homeostasis is mediated by 1,25(OH)2 D activation of the vitamin D receptor (VDR) to stimulate intestinal calcium and phosphate absorption, renal tubular calcium reabsorption, and skeletal calcium mobilization (47). Vitamin D deficiency leads to decreased calcium and phosphorus absorption and lower circulating blood calcium, which is secondary to hyperparathyroidism. Parathyroid hormone (PTH) increases renal tubular calcium reabsorption and inhibits phosphorus reabsorption in order to maintain blood calcium levels (48), and ultimately, insufficient calcium phosphate products lead to systemic bone mineralization, causing rickets in children and osteomalacia in adults (49). Vitamin D is essential for bone health, and supplementation is essential for patients at risk for fractures and/or vitamin D deficiency (50). Besides roles closely related to calcium and phosphate homeostasis and bone metabolism, vitamin D has many roles to play, especially in the immune response. It can act directly on immune cells to promote an anti-inflammatory state and maintain the balance between pro- and anti-inflammatory (51). However, although vitamin D can affect the immune system in a number of ways, it tends to be interconnected with the microbiome and influence each other and the immune system (52). Vitamin D plays an important role in the immune response and maintenance of intestinal homeostasis by influencing the number and pathways of innate lymphoid cells (ILCs), which affect the immune response in the gut (53, 54). Recent studies have shown that the composition of the gut microbiota is altered by vitamin D levels (55, 56). The gut microbiota also influences calcium and vitamin D absorption, regulates intestinal permeability, hormone secretion and immune response (57). The intestinal epithelial VDR regulates autophagy and innate immune function through genes such as ATG16L1, which may influence the microbiota profile in the gut (58). Vitamin D deficiency also plays a key role in airway microbiome composition, as weekly oral supplementation has an impact on cystic fibrosis patients (59). Therefore, it is extremely important to use vitamin D and probiotics to regulate the immune system (60).

Prediction tools are widely used in the medical field to predict clinical disease diagnosis and prognosis. Several prediction tools have been used to predict vitamin D deficiency. However, there are no prediction tools for predicting the risk of vitamin D deficiency in the general community, including young people. In addition, the sample size included in this study far exceeds that of similar previous studies. The machine learning prediction tools developed by Sluyter et al. (61) are similar to ours: both are tools developed using data that could have been collected in the community through simple inspection and interviews. However, Sluyter et al.'s study was only applicable to adults older than 50 and performed worse than the XGBoost method in this study: the best AUC value for Sluyter et al.'s prediction tool was only 0.73; whereas the AUC value for the XGBoost method in this study was 0.995. Carretero et al. (62) and Kheir et al. (63) on the other hand developed prediction tools applied to hypertensive population and ICU admitted population respectively. Their AUC values were 0.74 and 0.64, respectively. This study is the first predictive tool that can be widely applied to predict vitamin D deficiency in community populations. The best performing XGBoost method in this study had perfect predictive performance. The large number of subjects is one of the strengths of this study, which resulted in the high accuracy of the results. The results of this study show that an online web calculator using the XGBoost method can be a good predictor of vitamin D deficiency in the general population. Using this predictive tool, screening for vitamin D deficiency in the community or primary care settings can be achieved at almost no cost, avoiding much of the public health expenditure on unnecessary vitamin D testing and providing an intuitive and powerful scientific tool for health education and further testing. Based on the results of the online web calculator in this study, primary care providers can give appropriate clinical advice to their patients and make timely interventions for those at high risk of vitamin D deficiency, especially for children, pregnant women, and the elderly.

However, we need to recognize that there are still some limitations to this study. First, in order for the predictor tool to be widely applicable to various scenarios, the vast majority of the predictors used in this study were based on participants' self-reports, which may be subject to some bias. The NHANES database, on the other hand, has a strictly standardized process for data collection, and the large sample size of the studies included in this study can avoid these biases to a certain extent. Second, although internal validation was performed in this study by dividing the entire dataset into training and validation sets, we lacked external cohort studies to validate the performance of the prediction tool. All of the populations studied in this study were from the United States, and since vitamin D levels are related to factors such as race and latitude, the results of the study need to be viewed with caution when applied to populations in other regions. External validation of the study results using external datasets, especially from other continents, is necessary in the future.

5 Conclusion

The machine learning model constructed by the XGBoost method in this study possesses almost perfect performances. Based on this model, an online web calculator was further constructed, through which the risk of vitamin D deficiency in community populations can be predicted easily and quickly, and the public health expenditures caused by unnecessary vitamin D testing can be reduced.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), National Health and Nutrition Examination Survey (NHANES), https://wwwn.cdc.gov/nchs/nhanes/Default.aspx, NHANES 2001-2018.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics (NCHS) Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

JG: Formal analysis, Methodology, Software, Visualization, Writing – original draft. QH: Software, Visualization, Writing – review & editing. YL: Supervision, Writing – review & editing.

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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. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* (2008) 87(4):1080s–6s. doi: 10.1093/ajcn/87.4.1080S

2. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. *Review. Endocrine Rev* (2001) 22(4):477–501. doi: 10.1210/er.22.4.477

3. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* (2006) 81(3):353–73. doi: 10.4065/81.3.353

4. Sacerdote A, Dave P, Lokshin V, Bahtiyar G. Type 2 diabetes mellitus, insulin resistance, and vitamin D. . *Curr Diabetes Rep* (2019) 19(10):101. doi: 10.1007/s11892-019-1201-y

5. Remelli F, Vitali A, Zurlo A, Volpato S. Vitamin D deficiency and sarcopenia in older persons. *Nutrients* (2019) 11(12). doi: 10.3390/nu11122861

6. Cui X, McGrath JJ, Burne THJ, Eyles DW. Vitamin D and schizophrenia: 20 years on. *Mol Psychiatry* (2021) 26(7):2708–20. doi: 10.1038/s41380-021-01025-0

7. Illescas-Montes R, Melguizo-Rodríguez L, Ruiz C, Costela-Ruiz VJ. Vitamin D and autoimmune diseases. *Life Sci* (2019) 233:116744. doi: 10.1016/j.lfs.2019.116744

8. de la Guía-Galipienso F, Martínez-Ferran M, Vallecillo N, Lavie CJ, Sanchis-Gomar F, Pareja-Galeano H. Vitamin D and cardiovascular health. *Clin Nutr* (2021) 40 (5):2946–57. doi: 10.1016/j.clnu.2020.12.025

9. Carlberg C, Velleuer E. Vitamin D and the risk for cancer: A molecular analysis. *Biochem Pharmacol* (2022) 196:114735. doi: 10.1016/j.bcp.2021.114735

10. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. *Nutrients* (2020) 12(7). doi: 10.3390/nu12072097

11. Ismailova A, White JH. Vitamin D, infections and immunity. *Rev Endocr Metab Disord* (2022) 23(2):265–77. doi: 10.1007/s11154-021-09679-5

12. Villasis-Keever MA, López-Alarcón MG, Miranda-Novales G, Zurita-Cruz JN, Barrada-Vázquez AS, González-Ibarra J, et al. Efficacy and safety of vitamin D supplementation to prevent COVID-19 in frontline healthcare workers. A randomized clinical trial. *Arch Med Res* (2022) 53(4):423–30. doi: 10.1016/j.arcmed.2022.04.003

13. di Filippo L, Frara S, Nannipieri F, Cotellessa A, Locatelli M, Rovere Querini P, et al. Low vitamin D levels are associated with long COVID syndrome in COVID-19 survivors. *J Clin Endocrinol Metab* (2023) 108(10):e1106–16. doi: 10.1210/clinem/dgad207

14. De Niet S, Trémège M, Coffiner M, Rousseau AF, Calmes D, Frix AN, et al. Positive effects of vitamin D supplementation in patients hospitalized for COVID-19: A randomized, double-blind, placebo-controlled trial. *Nutrients* (2022) 14(15). doi: 10.3390/nu14153048

15. Woodford HJ, Barrett S, Pattman S. Vitamin D: too much testing and treating? *Clin Med (Lond).* (2018) 18(3):196–200. doi: 10.7861/clinmedicine.18-3-196

16. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* (2011) 96(7):1911–30. doi: 10.1210/jc.2011-0385

17. Zhang A, Xing L, Zou J, Wu JC. Shifting machine learning for healthcare from development to deployment and from models to data. *Nat BioMed Eng* (2022) 6 (12):1330–45. doi: 10.1038/s41551-022-00898-y

18. Caruana A, Bandara M, Musial K, Catchpoole D, Kennedy PJ. Machine learning for administrative health records: A systematic review of techniques and applications. *Artif Intell Med* (2023) 144:102642. doi: 10.1016/j.artmed.2023.102642

19. Li QY, Tang BH, Wu YE, Yao BF, Zhang W, Zheng Y, et al. Machine learning: A new approach for dose individualization. *Clin Pharmacol Ther* (2023). doi: 10.1002/cpt.3049

20. Bouillon R, Carmeliet G. Vitamin D insufficiency: Definition, diagnosis and management. *Best Pract Res Clin Endocrinol Metab* (2018) 32(5):669–84. doi: 10.1016/j.beem.2018.09.014

21. Altieri B, Cavalier E, Bhattoa HP, Pérez-López FR, López-Baena MT, Pérez-Roncero GR, et al. Vitamin D testing: advantages and limits of the current assays. *Eur J Clin Nutr* (2020) 74(2):231–47. doi: 10.1038/s41430-019-0553-3

22. Redelmeier DA, Bloch DA, Hickam DH. Assessing predictive accuracy: how to compare Brier scores. *J Clin Epidemiol.* (1991) 44(11):1141–6. doi: 10.1016/0895-4356(91)90146-z

23. Fitzgerald M, Saville BR, Lewis RJ. Decision curve analysis. *Jama* (2015) 313 (4):409–10. doi: 10.1001/jama.2015.37

24. Lundberg SM, Lee SI. A unified approach to interpreting model predictions. *Advances in Neural Information Processing Systems* (2017) 30.

25. Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, et al. Prevalence of vitamin D deficiency in Africa: a systematic review and meta-analysis. *Lancet Glob Health* (2020) 8(1):e134–42. doi: 10.1016/s2214-109x(19)30457-7

26. Mendes MM, Gomes APO, Araújo MM, Coelho ASG, Carvalho KMB, Botelho PB. Prevalence of vitamin D deficiency in South America: a systematic review and meta-analysis. *Nutr Rev* (2023) 81(10):1290–309. doi: 10.1093/nutrit/nuad010

27. Jiang Z, Pu R, Li N, Chen C, Li J, Dai W, et al. High prevalence of vitamin D deficiency in Asia: A systematic review and meta-analysis. *Crit Rev Food Sci Nutr* (2023) 63(19):3602–11. doi: 10.1080/10408398.2021.1990850

28. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* (2004) 80(6 Suppl):1689s–96s. doi: 10.1093/ajcn/80.6.1689S

29. Webb AR, Kift R, Durkin MT, O'Brien SJ, Vail A, Berry JL, et al. The role of sunlight exposure in determining the vitamin D status of the U.K. white adult population. *Br J Dermatol Nov* (2010) 163(5):1050–5. doi: 10.1111/j.1365-2133.2010.09975.x

30. Sutherland JP, Zhou A, Leach MJ, Hyppönen E. Differences and determinants of vitamin D deficiency among UK biobank participants: A cross-ethnic and socioeconomic study. *Clin Nutr* (2021) 40(5):3436–47. doi: 10.1016/j.clnu.2020.11.019

31. Hayden KE, Sandle LN, Berry JL. Ethnicity and social deprivation contribute to vitamin D deficiency in an urban UK population. *J Steroid Biochem Mol Biol* (2015) 148:253–5. doi: 10.1016/j.jsbmb.2014.11.014

32. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* (1982) 1(8263):74–6. doi: 10.1016/s0140-6736(82)90214-8

33. AlQuaiz AM, Kazi A, Fouda M, Alyousefi N. Age and gender differences in the prevalence and correlates of vitamin D deficiency. *Arch Osteoporos.* (2018) 13(1):49. doi: 10.1007/s11657-018-0461-5

34. Mo H, Zhang J, Huo C, Zhang M, Xiao J, Peng J, et al. The association of vitamin D deficiency, age and depression in US adults: a cross-sectional analysis. *BMC Psychiatry* (2023) 23(1):534. doi: 10.1186/s12888-023-04685-0

35. Nguyen HT, von Schoultz B, Nguyen TV, Dzung DN, Duc PT, Thuy VT, et al. Vitamin D deficiency in northern Vietnam: prevalence, risk factors and associations with bone mineral density. *Bone* (2012) 51(6):1029–34. doi: 10.1016/j.bone.2012.07.023

36. Golbahar J, Al-Saffar N, Altayab Diab D, Al-Othman S, Darwish A, Al-Kafaji G. Predictors of vitamin D deficiency and insufficiency in adult Bahrainis: a cross-sectional study. *Public Health Nutr* (2014) 17(4):732–8. doi: 10.1017/s136898001300030x

37. Middelkoop K, Walker N, Stewart J, Delport C, Jolliffe DA, Nuttall J, et al. Prevalence and determinants of vitamin D deficiency in 1825 cape town primary schoolchildren: A cross-sectional study. *Nutrients* (2022) 14(6). doi: 10.3390/nu14061263

38. Karagol C, Duyan Camurdan A. Evaluation of vitamin D levels and affecting factors of vitamin D deficiency in healthy children 0-18 years old. *Eur J Pediatr* (2023) 182(9):4123–31. doi: 10.1007/s00431-023-05096-9

39. Alloubani A, Akhu-Zaheya L, Samara R, Abdulhafiz I, Saleh A, Altowijri A. Relationship between vitamin D deficiency, diabetes, and obesity. *Diabetes Metab Syndr* (2019) 13(2):1457–61. doi: 10.1016/j.dsx.2019.02.021

40. Wakayo T, Whiting SJ, Belachew T. Vitamin D Deficiency is Associated with Overweight and/or Obesity among Schoolchildren in Central Ethiopia: A Cross-Sectional Study. *Nutrients* (2016) 8(4):190. doi: 10.3390/nu8040190

41. Shafinaz IS, Moy FM. Vitamin D level and its association with adiposity among multi-ethnic adults in Kuala Lumpur, Malaysia: a cross sectional study. *BMC Public Health* (2016) 16:232. doi: 10.1186/s12889-016-2924-1

42. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obes Rev Apr* (2015) 16 (4):341–9. doi: 10.1111/obr.12239

43. Huang YY, Zhang WS, Jiang CQ, Zhu F, Jin YL, Cheng KK, et al. Mendelian randomization on the association of obesity with vitamin D: Guangzhou Biobank Cohort Study. *Eur J Clin Nutr Feb* (2023) 77(2):195–201. doi: 10.1038/s41430-022-01234-y

44. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PloS Med* (2013) 10(2):e1001383. doi: 10.1371/journal.pmed.1001383

45. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* (2000) 72(3):690–3. doi: 10.1093/ajcn/72.3.690

46. Bergwitz C, Jüppner H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu Rev Med* (2010) 61:91–104. doi: 10.1146/annurev.med.051308.111339

47. Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D sources, metabolism, and deficiency: available compounds and guidelines for its treatment. *Metabolites* (2021) 11(4). doi: 10.3390/metabo11040255

48. Potts JT Jr. A short history of parathyroid hormone, its biological role, and pathophysiology of hormone excess. *J Clin Densitom.* (2013) 16(1):4–7. doi: 10.1016/j.jocd.2012.11.002

49. Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* (2006) 116(8):2062–72. doi: 10.1172/jci29449

50. Capozzi A, Scambia G, Lello S. Calcium, vitamin D, vitamin K2, and magnesium supplementation and skeletal health. *Maturitas* (2020) 140:55–63. doi: 10.1016/j.maturitas.2020.05.020

51. Yamamoto E, Jørgensen TN. Immunological effects of vitamin D and their relations to autoimmunity. *J Autoimmun* (2019) 100:7–16. doi: 10.1016/j.jaut.2019.03.002

52. Murdaca G, Gerosa A, Paladin F, Petrocchi L, Banchero S, Gangemi S. Vitamin D and microbiota: is there a link with allergies? *Int J Mol Sci* (2021) 22(8). doi: 10.3390/ijms22084288

53. Konya V, Czarnewski P, Forkel M, Rao A, Kokkinou E, Villablanca EJ, et al. Vitamin D downregulates the IL-23 receptor pathway in human mucosal group 3 innate lymphoid cells. *J Allergy Clin Immunol* (2018) 141(1):279–92. doi: 10.1016/j.jaci.2017.01.045

54. Ignacio A, Breda CNS, Camara NOS. Innate lymphoid cells in tissue homeostasis and diseases. *World J Hepatol* (2017) 9(23):979–89. doi: 10.4254/wjh.v9.i23.979

55. Luthold RV, Fernandes GR, Franco-de-Moraes AC, Folchetti LG, Ferreira SR. Gut microbiota interactions with the immunomodulatory role of vitamin D in normal individuals. *Metabolism* (2017) 69:76–86. doi: 10.1016/j.metabol.2017.01.007

56. Ooi JH, Li Y, Rogers CJ, Cantorna MT. Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis. *J Nutr* (2013) 143 (10):1679–86. doi: 10.3945/jn.113.180794

57. Locantore P, Del Gatto V, Gelli S, Paragliola RM, Pontecorvi A. The interplay between immune system and microbiota in osteoporosis. *Mediators Inflamm* (2020) 2020:3686749. doi: 10.1155/2020/3686749

58. Akimbekov NS, Digel I, Sherelkhan DK, Lutfor AB, Razzaque MS. Vitamin D and the host-gut microbiome: A brief overview. *Acta Histochem Cytochem* (2020) 53 (3):33–42. doi: 10.1267/ahc.20011

59. Sun J, Dietary vitamin D. vitamin D receptor, and microbiome. *Curr Opin Clin Nutr Metab Care* (2018) 21(6):471–4. doi: 10.1097/mco.0000000000000516

60. Murdaca G, Greco M, Borro M, Gangemi S. Hygiene hypothesis and autoimmune diseases: A narrative review of clinical evidences and mechanisms. *Autoimmun Rev* (2021) 20(7):102845. doi: 10.1016/j.autrev.2021.102845

61. Sluyter JD, Raita Y, Hasegawa K, Reid IR, Scragg R, Camargo CA. Prediction of vitamin D deficiency in older adults: the role of machine learning models. *J Clin Endocrinol Metab* (2022) 107(10):2737–47. doi: 10.1210/clinem/dgac432

62. Garcia Carretero R, Vigil-Medina L, Barquero-Perez O, Mora-Jimenez I, Soguero-Ruiz C, Ramos-Lopez J. Machine learning approaches to constructing predictive models of vitamin D deficiency in a hypertensive population: a comparative study. *Inform Health Soc Care* (2021) 46(4):355–69. doi: 10.1080/17538157.2021.1896524

63. Bou Kheir G, Khaldi A, Karam A, Duquenne L, Preiser JC. A dynamic online nomogram predicting severe vitamin D deficiency at ICU admission. *Clin Nutr* (2021) 40(10):5383–90. doi: 10.1016/j.clnu.2021.08.024

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Luigi Di Filippo,
San Raffaele Hospital (IRCCS), Italy
Luciano Colangelo,
Sapienza University of Rome, Italy
Manoj Chadha,
P.D. Hinduja Hospital, India

*CORRESPONDENCE
Juan J. Díez
juanjose.diez@salud.madrid.org

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Incident comorbidities in patients with chronic hypoparathyroidism after thyroidectomy: a multicenter nationwide study

Juan J. Díez [1,2*], Emma Anda [3], Begoña Pérez-Corral [4], Miguel Paja [5], Victoria Alcázar [6], Cecilia Sánchez-Ragnarsson [7], Aida Orois [8], Ana R. Romero-Lluch [9], Marcel Sambo [10], Amelia Oleaga [5], Águeda Caballero [11], María R. Alhambra [12], Virginia Urquijo [13], Ana M. Delgado-Lucio [14], José C. Fernández-García [15], Viyey Kishore-Doulatram [15], Suset Dueñas-Disotuar [9], Tomás Martín [16], Mercedes Peinado [16] and Julia Sastre [17]

[1]Department of Endocrinology, Hospital Universitario Puerta de Hierro Majadahonda, Instituto de Investigación Sanitaria Puerta de Hierro Segovia de Arana, Majadahonda, Spain, [2]Department of Medicine, Universidad Autónoma de Madrid, Majadahonda, Spain, [3]Department of Endocrinology, Hospital Universitario de Navarra, Pamplona, Spain, [4]Department of Endocrinology, Complejo Asistencial Universitario de León, León, Spain, [5]Department of Endocrinology, Hospital Universitario de Basurto, Universidad del País Vasco (UPV/EHU), Bilbao, Spain, [6]Department of Endocrinology, Hospital Severo Ochoa, Leganés, Spain, [7]Department of Endocrinology, Hospital Universitario Central de Asturias, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain, [8]Department of Endocrinology and Nutrition, Hospital Clínic, Barcelona, Spain, [9]Department of Endocrinology, Hospital Universitario Virgen del Rocío, Sevilla, Spain, [10]Department of Endocrinology, Hospital General Universitario Gregorio Marañón, Madrid, Spain, [11]Department of Endocrinology, Hospital Universitario de Canarias, Tenerife, Spain, [12]Department of Endocrinology, Hospital Universitario Reina Sofía, Córdoba, Spain, [13]Department of Endocrinology, Hospital Universitario de Cruces, Bilbao, Spain, [14]Department of Endocrinology, Hospital Universitario de Burgos, Burgos, Spain, [15]Department of Endocrinology, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga, Universidad de Málaga, Málaga, Spain, [16]Department of Endocrinology, Hospital Universitario Virgen Macarena, Sevilla, Spain, [17]Department of Endocrinology, Hospital Universitario de Toledo, Toledo, Spain

Purpose: Population-based and registry studies have shown that chronic hypoparathyroidism is accompanied by long-term complications. We aimed to evaluate the risk of incident comorbidity among patients with chronic postsurgical hypoparathyroidism in real-life clinical practice in Spain.

Methods: We performed a multicenter, retrospective cohort study including patients with chronic postsurgical hypoparathyroidism lasting ≥3 years with at least a follow-up visit between January 1, 2022 and September 15, 2023 (group H). The prevalence and incidence of chronic complications including chronic kidney disease, nephrolithiasis/nephrocalcinosis, hypertension, dyslipidemia, diabetes, cardiovascular disease, central nervous system disease, mental health disorders, eye disorders, bone mineral density alterations, fracture and cancer were evaluated. Patient data were compared with a group of patients who did not develop hypoparathyroidism, matched by gender, age, and follow-up time after thyroidectomy (group NH).

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Results: We included 337 patients in group H (median [IQR] age, 45 [36-56] years; median time of follow-up, 8.9 [6.0-13.0] years; women, 84.3%) and 669 in group NH (median age, 47 [37-55] years; median time of follow-up, 8.0 [5.3-12.0] years; women, 84.9%). No significant differences were found in the prevalence of comorbidities at the time of thyroidectomy between both groups. In multivariable adjusted analysis, patients with chronic hypoparathyroidism had significantly higher risk of incident chronic kidney disease (OR, 3.45; 95% CI, 1.72-6.91; P<0.001), nephrolithiasis (OR, 3.34; 95% CI, 1.55-7.22; P=0.002), and cardiovascular disease (OR, 2.03; 95% CI, 1.14-3.60; P=0.016), compared with patients without hypoparathyroidism. On the contrary, the risk of fracture was decreased in patients with hypoparathyroidism (OR, 0.09; 95% CI, 0.01-0.70; P=0.021).

Conclusion: This study demonstrates that, in the clinical practice of Spanish endocrinologists, a significant increase in the risk of chronic kidney disease, nephrolithiasis and cardiovascular disease, as well as a reduction in the risk of fractures is detected. These results are of interest for the development of new clinical guidelines and monitoring protocols for patients with hypoparathyroidism.

KEYWORDS

postsurgical hypoparathyroidism, comorbidity, incidence, prevalence, thyroidectomy

Introduction

Hypoparathyrodism is a rare endocrine disorder characterized by absence or inappropriately low levels of parathyroid hormone leading to hypocalcemia and hyperphosphatemia (1). In approximately 75% of cases it occurs as a complication of anterior neck surgery and is therefore seen more frequently in older adult women (2). Postsurgical hypoparathyroidism is a result of inadvertent removal or injury of the parathyroid glands during thyroid or parathyroid surgery. Hypoparathyroidism may be transient, lasting several days or weeks, protracted, lasting some months, or permanent (1, 3, 4). Hypoparathyroidism is considered definitive or permanent when the need for treatment with calcium and active vitamin D metabolites lasts more than 6 months (3, 4) or 12 months after surgery (5–7). The prevalence of permanent hypoparathyroidism varies depending on patient characteristics, diagnostic criteria, surgical experience and geographical area (8). Based on data from reviews and meta-analyses, the prevalence of postoperative persistent hypoparathyroidism had been estimated to be 0-3% (8). However, more recent studies conducted using national registries (9, 10) or multicenter cohort analyses (6, 11, 12) have shown prevalence values between 11-28%. In the particular case of Spain, a multicenter, nationwide study, carried out in the setting of clinical practice, in reference centers, showed that the prevalence of definitive hypoparathyroidism among patients with total thyroidectomy was 14.5% (6). All these data emphasize the potential importance of this hormonal deficiency in patients undergoing thyroid surgery.

Main goals of management of patient with hypoparathyroidism include normalization of calcium and phosphate metabolism parameters and preventing signs and symptoms of hypocalcemia and hypercalcemia (1). An additional objective in long-term follow-up should be the prevention of complications and comorbidities (3, 4). It has been reported that patients with hypoparathyroidism have a high risk of developing kidney failure, kidney stones, neuropsychiatric disease, epilepsy and cataracts in comparison with normal population (13–15). More recent studies have found that hypoparathyroidism is also associated with cardiovascular disease, infections and even an increased risk of mortality (16). The association of hypoparathyroidism with fractures is controversial, since variable results have been found in different studies (16, 17). Furthermore, a study showed that the risk of gastrointestinal cancer was significantly reduced in patients with postsurgical hypoparathyroidism (14).

Most authors who have associated chronic hypoparathyroidism with various comorbidities have carried out population-based studies or have used data from large national registries. To our knowledge, no clinical series of patients with hypoparathyroidism or studies that use data from routine clinical practice have been published to elucidate the risks of comorbidity in these patients in real life. Hence, in this study we aimed to compare the appearance of different comorbidities diagnosed after total thyroidectomy in a

group of patients with long-term definitive hypoparathyroidism with those found in a similar group of thyroidectomized patients without hypoparathyroidism.

Methods

Subjects

This is a multicenter, retrospective cohort study, with data from routine clinical practice, performed in patients treated by total thyroidectomy for any cause with a follow-up time of at least three years after surgery. We included all patients with permanent hypoparathyroidism lasting at least 3 years (group H) who attended the endocrinology clinics of the participating hospitals during the study period. For comparison purposes, we also analyzed a control group of patients who did not develop permanent hypoparathyroidism after surgery (group NH). For each patient, 1-3 controls matched by sex, age, and follow-up time after thyroidectomy were selected.

Inclusion criteria for patients with and without hypoparathyroidism were the following: age ≥18 years at the time of total thyroidectomy (one or two stages), availability of histological report, follow-up in the same hospital for a period ≥3 years, and date of last visit between January 1, 2022 and September 15, 2023.

Study design

This project was disseminated through the Thyroid Task Force of the Spanish Society of Endocrinology and Nutrition (Sociedad Española de Endocrinología y Nutrición, SEEN) composed of endocrinologists with special expertise and dedication to thyroid disease. Twenty investigators from 16 hospital centers agreed to participate in the study. A review of the medical records of all patients who met the inclusion criteria was performed. Each investigator selected patients with hypoparathyroidism who met the inclusion criteria and with at least one follow-up visit during the recruitment period.

We collected information on clinical and demographic data, initial surgery, pathological details, prevalent chronic diseases before thyroidectomy, follow-up time after surgery, and incident diseases detected in clinical practice until the last visit. For the study of prevalence and incidence, the following conditions were considered: chronic kidney disease (stage 3 or higher, i.e., estimated glomerular filtration rate <60ml/min/1.73m^2), nephrolithiasis/nephrocalcinosis, hypertension, dyslipidemia, diabetes, cardiovascular disease, central nervous system disease, mental health disorders, eye disorders, bone mineral density (BMD) alterations, fracture and cancer. The usual criteria were used for the diagnosis of these procedures in clinical practice and the presence of the diagnoses was verified in the patients' medical record. The glomerular filtration rate was estimated by the usual method in each of the participating hospitals (CDK-EPI equation (18) in 58% of the subjects and MDRD 4-variable equation (19) in 42%). For each patient we recorded all prevalent diseases at the time of thyroidectomy and all incident diseases, with the date of diagnosis, from thyroidectomy to the end of follow-up. We also registered the chronic pharmacological treatments used by patients both at the time of thyroidectomy and at the last follow-up visit.

All patient's data were obtained under the standard medical care conditions. The patient's confidential information was protected according to national law, and the study received favorable report from the ethics committee of the Hospital Universitario Puerta de Hierro Majadahonda (PI 253/22).

Statistical analysis

For quantitative variables, results are expressed as median (interquartile range, IQR). Categorical variables are described as absolute values, ratios, or percentages. For proportion comparisons, the chi-square test or Fisher's exact test was used. The McNemar test was used to compare the proportions of drug use at the time of thyroidectomy and at the last follow-up visit (paired data). For the analysis of incident comorbidities we estimated the values (with the 95% confidence interval) for the cumulative incidence and incidence rate in patients in group H. For the NH group we estimated the proportion of patients who developed incident diseases. For this analysis, the risk of each comorbidity was assessed among patients free of that condition during the baseline period.

Cumulative incidence (%) was calculated as the number of new cases of disease during follow-up divided by the total number of individuals in the population at risk at the beginning of follow-up. Incidence rate (cases per 100 patient-years) was estimated as the number of new cases of disease divided by the sum of the individual observation times of the at-risk population. To analyze the risk of developing comorbidities in patients with hypoparathyroidism we estimated the odds ratio (OR) as the ratio between the odds in group H and those obtained in the group NH, along with the corresponding 95% confidence interval.

To assess the association of hypoparathyroidism with the appearance of incident comorbidities, we selected the incident diseases in which a significant increase or decrease in odds ratio was detected in patients with hypoparathyroidism compared to group NH. In these cases, we performed a survival analysis using the Kaplan-Meier method, with the log-rank test used to compare groups. Multivariable logistic regression analysis was conducted to evaluate the relative importance of hypoparathyroidism as well as demographic and clinical characteristics of patients for the development of the different comorbidities. For the multivariable analysis, two models were used. Model 1 was adjusted for hypoparathyroidism, gender, age, thyroidectomy, histopathology, hypertension, dyslipidemia, diabetes, cardiovascular disease, and BMD alterations; model 2 was adjusted for the same covariates, and nephrolithiasis, central nervous system disease, mental health disorders, eye disorders, fracture, and cancer. All used tests were two-sided and differences were considered significant when $P < 0.05$. SPSS software version 21 was used to perform the statistical analysis.

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Results

Studied patients

Of the 366 patients with hypoparathyroidism initially selected for the study, 59 were excluded due to lack of clinical data during follow-up. Finally, in group H, 337 patients were included (284 women, 84.3%), aged between 18-80 years (median, 45[36-56] years). One hundred and twelve patients (33.2%) underwent thyroidectomy due to benign thyroid disease (follicular nodular disease in 65.2% of these cases, Graves' disease in 16.1%, chronic thyroiditis in 12.5% and others in 6.3%) and 225 (66.8%) due to thyroid cancer (papillary in 86.2% of these cases, follicular in 8.0%, oncocytic in 1.3% and others in 4.4%). The median follow-up time after thyroidectomy was 8.9 (6.0-13.0) years. The most common prevalent diseases at the time of thyroidectomy were dyslipidemia (19.3%), hypertension (16.3%), mental health disorders (13.1%), diabetes (4.5%), cancer (5.3%), eye disorders (3.3%), and cardiovascular disease (3.0%). The rest of the comorbidities were detected in less than 3% of the patients (Table 1).

The majority of patients in group H followed replacement treatment with oral calcium and calcitriol (n=191, 86.4%). There were 41 patients (12.2%) who only required calcitriol and 5 (1.5%) that were treated with only oral calcium. In addition, 138 patients (40.9%) were receiving treatment with vitamin D supplements (Supplementary Material, Supplementary Table 1). Control of hypoparathyroidism was generally adequate (Supplementary Material, Supplementary Table 2). 88.1% of the patients had serum calcium values equal to or greater than 8.0 mg/dl, 65.5% of them had serum phosphorus values equal to or less than 4.5 mg/dl and 98.5% had a serum calcium-phosphorus product less than 55 mg^2/dl^2. 24-hour urinary calcium excretion (quantified in 152 patients) was considered normal (<250 mg/24 h in women, <300 mg/24 h in men) in 73.7% of patients with this parameter available.

The group NH consisted of 669 patients (568 women, 84.9%) aged between 18 and 79 years (median 47[37-55] years). Demographic and clinical characteristics are shown in Table 1. We did not observe any differences between groups regarding gender, age, time of follow-up, and type of thyroidectomy. However, the percentage of patients with thyroid cancer was higher in group NH (82.1%) in relation to group H (66.8%; P<0.001). Differences between both groups in the prevalence of the different studied comorbidities were not observed.

Incident comorbidities during follow-up

The values of cumulative incidence and incidence rate in patients with hypoparathyroidism are summarized in Table 2 and row data are shown in Supplementary Table 3 (Supplementary Material). Incident comorbidities more commonly diagnosed during follow-up in patients in group H were dyslipidemia (incidence rate 2.14[1.57-2.70] cases per 100 patient-years), mental health disorders (1.61 [1.14-2.08] cases per 100 patient-years) and arterial hypertension (1.58[1.11-2.05] cases per 100 patient-years).

TABLE 1 Demographic and clinical characteristics of patients with and without hypoparathyroidism.

	Patients with hypoparathyroidism (Group H, n=337)	Patients without hypoparathyroidism (Group NH, n=669)
Gender		
Female	284 (84.3)	568 (84.9)
Male	53 (15.7)	101 (15.1)
Age (yr)	45 (36-56)	47 (37-55)
Time of follow-up (yr)	8.9 (6.0-13.0)	8.0 (5.3-12.0)
Total thyroidectomy		
One-stage	302 (89.6)	567 (84.8)
Two-stage	35 (10.4)	102 (15.2)
Thyroid histopathology		
Benign thyroid disease	112 (33.2)	120 (17.9)
Thyroid cancer	225 (66.8)	549 (82.1)
Benign thyroid disease		
Graves' disease	18 (16.1)	23 (19.2)
Follicular nodular disease	73 (65.2)	75 (62.5)
Chronic lymphocytic thyroiditis	14 (12.5)	13 (10.8)
Other	7 (6.3)	9 (7.5)
Thyroid cancer		
Papillary	194 (86.2)	476 (86.7)
Follicular	18 (8.0)	45 (8.2)
Oncocytic	3 (1.3)	9 (1.6)
Other	10 (4.4)	19 (3.5)
Chronic kidney disease	7 (2.1)	15 (2.2)
Nephrolithiasis	4 (1.2)	13 (1.9)
Arterial hypertension	55 (16.3)	121 (18.1)
Dyslipidemia	65 (19.3)	139 (20.8)
Diabetes	15 (4.5)	41 (6.1)
Cardiovascular disease	10 (3.0)	25 (3.7)
Coronary heart disease	3 (0.9)	8 (1.2)
Cerebrovascular disease	1 (0.3)	7 (1.0)
Other	7 (2.1)	12 (1.8)
Central nervous system disease	6 (1.8)	12 (1.8)
Mental health disorders	44 (13.1)	93 (13.9)
Eye disorders	11 (3.3)	14 (2.1)
Bone mineral density alterations	8 (2.4)	22 (3.3)

(Continued)

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TABLE 1 Continued

	Patients with hypoparathyroidism (Group H, n=337)	Patients without hypoparathyroidism (Group NH, n=669)
Fracture	3 (0.9)	6 (0.9)
Cancer	18 (5.3)	33 (4.9)

Data are the median (IQR) for quantitative variables. For categorical variables, the absolute value is represented and, in parentheses, the prevalence in patients with hypoparathyroidism and the proportion in patients without hypoparathyroidism.
The percentages of the different types of benign thyroid disease and thyroid cancer are calculated for the total of these histological types in patients with (112 benign and 125 malignant) and patients without hypoparathyroidism (120 benign and 549 malignant).

When analyzing the association of hypoparathyroidism with the appearance of incident diseases, we found that, in comparison with group NH, patients in group H exhibited a significantly higher OR for the incidence of chronic kidney disease (2.94[1.56-5.55]; P=0.001), nephrolithiasis (3.07[1.46-6.45]; P=0.003) and cardiovascular disease (1.98[1.15-3.42]; P=0.014), and a significantly lower relative risk for fracture (0.10[0.01-0.72]; P=0.023) (Table 2; Figure 1). When each of the considered cardiovascular diseases were studied individually (i.e., coronary heart disease, cerebrovascular disease, and others), no significant increase in the OR was observed in patients with hypoparathyroidism (Table 2).

Survival analysis

To assess the influence of hypoparathyroidism for the development of incident comorbidities, we performed a survival analysis using the Kaplan-Meier curves. Only the four incident comorbidities in which a significant increase or reduction in OR was detected in patients with hypoparathyroidism were analyzed (Figure 2). Survival free of incident disease was significantly lower in group H regarding chronic kidney disease (P=0.002), nephrolithiasis (P=0.004) and cardiovascular disease (P=0.020). However, incident fracture-free survival was significantly higher in group H compared to group NH (P=0.004).

Multivariable logistic regression analysis

Results of multivariable logistic regression analysis are shown in Supplementary Tables 4–7 (Supplementary Material). A summary of the most relevant findings is shown in Figure 3. This multivariable analysis, both in model 1 and model 2, showed statistically significant association of chronic kidney disease with the presence of hypoparathyroidism (OR in model 2, 3.45[1.72-6.91]; P<0.001), and advanced age (OR in model 2, 1.07[1.03-1.10]; P<0.001). In the case of nephrolithiasis, the only factor significantly related in the multivariate analysis was hypoparathyroidism (OR in model 2, 3.34[1.55-7.22]; P=0.002). Incident cardiovascular disease was significantly related to hypoparathyroidism (OR in model 2, 2.03[1.14-3.60]; P=0.016) and age (OR in model 2, 1.03[1.01-1.06]; P=0.010). Lastly, our analysis showed that the risk of fracture was significantly increased in patients with nephrolithiasis (OR in model 2, 6.86[1.06-44.37]; P=0.043) but reduced in patients with hypoparathyroidism (OR in model 2, 0.09[0.01-0.70]; P=0.021).

Pharmacological treatments

No significant differences were found between both groups in the drug categories studied before thyroidectomy. As expected, at the last visit, patients in group H had a significantly greater use of drugs related to the treatment of hypoparathyroidism, i.e., calcium, calcitriol, and thiazide diuretics, as well as a slight but significant lower use of angiotensin-converting enzyme (ACE) inhibitors. The proportions of use of most pharmacological categories increased significantly in both groups, without clinically relevant differences. However, the use of insulin, antiarrhythmic and antipsychotic agents did not increase significantly in any group. Finally, the use of non-thiazide diuretics and calcium antagonists increased significantly only in the NH group (Table 3).

Discussion

The results of the present study show that, in real-life clinical practice in Spain, patients with permanent postsurgical hypoparathyroidism have a higher risk of developing chronic kidney disease, nephrolithiasis, and cardiovascular disease, compared to thyroidectomized patients with normal parathyroid function. On the contrary, hypoparathyroidism seems to reduce the risk of incident fracture. Our data do not show any influence of hypoparathyroidism on other chronic conditions such as hypertension, dyslipidemia, diabetes, mental health disorders, eye disorders, BMD alterations or cancer.

Chronic hypoparathyroidism is associated with different complications that affect multiple organ systems. A recent systematic review (20) recognizes the presence of cataracts in 17% of patients, nephrocalcinosis/nephrolithiasis in 15%, renal failure in 12%, depression in 12%, seizures in 11%, infection in 11%, ischemic heart disease in 7% and arrhythmias in 7%. Most of these prevalence data have been obtained from registry or population-based studies (13–15, 21, 22). Our study has used a different methodology and should be understood as a real-life analysis of the comorbidities presented by patients with long-term chronic hypoparathyroidism and that are diagnosed in the clinical practice of Spanish endocrinologists. To correctly interpret and analyze these results, we must take into account that the clinical guidelines do not clearly establish the necessary examinations for the detection of comorbidities in hypoparathyroidism and that, therefore, most Spanish endocrinologists do not carry out a formal assessment of all possible complications in clinical practice. This fact has recently become evident in an international survey of expert endocrinologists that showed that most of them do not monitor intracerebral calcifications, ophthalmological examination for cataract or BMD on a regular basis (23).

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TABLE 2 Obtained values of cumulative incidence and incidence rates of incident comorbidities in patients with hypoparathyroidism and proportion of patients without hyperparathyroidism who develop incident comorbidities.

	Patients with hypoparathyroidism (group H)		Patients without hypoparathyroidism (group NH)	Odds ratio	
	Cumulative incidence (%)	Incidence rate (per 100 patient-year)	Proportion (%)	Value	P
Chronic kidney disease	7.27 (4.47-10.07)	0.73 (0.44-1.02)	2.60 (1.38-3.82)	2.94 (1.56-5.55)	0.001
Nephrolithiasis	5.41 (2.98-7.83)	0.54 (0.29-0.79	1.83 (0.80-2.85)	3.07 (1.46-6.45)	0.003
Hypertension	15.25 (11.05-19.44)	1.58 (1.11-2.05)	19.53 (16.21-22.84)	0.74 (0.50-1.09)	0.130
Dyslipidemia	20.22 (15.45-24.29)	2.14 (1.57-2.70)	22.08 (18.54-25.61)	0.90 (0.62-1.28)	0.545
Diabetes	7.14 (/4.33-9.96)	0.72 (0.43-1.02)	6.69 (4.73-8.64)	1.07 (0.63-1.82)	0.793
Cardiovascular disease	8.26 (5.27-11.24)	0.84 (0.52-1.16)	4.35 (2.27-5.92)	1.98 (1.15-3.42)	0.014
Coronary heart disease	2.10 (0.56-3.63)	0.21 (0.05-0.36)	1.21 (0.38-2.04)	1.75 (0.63-4.86)	0.285
Cerebrovascular disease	2.98 (1.16-4.79)	0.30 (0.11-0.48)	1.36 (0.48-2.24)	2.23 (0.90-5.53)	0.085
Other cardiovascular disease	4.24 (2.07-6.42)	0.42 (0.20-0.65)	2.89 (1.61-4.17)	1.49 (0.74-3.01)	0.268
Central nervous system disease	0.30 (0-0.89)	0.03 (0-0.09)	0.46 (0-0.97)	0.66 (0.07-6.38)	0.720
Mental health disorders	15.36 (11.23-19.49)	1.61 (1.14-2.08)	12.85 (10.11-15.58)	1.23 (0.82-1.84)	0.309
Eye disorders	4.60 (2.33-6.88)	0.45 (0.22-0.68)	3.66 (2.23-5.10)	1.27 (0.66-2.45)	0.480
BMD alterations	6.38 (3.74-9.02)	0.64 (0.37-0.92)	5.72 (3.93-7.51)	1.12 (0.65-1.95)	0.678
Fracture	0.30 (0-0.89)	0.03 (0-0.09)	3.02 (1.71-4.32)	0.10 (0.01-0.72)	0.023
Cancer	5.64 (3.11-8.17)	0.57 (0.31-0.83)	7.39 (5.36-9.42)	0.75 (0.43-1.31)	0.313

Data are the value of the parameter indicated with the 95% confidence interval in parentheses.
BMD, bone mineral density.

Renal conditions are among the most common complications in patients with hypoparathyroidism (21). The prevalence of chronic kidney disease in patients with hypoparathyroidism ranges from 2.5 to 41% (7, 24) and nephrolithiasis/nephrocalcinosis occurs in 19-31% of patients (13, 24, 25). Our data obtained in clinical practice differ from the findings of previous population-based and registry studies (16, 22, 26, 27). Our results in group H showed that 7.27% of patients developed chronic kidney disease and 5.41% presented with nephrolithiasis throughout the follow-up. In patients with normal parathyroid function these values were only 2.60% for renal failure and 1.83% for nephrolithiasis. The incidence rate was 0.73 cases per 100 patient-years for chronic kidney disease and 0.54 cases per 100 patient-years for nephrolithiasis. Similar to that reported in other studies (13), our multivariate analysis shows that patients with hypoparathyroidism, compared to the NH group, have a three-fold increased risk for chronic kidney disease (OR 3.45; 95% CI, 1.72-6.91) and nephrolithiasis (OR 3.34; 95% CI, 1.55-7.22). Age was also a factor significantly related to chronic kidney disease, while for nephrolithiasis the only factor was hypoparathyroidism.

Hypoparathyroidism may increase the risk of hypercalciuria due to the lack of tubular calcium reabsorption by PTH. Furthermore, treatment with large doses of calcium and active vitamin D can also increase the risk of hypercalciuria, renal stones, and renal insufficiency (25, 28). An increase in the calcium-phosphorus product, together with the deposit of calcium

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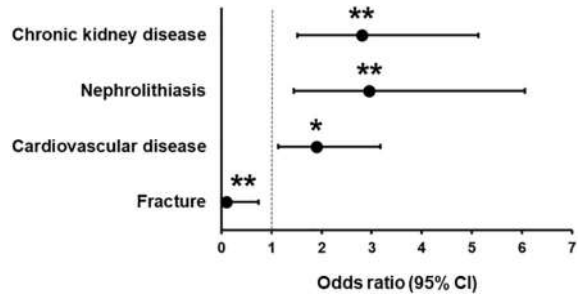

FIGURE 1
Odds ratio (with 95% confidence intervals) in patients with hypoparathyroidism in comparison with patients without hypoparathyroidism for chronic kidney disease, nephrolithiasis, cardiovascular disease, and fracture. *P < 0.05, **P < 0.01.

phosphate has been implicated in the increase of renal failure and nephrolithiasis in patients with hypoparathyroidism (13, 27). The number of episodes of hypercalcemia and the duration of disease have also been considered risk factors associated with kidney disease (26).

One of the most noteworthy findings of our study is the increased risk of overall cardiovascular disease in patients with hypoparathyroidism (OR in multivariable analysis, 2.03; 95% CI, 1.14-3.60). Although the subgroup analysis did not show an increase in risk in each of the considered conditions, 8.26% of our patients developed some type of cardiovascular disease during follow-up. Our results contrast with those found in a study of 688 patients with postsurgical hypoparathyroidism identified in a Danish national registry by Underbjerg et al. (13). Compared with controls, these patients did not have an increased risk of cardiovascular disease, cardiac arrhythmias or death. However, in a subsequent study, conducted in 180 patients with nonsurgical hypoparathyroidism, these authors (15) found a significantly increased risk of cardiovascular disease (HR 1.91; 95% CI, 1.29-2.81), similar to that found in the present survey. The population-based study by Vadiveloo et al. (21) showed that nonsurgical hypoparathyroid patients had increased risk of cardiovascular (HR 2.18; 95% CI, 1.41-3.39) and cerebrovascular disease (HR, 2.95, 95% CI, 1.46-5.97). A national population-based Korean study (22) also showed that patients with nonsurgical hypoparathyroidism had a higher risk of cardiovascular disease, especially arrhythmia (HR, 2.03; 95% CI, 1.11-3.70) and heart failure (HR, 2.43; 95% CI, 1.22-4.83). Similarly, a retrospective cohort study using a large medical insurance database in USA showed that patients with chronic hypoparathyroidism had significantly higher risk of incident cardiovascular conditions compared with those without hypoparathyroidism (HR 1.63; 95% CI, 1.52-1.75) (29).

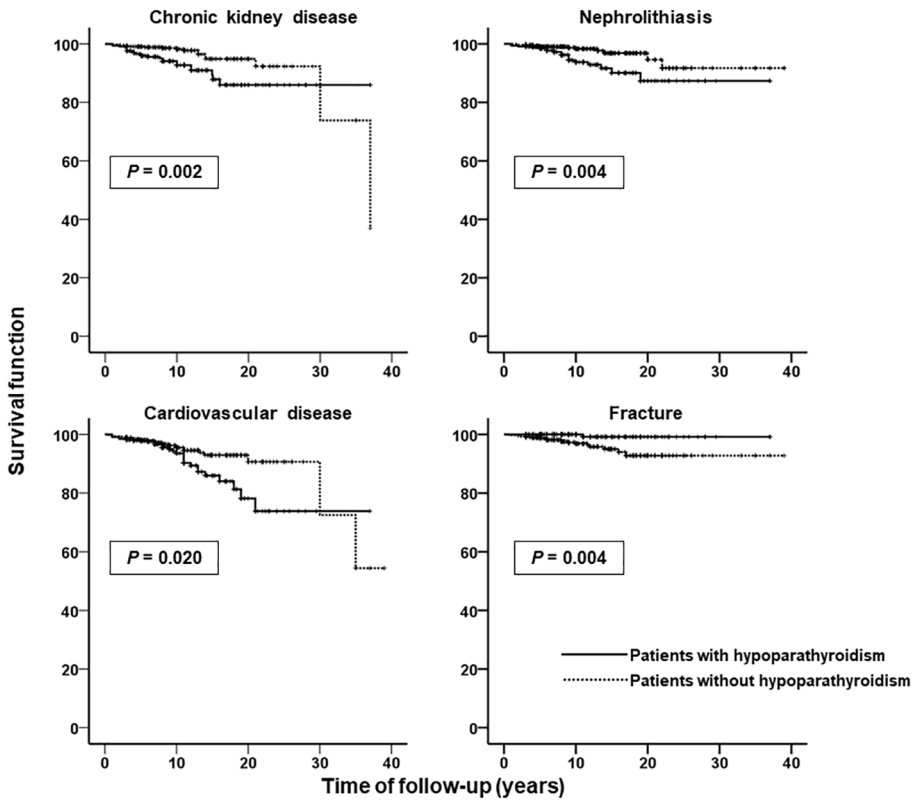

FIGURE 2
Kaplan-Meier curves for time of follow-up without developing incident chronic kidney disease, nephrolithiasis, cardiovascular disease, and fracture in patients with (solid lines) and without (dashed lines) hypoparathyroidism. Ordinate scale: survival function (proportion of patients not developing incident disease). Abscissa scale: time of follow-up (years).

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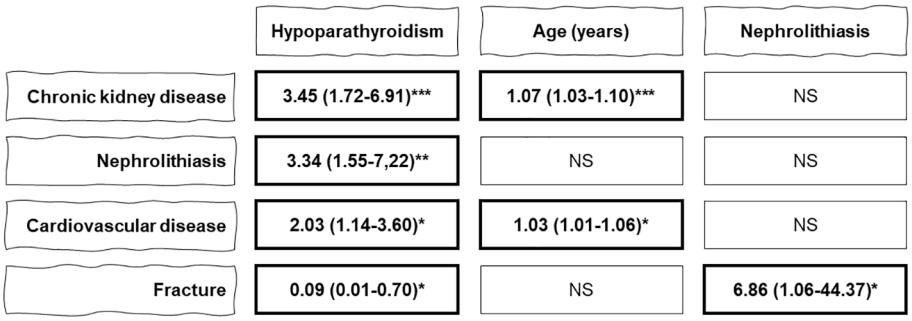

FIGURE 3
Summary of the results of the multivariable logistic regression analysis (model 2) to study the influence of hypoparathyroidism and different variables on the development of incident comorbidities (chronic kidney disease, nephrolithiasis, cardiovascular disease, and facture). Data are the odds ratio with 95% confidence intervals. NS, non-significant; *P < 0.05, **P < 0.01, ***P < 0.001.

Chronic hypocalcemia and the lack of action of PTH at the cardiac and vascular levels have been implicated as causal factors in cardiovascular complications. In an elegant study, Underbjerg et al. (26) have shown that disturbances in calcium-phosphate homeostasis are significantly associated with risk of complications. In particular, the increased cardiovascular risk was associated with an increased number of hypercalcemic episodes, lower time-weighted serum ionized calcium, and longer duration of hypoparathyroidism.

We analyzed changes in the drug therapies of the two groups of patients. The use of drugs in the baseline situation prior to surgery was very similar between both groups. As expected, at last visit, the use of drugs related to hypoparathyroidism was significantly higher in group H. However, the use of remaining drugs at last visit was

TABLE 3 Pharmacological treatments used by patients before thyroidectomy and at the last follow-up visit.

	Before thyroidectomy			Last visit			Comparison last visit vs. before surgery	
	Group H	Group NH		Group H	Group NH		Group H	Group NH
			P			P	P	P
Calcium	9 (2.7)	17 (2.5)	0.526	296 (87.8)	57 (8.5)	<0.001	<0.001	<0.001
Calcitriol				332 (98.5)	0 (0)	<0.001		
Levothyroxine	32 (9.5)	44 (6.6)	0.066	337 (100)	669 (100)			
Vitamin D	21 (6.2)	26 (3.9)	0.068	138 (40.9)	238 (35.6)	0.098	<0.001	<0.001
Hypolipidemic drugs	48 (14.2)	101 (15.1)	0.394	91 (27.0)	200 (29.9)	0.337	<0.001	<0.001
Oral antidiabetics	13 (3.9)	35 (5.2)	0.211	32 (9.5)	67 (10.0)	0.824	<0.001	<0.001
Injectable antidiabetics	2 (0.6)	4 (0.6)	0.677	10 (3.0)	18 (2.7)	0.840	0.008	0.001
Insulin	3 (0.9)	9 (1.3)	0.387	5 (1.5)	15 (2.2)	0.482	0.500	0.109
Thiazide diuretics	16 (4.7)	32 (4.8)	0.562	65 (19.3)	93 (13.9)	0.028	<0.001	<0.001
Non thiazide diuretics	11 (3.3)	12 (1.8)	0.108	19 (5.6)	37 (5.5)	1.000	0.057	<0.001
Beta-blockers	11 (3.3)	27 (4.0)	0.339	29 (8.6)	51 (7.6)	0.622	<0.001	<0.001
Calcium antagonists	20 (5.9)	28 (4.2)	0.142	30 (8.9)	64 (9.6)	0.819	0.064	<0.001
ACE inhibitors	36 (10.7)	91 (13.6)	0.111	70 (20.8)	181 (27.1)	0.031	<0.001	<0.001
Antiarrhythmics	1 (0.3)	4 (0.6)	0.457	4 (1.2)	9 (1.3)	1.000	0.375	0.227
Oral anticoagulants	1 (0.3)	6 (0.9)	0.259	8 (2.4)	14 (2.1)	0.821	0.016	0.039
Anxiolytics	35 (10.4)	63 (9.4)	0.350	62 (18.4)	130 (19.4)	0.734	<0.001	<0.001
Antidepressants	18 (5.3)	43 (6.4)	0.298	38 (11.3)	87 (13.0)	0.479	0.001	<0.001
Antipsychotics	4 (1.2)	9 (1.3)	0.547	5 (1.5)	14 (2.1)	0.627	1.000	0.125

Data are the number of patients (percentage) in each pharmacological group.
ACE, angiotensin-converting enzyme.

similar in both groups, with the exception of a higher proportion of ACE inhibitor users in group NH (group (27.1 vs. 20.8%; P=0.031). The use of calcium and vitamin D has been linked to a possible increase in cardiovascular risk, although study results have been conflicting (13). Our data suggest, but do not prove, that the lower use of ACE inhibitors in group H could be related to the increased risk of cardiovascular disease, although this relationship is uncertain.

On the other hand, our study did not show any relationship between hypoparathyroidism and some of the classic risk factors for cardiovascular disease, such as diabetes, dyslipidemia, and hypertension. We have not found any previous studies showing associations of chronic hypoparathyroidism with hypertension or dyslipidemia. Nevertheless, a recent retrospective database report suggests that chronic hypoparathyroidism is associated with an increased risk of type 2 diabetes (HR 1.80; 95% CI, 1.64-1.96) (30). Further research is needed to confirm these results and understand the potential mechanisms of this association.

It has been well established that PTH deficiency is accompanied by a reduction in bone turnover and abnormalities in skeletal microstructure, both in cortical and cancellous compartments (31–33). An increase in BMD in patients with hypoparathyroidism compared to individuals matched for age and sex has been reported (25, 32, 34). This increase in BMD, in general, affects all skeletal sites, with higher values in the lumbar spine (33). It is not, however, well established whether this increase in BMD is accompanied by a decrease in the risk of fractures, since the available studies have shown contradictory results (14–16, 21, 22, 35, 36). Apart from BMD, other risk factors for fractures in patients with hypoparathyroidism, such as impairment in the trabecular microarchitecture, should be considered. A recent study has shown that bone marrow adipose tissue is increased in postmenopausal women with postsurgical hypoparathyroidism and negatively associated with trabecular microarchitecture (37).

On the other side, it should be emphasized that the evaluation of clinical fractures is not a sensitive method and, therefore, is not the ideal procedure to study the incidence of skeletal health problems in patients with hypoparathyroidism. Recent data showed that a morphometric approach is essential for evaluating bone health in patients with endocrine disorders known to affect skeletal health (38). Therefore, the proactive search of clinically asymptomatic fractures by this method has been recognized as one of the most useful tools in these patients (38). In fact, in a study carried out on 50 postmenopausal women with chronic hypoparathyroidism and 40 age-matched healthy postmenopausal women, Cipriani et al. (36) demonstrated that, although BMD values were higher in the hypoparathyroid group in comparison to healthy controls, patients exhibited a higher incidence of asymptomatic skeletal fractures at vertebral spine.

Furthermore, clinical guidelines do not give precise indications (4) or recommend against routine BMD monitoring (3). However, the detection of incident alterations in BMD in 6.38% of patients in group H and 5.72% of patients in group NH suggests that this examination is frequently used in the clinical practice of Spanish endocrinologists. Although our data did not show statistically different changes in BMD between both groups, a lower risk of incident fracture in patients with hypoparathyroidism compared to subjects with normal calcium metabolism was found. This finding is in line with a recent study that have shown that prevalence of fragility fractures was low in women with hypoparathyroidism and compatible with low fracture risk estimated by the FRAX tool (39). Taken together, these data suggest, although they do not demonstrate, that hypoparathyroidism protects against fracture risk (32). Nonetheless, these data should be taken with caution, because the incidence of fractures was not actively sought by the researchers of this retrospective study and it is possible that some cases of fractures were not detected in clinical practice. Our study also showed a strong association of nephrolithiasis with the incidence of fractures. This data is difficult to explain and could be related to the small number of events recorded in group H.

Mental health disorders, including depression, anxiety and bipolar affective disorder have been reported in patients with hypoparathyroidism (14, 21, 22). Our survey detected that 15% of patients with hypoparathyroidism had mental health disorders throughout the follow-up. This cumulative incidence was slightly higher than that found in group NH, although it did not reach statistical significance. The detection of psychiatric problems is common in patients with hypoparathyroidism and has been related to a decrease in the quality of life of these patients. Different studies have been able to demonstrate a significant negative impact on mental and emotional health using instruments validated for chronic diseases (40–42) and also disease-specific instrument developed and validated for hypoparathyroidism (43). As with tumor development, a protective role of vitamin D for neurocognitive disorders is possible. In fact, some data suggest that vitamin D is important for normal brain development and function in rodents and humans (44). In a recent study, carried out in coronoavirus disease-19 (COVID) survivors with long COVID, lower 25(OH)-vitamin D levels were observed in those with neurocognitive symptoms at follow-up than those without (45). However, vitamin D supplements have produced conflicting results on neurocognitive performance (44).

Although our patients with hypoparathyroidism had a higher proportion of eye disorders during follow-up (4.60%), the difference with patients in the NH group (3.66%) was not significant. The increased risk of cataract in hypoparathyroidism has been well documented (15, 20–22) and has been related to the duration of disease (16, 22). Similarly, we have also not found an increased risk of central nervous system diseases even though the prevalence of basal ganglia calcifications has been reported in 37% of nonsurgical patients and in 15% of postsurgical patients (46), and the risk of epilepsy has been found to be elevated in nonsurgical and surgical hypoparathyroidism (13, 20–22).

The detection of complications is clearly dependent on carrying out an active search. In the study by Mitchell et al. (25), of those patients with renal imaging, 31% had renal calcifications, and 52% of those with head imaging had basal ganglia calcifications. The lack of detection of an increased risk of cataract or central nervous system conditions can be explained because this is a retrospective study of routine clinical practice and, in our country, there are no protocols or clinical guidelines on screening for these chronic

complications of hypoparathyroidism. Unfortunately, our data suggest that most of Spanish endocrinologists do not actively search for cases of cataract or central nervous system disease (47). Nevertheless, it is worth mentioning that basal ganglia calcification was not identified as one of the common complications of hypoparathyroidism in a recent systematic review of observational studies (7).

Our data do not provide evidence of a higher incidence of malignancies in patients with hypoparathyroidism. We must assume that in our study population no specific detection tests are performed on these patients, but only general population cancer screening. Our results agree with those from the Danish registry study, which showed that the risk of overall malignant diseases did not differ between patients with postsurgical hypoparathyroidism and controls (14). Nonetheless, the risk of gastrointestinal cancers was significantly lower in patients in this study (14) and the risk of overall malignancy was decreased among patients with nonsurgical hypoparathyroidism (15). This cancer risk reduction has been attributed to the use of calcium and vitamin D in these patients, since there is an inverse association between vitamin D status, calcium intake and the risk of digestive cancer (14)

Our results may have implications for clinical practice. Prevalence of complications of chronic hypoparathyroidism may vary among patient populations and the methodology used. Our findings, based on clinical practice, could provide useful information for future guidelines and consensus on the practical management of patients with hypoparathyroidism. The increased risk of cardiovascular disease registered in our analysis might explain the increased mortality in hypoparathyroidism reported in some epidemiological studies (21, 48). However, this aspect is not conclusive, since other studies have not detected an increase in mortality (13, 15, 22).

Monitoring of complications of chronic hypoparathyroidism is not well established and the recommendations offered by the guidelines are based on expert opinions and consensus statements (23). Our study highlights the long-term morbidity associated with hypoparathyroidism found in real clinical practice by Spanish endocrinologists. We suggest that a more active and rigorous monitoring of hypoparathyroidism comorbidities will lead to greater detection of complications and will have an impact on the epidemiology of the disease and the prognosis of patients.

The main strengths of our study include the high sample size, taking into account the rarity of the disease, and its multicenter and nationwide design, as well as the non-inclusion of patients with hypoparathyroidism of short duration (<3 years). Our investigation includes diagnoses made in real clinical practice by expert specialists. Although all diagnoses are reliable and are recorded in the patients' medical records, it is possible that there are unrecorded diagnoses and, therefore, comorbidities not detected in this study. Additionally, in our study, the two groups studied were comparable at baseline not only in age, sex and time of evolution, but also in prevalent disease burden and use of drugs.

Among the limitations, we must point out that our study included a cohort with selection of a non-exposed group that is not representative of the total. However, our non-exposed group (group NH) can be considered at higher risk of developing comorbidities, since they are patients with hospital follow-up. Our study required the included patients to be alive at the time of the study, that is, it presents an immortal time bias. However, our study did not aim to analyze mortality and, furthermore, patients have a mean age (45 years) at which deaths are not expected in the short term. Although our sample size is noteworthy, it may not have a sufficient size to detect comorbidities with a low incidence. In the particular case of fractures, we have to recognize that the use of anamnesis or records of fractures with clinical manifestation are not the most appropriate procedures to investigate the impact of hypoparathyroidism on the skeleton. We do not have data on quality of life or the incidence of infections, aspects of clinical interest in these patients. Another limitation is that our study design did not include smoking and, therefore, we cannot analyze the effect of smoking on incident comorbidities in patients with hypoparathyroidism. Further to this, our study was limited to the setting of specialized medical care in Spain, so the results could vary in different settings or countries.

In summary, to our knowledge, this is the first study that analyzes a large number of incident comorbidities in patients with chronic hypoparathyroidism using clinical practice data. The results are consistent with the associations found in large-scale database and registry analysis. However, some results from clinical practice do not agree with registry studies. This may be due to the lack of agreed criteria for exhaustive screening of complications in chronic hypoparathyroidism. We believe that further real-life studies are necessary to inform the writing of future clinical guidelines and monitoring protocols.

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 patient's confidential information was protected according to national law, and the study received favorable report from the ethics committee of the Hospital Universitario Puerta de Hierro Majadahonda (PI 253/22). Full name and affiliation: Belén Ruiz Antorán, Hospital Universitario Puerta de Hierro Majadahonda, Calle Joaquín Rodrigo 2, 28222 Majadahonda (Madrid, Spain). Phone: +34911916000. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because all patient's data were obtained under the standard medical care conditions. This is a retrospective study without participation of any patients.

Author contributions

JD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EA: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. BP-C: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. MPa: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. VA: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. CS-R: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. AOr: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. AR-L: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. MS: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. AOl: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. ÁC: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. MA: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. VU: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. AD-L: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. JF-G: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. VK-D: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. SD-D: Writing – review & editing. TM: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. MPe: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. JS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1348971/full#supplementary-material

References

1. Mannstadt M, Bilezikian JP, Thakker RV, Hannan FM, Clarke BL, Rejnmark L, et al. Hypoparathyroidism. *Nat Rev Dis Primers* (2017) 3:17080. doi: 10.1038/nrdp.2017.80

2. Gafni RI, Collins MT. Hypoparathyroidism. *N Engl J Med* (2019) 380(18):1738–47. doi: 10.1056/NEJMcp1800213

3. Bollerslev J, Rejnmark L, Marcocci C, Shoback DM, Sitges-Serra A, van Biesen W, et al. European Society of Endocrinology. European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. *Eur J Endocrinol* (2015) 173(2):G1–20. doi: 10.1530/EJE-15-0628

4. Brandi ML, Bilezikian JP, Shoback D, Bouillon R, Clarke BL, Thakker RV, et al. Management of hypoparathyroidism: summary statement and guidelines. *J Clin Endocrinol Metab* (2016) 101(6):2273–83. doi: 10.1210/jc.2015-3907

5. Stack BC Jr, Bimston DN, Bodenner DL, Brett EM, Dralle H, Orloff LA, et al. American association of clinical endocrinologists and American College of Endocrinology disease state clinical review: Postoperative Hypoparathyroidism–Definitions And ManagemenT. *Endocr Pract* (2015) 21(6):674–85. doi: 10.4158/EP14462.DSC

6. Díez JJ, Anda E, Sastre J, Pérez Corral B, Álvarez-Escolá C, Manjón L, et al. Prevalence and risk factors for hypoparathyroidism following total thyroidectomy in Spain: A multicentric and nation-wide retrospective analysis. *Endocrine* (2019) 66(2):405–15. doi: 10.1007/s12020-019-02014-8

7. Khan AA, Bilezikian JP, Brandi ML, Clarke BL, Gittoes NJ, Pasieka JL, et al. Evaluation and management of Hypoparathyroidism summary statement and guidelines from the second international workshop. *J Bone Miner Res* (2022) 37(12):2568–85. doi: 10.1002/jbmr.4691

8. Edafe O, Antakia R, Laskar N, Uttley L, Balasubramanian SP. Systematic review and meta-analysis of predictors of post-thyroidectomy hypocalcaemia. *Br J Surg* (2014) 101(4):307–20. doi: 10.1002/bjs.9384

9. Annebäck M, Hedberg J, Almquist M, Stålberg P, Norlén O. Risk of permanent Hypoparathyroidism after total thyroidectomy for benign disease: A nationwide

population-based cohort study from Sweden. *Ann Surg* (2021) 274(6):e1202–8. doi: 10.1097/SLA.0000000000003800

10. Takahashi T, Yamazaki K, Shodo R, Ueki Y, Horii A. Actual prevalence of hypoparathyroidism after total thyroidectomy: A health insurance claims-database study. *Endocrine* (2022) 78(1):151–8. doi: 10.1007/s12020-022-03153-1

11. Lui DTW, Fung MMH, Lee CH, Fong CHY, Woo YC, Lang BHH. A territory-wide assessment of the incidence of persistent hypoparathyroidism after elective thyroid surgery and its impact on new fracture risk over time. *Surgery* (2021) 170 (5):1369–75. doi: 10.1016/j.surg.2021.05.004

12. Loncar I, Noltes ME, Dickhoff C, Engelsman AF, Schepers A, Vriens MR, et al. Persistent postthyroidectomy hypoparathyroidism in the Netherlands. *JAMA Otolaryngol Head Neck Surg* (2021) 147(11):959–65. doi: 10.1001/jamaoto.2021.2475

13. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Cardiovascular and renal complications to postsurgical hypoparathyroidism: a Danish nationwide controlled historic follow-up study. *J Bone Miner Res* (2013) 28(11):2277–85. doi: 10.1002/jbmr.1979

14. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Postsurgical hypoparathyroidism–risk of fractures, psychiatric diseases, cancer, cataract, and infections. *J Bone Miner Res* (2014) 29(11):2504–10. doi: 10.1002/jbmr.2273

15. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. The epidemiology of nonsurgical hypoparathyroidism in Denmark: A nationwide case finding study. *J Bone Miner Res* (2015) 30(9):1738–44. doi: 10.1002/jbmr.2501

16. Vadiveloo T, Donnan PT, Leese CJ, Abraham KJ, Leese GP. Increased mortality and morbidity in patients with chronic hypoparathyroidism: A population-based study. *Clin Endocrinol (Oxf)* (2019) 90(2):285–92. doi: 10.1111/cen.13895

17. Clarke BL. Epidemiology and complications of hypoparathyroidism. *Endocrinol Metab Clin North Am* (2018) 47(4):771–82. doi: 10.1016/j.ecl.2018.07.004

18. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med* (2021) 385(19):1737–49. doi: 10.1056/NEJMoa2102953

19. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* (1999) 130(6):461–70. doi: 10.7326/0003-4819-130-6-199903160-00002

20. Yao L, Hui X, Li M, Li J, Ahmed MM, Lin C, et al. Complications, symptoms, presurgical predictors in patients with chronic hypoparathyroidism: A systematic review. *J Bone Miner Res* (2022) 37(12):2642–53. doi: 10.1002/jbmr.4673

21. Vadiveloo T, Donnan PT, Leese GP. A population-based study of the epidemiology of chronic hypoparathyroidism. *J Bone Miner Res* (2018) 33(3):478–85. doi: 10.1002/jbmr.3329

22. Kim SH, Rhee Y, Kim YM, Won YJ, Noh J, Moon H, et al. Prevalence and complications of nonsurgical hypoparathyroidism in Korea: A nationwide cohort study. *PloS One* (2020) 15(5):e0232842. doi: 10.1371/journal.pone.0232842

23. Van Uum S, Shrayyef M, M'Hiri I, Dandurand K, Ali DS, Bilezikian JP, et al. Initial assessment and monitoring of patients with chronic hypoparathyroidism: A systematic current practice survey. *J Bone Miner Res* (2022) 37(12):2630–41. doi: 10.1002/jbmr.4698

24. Gosmanova EO, Houillier P, Rejnmark L, Marelli C, Bilezikian JP. Renal complications in patients with chronic hypoparathyroidism on conventional therapy: A systematic literature review : Renal disease in chronic hypoparathyroidism. *Rev Endocr Metab Disord* (2021) 22(2):297–316. doi: 10.1007/s11154-020-09613-1

25. Mitchell DM, Regan S, Cooley MR, Lauter KB, Vrla MC, Becker CB, et al. Long-term follow-up of patients with hypoparathyroidism. *J Clin Endocrinol Metab* (2012) 97 (12):4507–14. doi: 10.1210/jc.2012-1808

26. Underbjerg L, Sikjaer T, Rejnmark L. Long-term complications in patients with hypoparathyroidism evaluated by biochemical findings: A case-control study. *J Bone Miner Res* (2018) 33(5):822–31. doi: 10.1002/jbmr.3368

27. Ketteler M, Chen K, Gosmanova EO, Signorovitch J, Mu F, Young JA, et al. Risk of nephrolithiasis and nephrocalcinosis in patients with chronic hypoparathyroidism: A retrospective cohort study. *Adv Ther* (2021) 38(4):1946–57. doi: 10.1007/s12325-021-01649-2

28. Bilezikian JP, Khan A, Potts JT Jr, Brandi ML, Clarke BL, Shoback D, et al. Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Miner Res* (2011) 26(10):2317–37. doi: 10.1002/jbmr.483

29. Gosmanova EO, Chen K, Ketteler M, Rejnmark L, Mu F, Swallow E, et al. Risk of cardiovascular conditions in patients with chronic hypoparathyroidism: A retrospective cohort study. *Adv Ther* (2021) 38(8):4246–57. doi: 10.1007/s12325-021-01787-7

30. Chen K, Gosmanova EO, Curhan G, Mu F, Macheca M, Warchol M, et al. Risk of type 2 diabetes (T2D) in patients with chronic hypoparathyroidism (HypoPT): A retrospective cohort study. *Endocrine Abstracts* (2019) 63:GP1038. doi: 10.1530/endoabs.63.P1038

31. Chen Q, Kaji H, Iu MF, Nomura R, Sowa H, Yamauchi M, et al. Effects of an excess and a deficiency of endogenous parathyroid hormone on volumetric bone mineral density and bone geometry determined by peripheral quantitative computed tomography in female subjects. *J Clin Endocrinol Metab* (2003) 88(10):4655–8. doi: 10.1210/jc.2003-030470

32. Silva BC, Rubin MR, Cusano NE, Bilezikian JP. Bone imaging in hypoparathyroidism. *Osteoporos Int* (2017) 28(2):463–71. doi: 10.1007/s00198-016-3750-0

33. Rubin MR, Dempster DW, Zhou H, Shane E, Nickolas T, Sliney J Jr, et al. Dynamic and structural properties of the skeleton in hypoparathyroidism. *J Bone Miner Res* (2008) 23(12):2018–24. doi: 10.1359/jbmr.080803

34. Cusano NE, Nishiyama KK, Zhang C, Rubin MR, Boutroy S, McMahon DJ, et al. Noninvasive assessment of skeletal microstructure and estimated bone strength in hypoparathyroidism. *J Bone Miner Res* (2016) 31(2):308–16. doi: 10.1002/jbmr.2609

35. Pal R, Bhadada SK, Mukherjee S, Banerjee M, Kumar A. Fracture risk in hypoparathyroidism: a systematic review and meta-analysis. *Osteoporos Int* (2021) 32 (11):2145–53. doi: 10.1007/s00198-021-05966-8

36. Cipriani C, Minisola S, Bilezikian JP, Diacinti D, Colangelo L, Piazzolla V, et al. Vertebral fracture assessment in postmenopausal women with postsurgical hypoparathyroidism. *J Clin Endocrinol Metab* (2021) 106(5):1303–11. doi: 10.1210/clinem/dgab076

37. Diacinti D, Diacinti D, Iannacone A, Pepe J, Colangelo L, Nieddu L, et al. Bone marrow adipose tissue is increased in postmenopausal women with postsurgical hypoparathyroidism. *J Clin Endocrinol Metab* (2023) 108(9):e807–15. doi: 10.1210/clinem/dgad116

38. Uygur MM, di Filippo L, Giustina A. New tools for bone health assessment in secreting pituitary adenomas. *Trends Endocrinol Metab* (2023) 34(4):231–42. doi: 10.1016/j.tem.2023.01

39. Santa Rosa RG, Polonine S, Pichone A, Gomes CP, Lima LFC, de Paula Paranhos Neto F, et al. Chronic hypoparathyroidism is associated with increased cortical bone density evaluated using high-resolution peripheral quantitative computed tomography. *Endocrine* (2023) 82(3):673–80. doi: 10.1007/s12020-023-03495-4

40. Cusano NE, Rubin MR, McMahon DJ, Irani D, Tulley A, Sliney J Jr, et al. The effect of PTH(1-84) on quality of life in hypoparathyroidism. *J Clin Endocrinol Metab* (2013) 98(6):2356–61. doi: 10.1210/jc.2013-1239

41. Büttner M, Locati LD, Pinto M, Araújo C, Tomaszewska IM, Kiyota N, et al. Quality of life in patients with Hypoparathyroidism after treatment for thyroid cancer. *J Clin Endocrinol Metab* (2020) 105(12):dgaa597. doi: 10.1210/clinem/dgaa597

42. Hepsen S, Akhanli P, Sakiz D, Sencar ME, Ucan B, Unsal IO, et al. The effects of patient and disease-related factors on the quality of life in patients with hypoparathyroidism. *Arch Osteoporos* (2020) 15(1):75. doi: 10.1007/s11657-020-00759-8

43. Brod M, Waldman LT, Smith A, Karpf D. Living with hypoparathyroidism: development of the Hypoparathyroidism patient experience Scale-Impact (HPES-Impact). *Qual Life Res* (2021) 30(1):277–91. doi: 10.1007/s11136-020-02607-1

44. Gáll Z, Székely O. Role of vitamin D in cognitive dysfunction: New molecular concepts and discrepancies between animal and human findings. *Nutrients* (2021) 13 (11):3672. doi: 10.3390/nu13113672

45. di Filippo L, Nannipieri F, Cotellessa A, Locatelli M, Rovere Querini P, Giustina A. Low vitamin D Levels are associated with long COVID syndrome in COVID-19 survivors. *J Clin Endocrinol Metab* (2023) 108(10):e1106–16. doi: 10.1210/clinem/dgad207

46. Khan AA, AbuAlrob H, Punthakee Z, Shrayyef M, Werfalli RE, Kassem HA, et al. Canadian national hypoparathyroidism registry: an overview of hypoparathyroidism in Canada. *Endocrine* (2021) 72(2):553–61. doi: 10.1007/s12020-021-02629-w

47. Burillo E, Vila J, Oguiza A. Characteristics of patients with chronic hypoparathyroidism not adequately controlled with conventional treatment and management patterns in Spain. *Endocrinol Diabetes Nutr (Engl Ed).* (2021) 68 (5):304–11. doi: 10.1016/j.endien.2021.08.008

48. Amquist M, Ivarsson K, Nordenström E, Bergenfelz A. Mortality in patients with permanent hypoparathyroidism after total thyroidectomy. *Br J Surg* (2018) 105 (10):1313–8. doi: 10.1002/bjs.10843

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Philippe Rondard,
Centre National de la Recherche Scientifique (CNRS), France
Miles Douglas Thompson,
University Health Network (UHN), Canada

*CORRESPONDENCE
Shih-Hua Lin
l521116@ndmctsgh.edu.tw;
l521116@gmail.com

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Identification and characterization of a novel CASR mutation causing familial hypocalciuric hypercalcemia

Chien-Ming Lin[1], Yi-Xuan Ding[1], Shih-Ming Huang[2], Ying-Chuan Chen[3], Hwei-Jen Lee[2], Chih-Chien Sung[4] and Shih-Hua Lin[4]*

[1]Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, [2]Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan, [3]Department of Physiology and Biophysics, National Defense Medical Center, Taipei, Taiwan, [4]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

Context: Although a monoallelic mutation in the calcium-sensing receptor (*CASR*) gene causes familial hypocalciuric hypercalcemia (FHH), the functional characterization of the identified *CASR* mutation linked to the clinical response to calcimimetics therapy is still limited.

Objective: A 45-year-old male presenting with moderate hypercalcemia, hypocalciuria, and inappropriately high parathyroid hormone (PTH) had a good response to cinacalcet (total serum calcium (Ca^{2+}) from 12.5 to 10.1 mg/dl). We identified the genetic mutation and characterized the functional and pathophysiological mechanisms, and then linked the mutation to calcimimetics treatment *in vitro*.

Design: Sanger sequencing of the *CASR*, *GNA11*, and *AP2S1* genes was performed in his family. The simulation model was used to predict the function of the identified mutant. *In vitro* studies, including immunoblotting, immunofluorescence, a cycloheximide chase study, Calbryte™ 520 Ca^{2+} detection, and half-maximal effective concentration (EC_{50}), were examined.

Results: This proband was found to carry a *de novo* heterozygous missense *I554N* in the cysteine-rich domain of *CASR*, which was pathogenic based on the different software prediction models and ACGME criteria. The simulation model showed that *CASR I554N* mutation decreased its binding energy with Ca^{2+}. Human *CASR I554N* mutation attenuated the stability of CASR protein, reduced the expression of p-ERK 1/2, and blunted the intracellular Ca^{2+} response to gradient extracellular Ca^{2+} (eCa^{2+}) concentration. The EC_{50} study also

demonstrated the correctable effect of calcimimetics on the function of the *CASR I554N* mutation.

Conclusion: This novel *CASR I554N* mutation causing FHH attenuates CASR stability, its binding affinity with Ca^{2+}, and the response to eCa^{2+} corrected by therapeutic calcimimetics.

KEYWORDS

calcium-sensing receptor, familial hypocalciuric hypercalcemia, calcimimetics, parathyroid hormone, half-maximal effective concentration

Introduction

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disorder characterized by a lifelong elevation of serum calcium (Ca^{2+}) level with hypocalciuria and inappropriately normal or high parathyroid hormone (PTH) concentrations (1, 2). Based on the causative genetic mutations, FHH type 1 (FHH1; OMIM #145980) caused by a heterozygous loss-of-function mutation in the Ca^{2+}-sensing receptor (*CASR*) gene is the most common and estimated to represent approximately 85-90% of all FHH cases, followed by the *AP2S1* gene (FHH3), at approximately 5-10%, and the *GNA11* gene (FHH2), at less than 5% (3–7). Although most FHH patients are usually asymptomatic or mildly symptomatic, e.g., fatigue, weakness, or thought disturbances (8), the increased risk of chronic kidney disease (CKD), coronary heart disease, pancreatitis, femoral fracture, and chondrocalcinosis with advancing age has been reported (9–11). Additionally, patients with FHH may be misdiagnosed as primary hyperparathyroidism, receiving an unnecessary parathyroidectomy, which typically does not successfully normalize hypercalcemia (12).

The majority of the inactivating mutations in the *CASR* gene are missense and are scattered throughout the protein sequence with some clustering in the first half of the extracellular domain (ECD) (venus flytrap (VFT) domain and closely associated with the Ca^{2+}-binding sites) and the latter part of the ECD (cysteine-rich region and parts of the transmembrane-spanning region) (Supplementary Figure 1) (13). With respect to treatment, calcimimetics act as positive allosteric modulators of the CASR that increases the receptor's response to extracellular Ca^{2+} (eCa^{2+}) levels (8), thus they have been used to treat symptomatic hypercalcemia in certain cases of FHH (14–16). To date, the use of calcimimetics in FHH is still a matter of debate, and the long-term effects of calcimimetic therapy in FHH are not yet fully understood (8). Considering the different mutant sites of the *CASR* gene for biodiversity, the comprehensive identification and characterization of *de novo* variants in CASR will provide valuable insights into the pathogenesis and elucidate the role of calcimimetics in the treatment of FHH (17).

We have encountered an adult FHH patient with moderate hypercalcemia, hypocalciuria, inappropriately high PTH levels, and progressive deteriorated renal function who had a good response to 25 mg oral cinacalcet daily for 6 months. In this study, our objective was to identify his responsible genetic mutation and assess the functional analysis of the identified mutation in relation to calcimimetic response. The results revealed a *de novo* heterozygous mutation (c.T1661A, *I554N*) in the cysteine-rich domain of the *CASR* gene responsible for his FHH. This missense mutation disrupted the binding of CASR I554N with eCa^{2+} in simulation models, leading to a decrease in the stability of CASR protein, a reduction in the expression of p-ERK 1/2, and a diminished response to eCa^{2+} concentration. The half-maximal effective concentration (EC_{50}) study also demonstrated the correctable effect with the use of calcimimetics.

Materials and methods

Human subjects

This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Institutional Review Board of the Tri-Service General Hospital (TSGH), National Defense Medical Center (TSGHIRB No.:A202105016). All methods were performed in accordance with approved guidelines. Written informed consent was obtained from the participants after a detailed description of the study.

Index case

The 45-year-old man was referred because of hypercalcemia of unknown causes for more than 5 years and a progressive decline in the estimated glomerular filtration rate (eGFR). There were no obvious symptoms related to hypercalcemia, but easy fatigue, constipation, and worsening renal function were noticed. Family and personal histories were unremarkable. The most striking laboratory abnormality was hypercalcemia (12.5 mg/dL; range, 8.6-10.2) with hypocalciuria (spot urine Ca^{2+}/Cr ratio 0.019 mg/mg, 24-hour urinary Ca^{2+} excretion 32 mg/day) and an inappropriately increased iPTH (65 pg/mL; range,

10.0-69.0) (Table 1). A sonography of the parathyroid gland and abdomen were normal. Under the diagnosis of FHH, an allosteric modulator of the CASR, oral cinacalcet (25 mg/day), was given. It reduced his serum Ca^{2+} concentration to 10.1 mg/dL and iPTH level to 25 pg/mL, coupled with an increased urine Ca^{2+} excretion (spot urine Ca^{2+}/Cr ratio of 0.06) and improved eGFR after the use of calcimimetic agent 6 months later.

Molecular screening of FHH and confirmation of CASR mutation

Peripheral blood was collected and deoxyribonucleic acid (DNA) extracted following a classic phenol–chloroform protocol (QIAamp Blood Kit; Qiagen, Dusseldorf, Germany). Molecular screening of the entire *CASR*, *GNA11*, and *AP2S1* coding sequences (18 exons–21 amplicons, including exon–intron boundaries) was performed. Furthermore, *CASR* mutation was confirmed by sequencing in both directions on the original amplicon and on a different polymerase chain reaction (PCR) product. Nine primer pairs were used to amplify exons 2–7 (which encode the receptor protein) of the CASR gene, as described previously (18). Forward and reverse primers were modified at their 5′-ends by the addition of a T7 or T3 promoter sequence, respectively, to aid in the subsequent nucleotide sequencing of the PCR product.

Simulation models of CASR mutation

The resolved structures of the extracellular domain of human CASR (PDB code: 5K5S and 5K5T) were used as a template to build the models of WT-CASR using the Homology Modeling protocol (Biovia Discovery Studio 2019). The simulation includes the loop refinement at a high optimization level. The mutant I554N model was generated using the Built Mutants protocol followed by energy minimization. The geometries of the models were optimized using the algorithm of smart minimization in the CHARMM force field including the generalized born implicit solvent model in the calculation.

Evaluating the effect of the change in mutation energy caused by mutations on the stability of CASR

AlphaFold is a computational method for predicting protein structures with atomic accuracy, even in cases in which no similar structure is known. Additionally, it can be used to evaluate and compare the similarity of these two predictive models and then utilize the results in additional advanced bioinformatic analyses. The effect of residue substitution on the stability of CASR was determined from the predictive Alphafold model using Discovery studio visualizer version v19.1.0.18287 (BIOVIA, San Diego, CA, USA). As for the calculation of the change in mutation, the energy is normalized to CASR-WT.

cDNA expression vectors and mutagenesis

The *CASR* variants of interest were introduced into a pCMV6 vector expressing Myc-DDK-tagged human WT CASR cDNA (RC211229, OriGene) through site-directed mutagenesis (QuikChange, Stratagene, La Jolla, CA, USA) and confirmed by

TABLE 1 Blood and urine data before and after cinacalcet treatment.

	Normal ranges	Before	After
Blood biochemistries			
BUN (mg/dL)	7-25	16	15
Cre (mg/dL)	0.7-1.2	1.1	0.8
eGFR (mL/min/1.73m^2)	100-120	76	111
Sodium (mmol/L)	136-145	139	138
Potassium (mmol/L)	3.5-5.1	4.3	4.1
Chloride (mmol/L)	98-107	102	104
Total Ca^{2+} (mg/dL)	8.6-10.2	12.5	10.1
P (mg/dL)	2.7-4.5	2.6	2.8
Mg^{2+} (mg/dL)	1.7-2.55	2.3	2.2
iPTH (pg/mL)	10.0-69.0	65	25
Urine biochemistries			
Ca^{2+}/Cr (mg/mg)		0.019	0.060
$FE_{Ca}{}^{2+}$ (%)		0.17	0.48

BUN, blood urea nitrogen; Cre, creatinine; Ca^{2+}, calcium; P, inorganic phosphorus; Mg^{2+}, magnesium; iPTH, intact parathyroid hormone; $FE_{Ca}{}^{2+}$ represents the fractional excretion of Ca^{2+}.

DNA sequencing analysis. Briefly, the mutagenesis reaction was carried out to generate mutant pCMV6-CASR **I554N**-Myc-DDK and pCMV6-CASR **R220W**-Myc-DDK constructs using the following primers: p. I554N, For: 5′-GCAGGGACCAGGAAAGGG **AAC**ATTGAGGGGGAGCCCACC-3′ and Rev: 5′-GGTGGGCT CCCCCTCAATGTTCCCTTTCCTGGTCCCTGC-3′; p. R220W, For: 5′- GCTGATGACGACTATGGG**TGG**CCGGGGATTGAGA AATTC-3′ and Rev: 5′- GAATTTCTCAATCCCCGGCCACCCAT AGTCGTCATCAGC-3′, the mutated bases are underlined).

Cell culture, plasmid transfection, and protein stability assay

HEK-293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37°C in a humidified 5% CO_2 incubator. HEK-293 cells (4×10^5 cells per 6-well plate) were transfected with the indicated amount of plasmid DNA using Lipofectamine 3000 reagent (Invitrogen). For each transfection, 5 μg of expression vectors was used, and the total amount of plasmid DNA was adjusted by adding empty vectors. Cells were visualized using a fluorescence microscope (Carl Zeiss, Inc., Oberkochen, Germany) with an epifluorescence filter, and images were captured using Openlab software (Improvision Inc. Lexington, MA, USA).

For protein stability analysis, transfected cells were treated with 50 mg/mL cycloheximide (CHX) for the indicated time and harvested for IB, which was performed using mouse anti-Myc monoclonal antibody (TA150121-1; OriGene). Phospho-ERK1/2 (Thr202 and Tyr204) antibody (#9101; Cell Signaling Technology) was used for IB.

Fluorescence measurements of iCa2+ in the whole cell population

The iCa^{2+} was measured in CASR-expressing HEK293 cells (approximately 5×10^5 cells/ml for each experiment). HEK293 cells were loaded with 10 mM Calbryte™ 520 (NC1424566; Fisher Scientific), incubated for 120 min in a 5% CO_2 incubator at 37°C, as described previously (19). The dye loading solution was removed, and fresh cell culture medium was added to the plate. To study the response of mutant CASR variants to eCa^{2+} stimulation, the eCa^{2+} was increased stepwise by the addition of $CaCl_2$ at concentrations between 0 mmol/l and 10.0 mmol/l. The cells were collected at the indicated eCa^{2+} concentrations and washed once with Ca^{2+}- and magnesium-free Hanks' balanced salt solution (HBSS) (Invitrogen) before analysis.

For the *in vitro* rescue study, the calcimimetic NPS R-568 (SI-SML2160; Sigma-Aldrich) was added at a concentration range of 1.0 to 100 μmol/l in the presence of the same $CaCl_2$ concentration (0.6 mmol/l) (20). The iCa^{2+} was calculated from the ratio of the fluorescence emission recorded at the two-excitation wavelengths (19). The EC_{50} (i.e., iCa^{2+} required for 50% of the maximal response) for each normalized concentration–response curve was determined.

Statistical analysis

The results were presented as the mean ± standard deviation (SD) for continuous variables. Comparisons between groups were carried out using the Wilcoxon test or $\chi 2$ test as appropriate. The mean EC_{50} was calculated, and statistical analysis performed using the Mann–Whitney U test. All analyses were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL). A *p*-value less than 0.05 was considered statistically significant.

Results

Identification of a novel *CASR I554N* mutation

Direct sequence analysis of the relevant genes, including *CASR*, *GNA11*, and *AP2S1*, revealed a heterozygous missense T>A nucleotide substitution in exon 6 of the *CASR* gene at codon 1661. This substitution resulted in an amino acid change from isoleucine to asparagine (ATC to AAC, *I554N*) (Figures 1A, B). The missense *I554N* mutation was located in the cysteine-rich (CR) domain (residues 542–612) of the C-terminus of *CASR*. The mutated residue (*I554*) is highly conserved across different species (Figure 1C) and was not inherited from his parents. The *de novo I554N* mutation was not identified in 1517 healthy Taiwanese subjects according to the Taiwan Biobank database. Furthermore, it was predicted to be a pathogenic variant based on PROVEAN and Polyphen-2 scores.

The effects of *CASR I554N* mutation on binding energy

According to The Human Gene Mutation Database in April 2023, 399 *CASR* mutations causing FHH1 have been reported, with the majority (88.1%) being missense/nonsense mutations. However, the pathogenic mechanism of the newly identified missense *CASR I554N* mutation has not been thoroughly investigated *in vitro*, and the therapeutic response to calcimimetic treatment has not been validated in affected patients. Fundamentally, *CASR I554* was localized in the CR region (Figure 1D; Supplementary Figure 1), which may impact CASR signal transduction (21). In line with this, the simulation model predicted that the *I554N* mutation affects the binding energy between CASR and Ca^{2+} (*WT*, -720 kcal/mol vs. *I554N*, -664 kcal/mol) (Figure 2). The impact of FHH1-associated mutations on the stability of our predicted CSB structures was calculated using BIOVIA Discovery Studio 2019 software. As for the calculation of the change in mutation, the corresponding mutation energy was normalized by CASR-WT protein. The result showed that *I554N* mutation exerted a destabilizing effect on Alphafold's model (Figure 2).

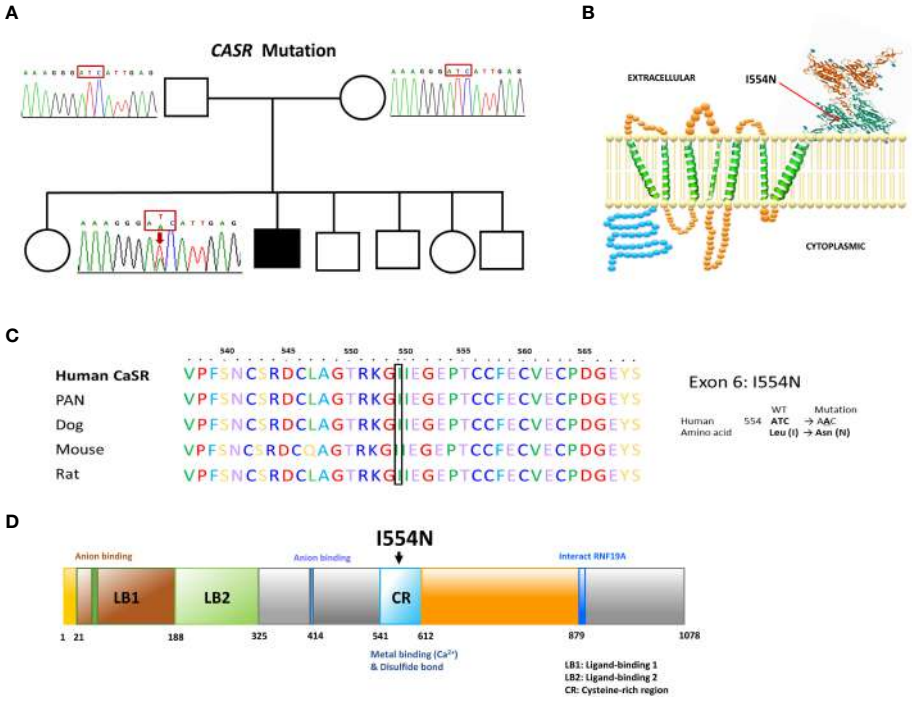

FIGURE 1
Our FHH1 patient with a *de novo I544N* mutation localized the cysteine-rich region of CASR. **(A)** Pedigree of the family. The squares and circles indicate male and female, respectively. The black shading indicates the affected FHH1 patient. Sanger sequencing identified the heterozygous *de novo* mutation in *CASR*. The arrow indicates the mutation site (c.T1661A, *p.I554N*). **(B)** Schematic representation of human CASR structure and the corresponding site of *I554N* mutation (exon 6). **(C)** Conservation of the local amino acid sequence by multiple alignment of CASR from different species; *I554* is a highly conserved position. **(D)** *CASR I554N* mutation localized in the cysteine-rich region, which was critical for Ca^{2+} binding.

The instability of *CASR I554N* protein and decreased p-ERK1/2 levels

To substantiate the above prediction, we generated mutant *CASR* constructs of interest for subsequent *in vitro* functional studies, and they expressed well in HEK-293 cells (Supplementary Figure 2). The time-course of cycloheximide (CHX) chase analysis showed that CASR I554N protein was more unstable than CASR-WT (Figures 3A, B). In addition, we selected the previously reported *CASR R220W* mutation as a positive control. The R220 residue is positioned within the VFT domain of the CASR and is crucial for ligand binding and receptor activation (22). It has been demonstrated that the *CASR R220W* mutation hinders the normal conformational changes in the VFT domain upon Ca^{2+} binding, leading to decreased sensitivity to eCa^{2+} levels and impairing the transduction of Ca^{2+} signaling (22). In comparison with CASR-WT, the levels of CASR and p-ERK1/2 proteins, both basal and Ca^{2+}-stimulated, were decreased in the *CASR I554N* and *CASR R220W* mutations, indicating the abrogation of the MAPK pathway (Figures 3C, D).

Blunt iCa^{2+} response to eCa^{2+} in the *CASR I554N* mutation

CASR-WT showed a brisk intracellular Ca^{2+} (iCa^{2+}) response after the eCa^{2+} concentration was slightly increased (0.2 mM), and a plateaued iCa^{2+} response was noted when eCa^{2+} reached 1.0 mM (Figure 4). In contrast, both the *CASR I554N* mutation and inactivating *CASR R220W* mutation exhibited a blunt response to the addition of eCa^{2+}, indicating the inactivating function of the *CASR I554N* mutant.

Calcimimetics rescue CASR function impaired by the *I554N* mutation

As the simulation model showed that *I554* is localized in the CR region, the issue of whether calcimimetics (positive allosteric modulators) can rescue the dysfunction of *CASR I544N* should be clarified. Therefore, a fluorescence-based assay of Calbryte™ 520 was used to detect iCa^{2+}. HEK-*CASR* WT and HEK-*CASR I554N* cells were stimulated with increasing concentrations of calcimimetic NPS R-568 (ranging from 1.0 to 100 μmol/l, Figure 5) in the presence of the same $CaCl_2$ concentration (0.6 mmol/l). There was no significant difference in NPS R-568-EC_{50} between HEK-*CASR I554N* and HEK-CASR WT cells (NPS R-568-EC_{50}: 10.02± 0.27 μmol/l vs. 7.10 ± 0.18 μmol/l, respectively), indicating calcimimetics can correct CASR function impaired by the *I554N* mutation. These findings also support the clinical presentation of our case, which showed an excellent therapeutic response to cinacalcet and the rapid correction of hypercalcemia within 6 months.

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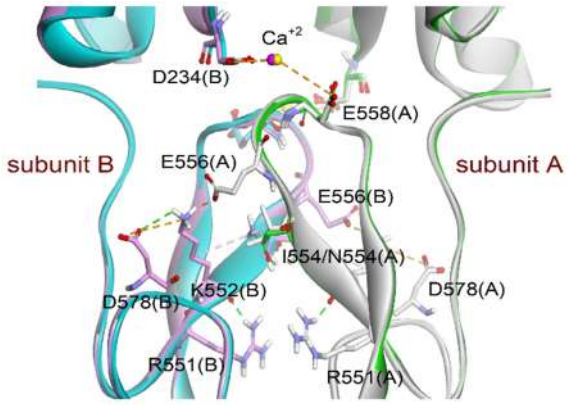

Calculated binding energy of CBS module of CASR with Ca^{2+}		
CASR	**Binding energy (kcal/mol)**	
Wild-type	-720	
I554N	-664	
The effect of mutant CASR on the its stability*		
CASR	**Mutation energy (Stability) (kcal/mol)**	**Effect**
I554N	1.93	Destabilizing

* Mutation on the stability of human CSB protein was calculated using the predicated model from AlphaFold.

FIGURE 2
Simulation of the CASR models (PDB codes: 5K5S and 5K5T). Superimposition of wild-type (WT) and mutant CASR *I554N* models. The dimeric proteins were presented as a ribbon model and colored white and green for subunit A and pink and cyan for subunit B in WT and mutant CASR, respectively. The residues involved in interactions are shown as a stick model. The magenta and yellow spheres represent the calcium ions in WT CASR and mutant CASR, respectively. The hydrogen bond and electrostatic and hydrophobic interactions are shown as dashed green, orange, and pink lines, respectively. The *CASR I554N* mutation showed decreased binding ability to eCa^{2+} and its stability based on the CBS module and AlphaFold model.

Discussion

The main findings regarding the *CASR I554N* mutation

A *de novo* heterozygous *CASR I554N* mutation was identified in our FHH1 patient. Simulation models showed decreased binding energy between the mutant *CASR I554N* and Ca^{2+}, supporting pathogenic predictions based on PROVEAN and PolyPhen-2 scores. The *CASR I554N* mutation exhibited protein instability and reduced pERK1/2 expression, suggesting it could abrogate the MAPK pathway (Figure 6). The notion of defective CASR I554N protein was further supported by a decreased iCa^{2+} response to the gradient eCa^{2+} concentrations in a Calbryte 520 staining study. *In vitro* EC_{50} analysis revealed that the mutant *CASR I544N* responded well to the calcimimetic compound NPS R-568, restoring its function to a level comparable with *CASR WT*. These findings consistently demonstrated that our patient carrying the *CASR I554N* mutation presented with a dramatic response of cinacalcet to correct hypercalcemia in a short time.

Decreased expression and stability of CASR and disruption of the downstream MAPK pathway

In vitro study showed that the *I554N* mutation contributed to the decreased CASR expression and the instability of CASR protein. It was reported that approximately 50% of the *CASR* mutations associated with FHH1 can reduce CASR expression due to defective trafficking to the plasma membrane (5, 15, 23). In short, mutant CASR is often retained intracellularly and is unable to exit the endoplasmic reticulum or Golgi apparatus (5, 15, 23). These previous findings align with our results, which demonstrate that the I554N mutation attenuates CASR protein expression and its downstream substrate phosphorylated ERK1/2, leading to a reduction in CASR function through the MAPK pathway (15). In addition to unstable expression, whether *CASR I554N* mutation can affect the binding affinity with eCa^{2+} should be investigated to gain new insight into the molecular mechanism of FHH1.

The *I554N* mutation affected CASR dimerization and decreased the binding affinity to eCa^{2+}

The human CASR is a dimeric cell-surface protein consisting of 1078 amino acids (5). It has a large ECD comprising 612 amino acids, which forms two globular lobes adopting a venus flytrap (VFT) conformation (5). A previous study examining FHH1-causing CASR mutations found that these mutations tend to cluster around the predicted calcium-binding sites, primarily located within the cleft region of the bilobed VFT domain (3). These mutated residues can directly disrupt the binding of eCa^{2+} or indirectly affect the conformational changes that occur upon eCa2+ binding, ultimately leading to the impairment of intracellular signaling cascades (3, 24–26). In our simulation model, the *CASR I554* mutation was observed to impact the dimerization structure of CASR. Additionally, the *CASR I554* mutation is situated in a cysteine-rich (CR) region, which potentially serves as an intramolecular switch regulating the entry and binding of eCa^{2+}. Consistent with this notion, our *in vitro* study demonstrated that the *CASR I554N* variant exhibited a diminished response to increased eCa^{2+} concentrations, similar to the previously identified inactivating *CASR R220W* mutation (1).

Limited calcimimetic functional study of missense CASR mutations

To date, FHH1 has been associated with 399 different mutations of *CASR*, with missense substitutions accounting for over 85% of cases, while nonsense, deletion, insertion, and splice-site mutations leading to truncated CASR proteins have been reported in less than 15% of cases (12, 27). Furthermore, *CASR* mutations have been found to cluster in three regions: the second peptide loop of the

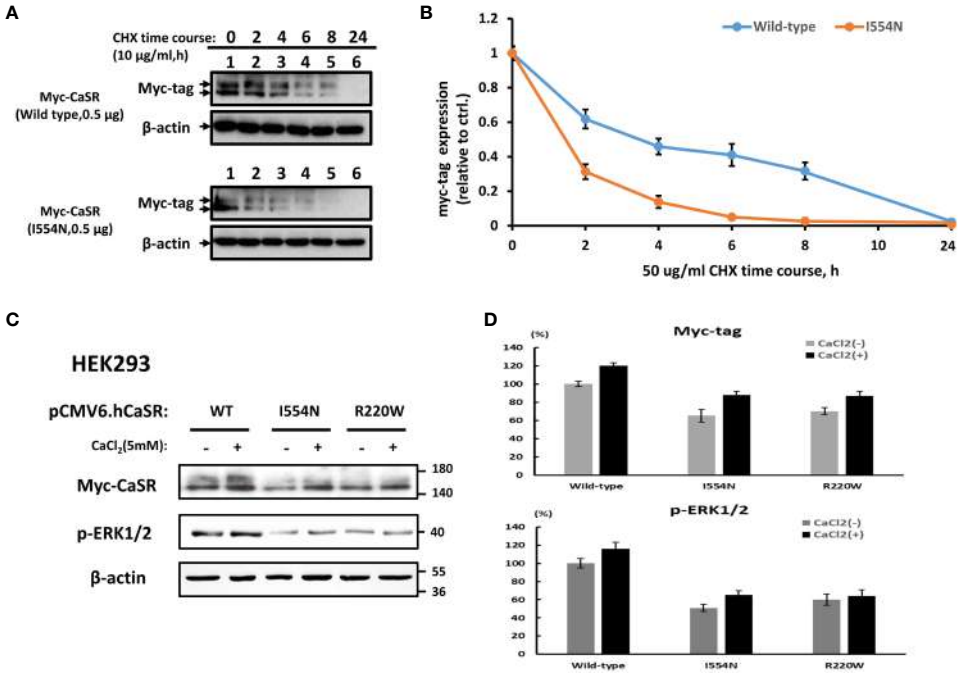

FIGURE 3
Analysis of the stability of CASR proteins and downstream p-ERK1/2 levels. A cycloheximide (CHX) chase study. **(A)** CASR protein expression was examined at different time points in HEK-293 cells transfected with CASR-**WT**-Myc and CASR-**I554N**-Myc vectors. **(B)** Representative quantified data expressed as the CASR/β-actin ratio. Results were normalized to β-actin control levels and expressed as the ratio change based on the level at time zero, which was set to 1.0. **(C, D)** Representative immunoblots and densitometry plots. **(C)** Representative IB analyses of the levels of CASR and p-ERK1/2 proteins in the HEK-293 cells transfected with CASR-**WT**-Myc, CASR-**I554N**-Myc, and CASR-**R220W**-Myc (inactivating CASR mutant: positive control) vectors. Compared with CASR-WT, the *CASR I554N* and *CASR R220W* mutations exhibited reduced CASR and p-ERK1/2 protein levels under basal and Ca^{2+}-stimulated conditions. **(D)** The densitometry plots reflect the results of semi-quantification by densitometry (expressed as percentages and means ± SDs). Mouse Anti-Myc monoclonal antibody (TA150121-1; OriGene) was used to detect CASR expression in **(A, C)**.

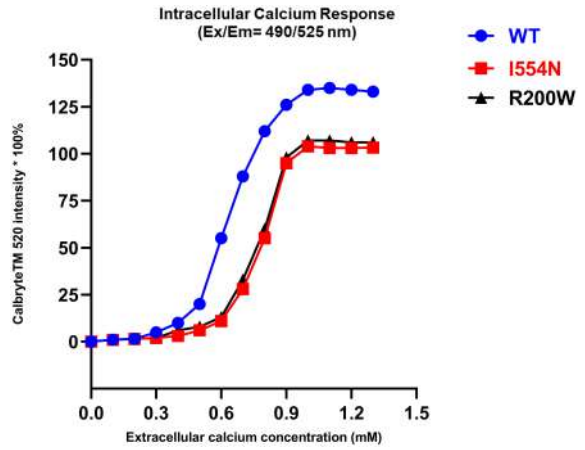

FIGURE 4
The intracellular calcium response in various CASR mutations. Calbryte™ 520 (Ex/Em=490/525 nm) is a new fluorescent and cell-permeable calcium indicator, which produces a bright fluorescence signal in the presence of iCa^{2+} at a high concentration. In contrast to CASR-WT, the CASR I554N mutation and the inactivating CASR R220W mutation exhibited a diminished response of iCa^{2+} to the increase in eCa^{2+} concentration.

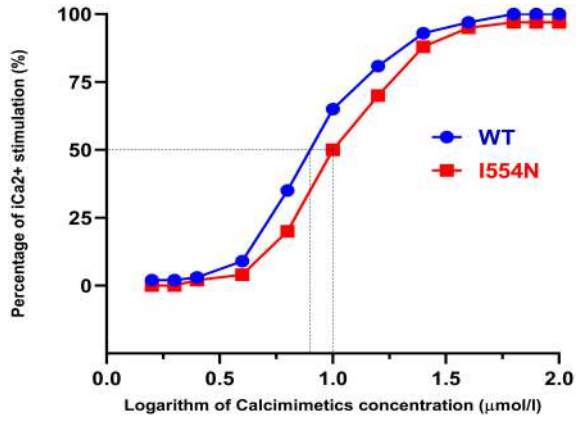

FIGURE 5
Response to calcimimetic NPS R-568 in CASR-WT and CASR-I554N. Calbryte™ 520 intensity [Percentage of iCa^{2+}stimulation (%)] was examined at different calcimimetic NPS R-568 concentrations in HEK-293 cells transfected with CASR-**WT**-Myc and CASR-**I554N**-Myc vectors. HEK-WT and HEK-I554N cells were loaded with Calbryte™ 520 and stimulated by increasing NPS R-568 concentrations in the presence of 0.6 mmol/l $CaCl_2$. The response was normalized and the percentage of iCa^{2+} stimulation was plotted against the logarithm of NPS R-568 concentrations. The dotted lines indicate the logarithmic values of NPS R-568 EC_{50}.

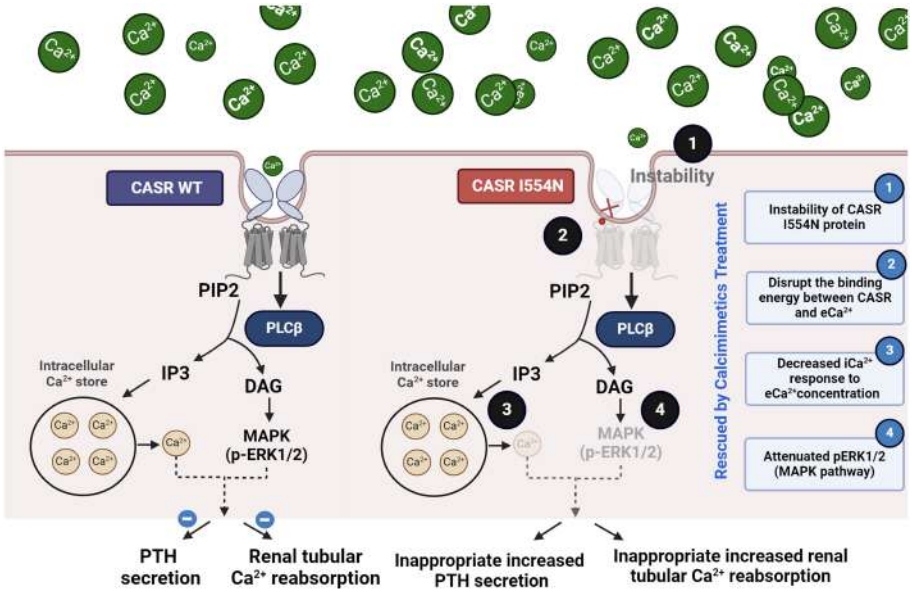

FIGURE 6
Summary of the mechanism of the pathogenic *CASR I554N* mutation.

ECD, the VFT cleft region (eCa^{2+}_o-binding site), and the region encompassing transmembrane domains (TMD) 6 and 7 (5, 12). Intriguingly, FHH1 patients with *CASR* mutations in different domains exhibit distinct and variable phenotypic severities (1, 11). Although one *in vitro* study showed that calcimimetics can correct the expression in some *CASR* mutations (11), research on personalized treatment using calcimimetics for different CASR variants is limited (28–30). In addition, cinacalcet-unresponsive patients might harbor the missense mutations or in-frame deletions of *CASR* exon 5 encoding amino acids 460-536 in the extracellular domain (ECD) (31, 32), indicating that the different CASR mutations are likely responsible for the biodiversity in calcimimetics treatment. A better understanding of the effect of calcimimetics on heterogeneous FHH1 patients could greatly contribute to the development of novel therapeutics targeting the CASR-regulated MAPK pathway (33–36).

A calcimimetic restored *CASR I554N* dysfunction in an EC_{50} study

Although FHH1 is considered the least severe form of FHH (3, 5–7), it still can result in unfavorable CKD or severe hypercalcemia associated with nephrolithiasis, for which surgical treatment is ineffective (9). To prevent these complications, calcimimetics may act as pharmacochaperones and provide a promising treatment option for FHH1 patients. They promote proper folding and/or increase the plasma membrane targeting of CASR mutants, as well as activate the CASR signaling pathway (MAPK pathway) (14, 23, 37–41). Of note, our *in vitro* study demonstrated that the EC_{50} value of NPS R-568 for the *CASR I554N* variant was similar to that of *CASR WT*, indicating that the calcimimetic corrected the dysfunction of the *CASR I554N* mutant by increasing iCa^{2+} mobilization (28). Although *CASR* mutations can exhibit different responses to calcimimetics due to the diverse types and locations of mutant variants in affected individuals, our study showed an excellent therapeutic response to the calcimimetic in our FHH1 patient. This suggests the calcimimetic could restore the function of the missense *CASR I554N* mutation localized in the CR region to a certain extent.

Conclusion

Our study identified a *de novo* heterozygous pathogenic *CASR I554N* mutation that decreased CASR protein expression and stability, impaired binding to eCa^{2+}, and attenuated pERK1/2 expression. The calcimimetics effectively corrected these dysfunctions *in vitro*. These findings have significant implications for FHH1 patients with CR region mutations, offering a practical approach to modulate CASR signal transduction.

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.

Author contributions

C-ML: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project

administration, Writing – original draft. Y-XD: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. S-MH: Supervision, Writing – review & editing. Y-CC: Supervision, Writing – review & editing. H-JL: Supervision, Writing – review & editing. C-CS: Writing – review & editing. S-HL: Supervision, Writing – review & editing.

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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.2024.1291160/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Summary of CASR pathogenic variants and corresponding locations among FHH1 patients. **(A)** The percentage of reported pathogenic variants in CASR, categorized by mutation types. **(B)** The percentage of missense/nonsense mutations in CASR, categorized by location. **(C)** The missense/nonsense mutations in CASR plotted according to their corresponding locations.

SUPPLEMENTARY FIGURE 2
Validation of mutant CASR construct transfection. Representative immunofluorescence micrographs of CASR proteins in the HEK-293 cells transfected with pCMV6-AN-mRFP-vector (negative control), pCMV6-CASR-**WT**-Myc (RC211229, OriGene), pCMV6-CASR-**I554N**-Myc, and pCMV6-CASR-**R220W**-Myc (inactivating CASR R220W: positive control). WT-CASR and mutant CASR constructs all expressed well in HEK-293 cells. Original magnification 400× **(A)** and 1000× **(B)**. Scale bars = 10 μm.

References

1. Mayr B, Schnabel D, Dörr HG, Schöfl C. GENETICS IN ENDOCRINOLOGY: Gain and loss of function mutations of the calcium-sensing receptor and associated proteins: current treatment concepts. *Eur J Endocrinol*. (2016) 174(5):R189–208. doi: 10.1530/EJE-15-1028

2. Hannan FM, Kallay E, Chang W, Brandi ML, Thakker RV. The calcium-sensing receptor in physiology and in calcitropic and noncalcitropic diseases. *Nat Rev Endocrinol*. (2018) 15(1):33–51. doi: 10.1038/s41574-018-0115-0

3. Hannan FM, Nesbit MA, Zhang C, Cranston T, Curley AJ, Harding B, et al. Identification of 70 calcium-sensing receptor mutations in hyper- and hypo-calcaemic patients: evidence for clustering of extracellular domain mutations at calcium-binding sites. *Hum Mol Genet*. (2012) 21(12):2768–78. doi: 10.1093/hmg/dds105

4. Shinall MC Jr., Dahir KM, Broome JT. Differentiating familial hypocalciuric hypercalcemia from primary hyperparathyroidism. *Endocrine Pract Off J Am Coll Endocrinol Am Assoc Clin Endocrinologists*. (2013) 19(4):697–702. doi: 10.4158/EP12284.RA

5. Hannan FM, Babinsky VN, Thakker RV. Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Mol Endocrinol*. (2016) 57(3):R127–142. doi: 10.1530/JME-16-0124

6. Nissen PH, Christensen SE, Heickendorff L, Brixen K, Mosekilde L. Molecular genetic analysis of the calcium sensing receptor gene in patients clinically suspected to have familial hypocalciuric hypercalcemia: phenotypic variation and mutation spectrum in a Danish population. *J Clin Endocrinol Metab*. (2007) 92(11):4373–9. doi: 10.1210/jc.2007-0322

7. Guarnieri V, Canaff L, Yun FH, Scillitani A, Battista C, Muscarella LA, et al. Calcium-sensing receptor (CASR) mutations in hypercalcemic states: studies from a single endocrine clinic over three years. *J Clin Endocrinol Metab*. (2010) 95(4):1819–29. doi: 10.1210/jc.2008-2430

8. Marx SJ. Calcimimetic use in familial hypocalciuric hypercalcemia-A perspective in endocrinology. *J Clin Endocrinol Metab*. (2017) 102(11):3933–6. doi: 10.1210/jc.2017-01606

9. Stratta P, Merlotti G, Musetti C, Quaglia M, Pagani A, Izzo C, et al. Calcium-sensing-related gene mutations in hypercalcaemic hypocalciuric patients as differential diagnosis from primary hyperparathyroidism: detection of two novel inactivating mutations in an Italian population. *Nephrol Dial Transplant*. (2014) 29(10):1902–9. doi: 10.1093/ndt/gfu065

10. Gorvin CM, Cranston T, Hannan FM, Rust N, Qureshi A, Nesbit MA, et al. A G-protein subunit-α11 loss-of-function mutation, Thr54Met, causes familial hypocalciuric hypercalcemia type 2 (FHH2). *J Bone Miner Res*. (2016) 31(6):1200–6. doi: 10.1002/jbmr.2778

11. Josephs TM, Zhang F, Dinh LV, Keller AN, Conigrave AD, Capuano B, et al. Personalised medicines for familial hypercalcemia and hyperparathyroidism. *J Mol Endocrinol*. (2022) 69(1):243–57. doi: 10.1530/JME-21-0263

12. Hannan FM, Thakker RV. Calcium-sensing receptor (CaSR) mutations and disorders of calcium, electrolyte and water metabolism. *Best Pract Res Clin Endocrinol Metab*. (2013) 27(3):359–71. doi: 10.1016/j.beem.2013.04.007

13. Thompson MD, Hendy GN, Percy ME, Bichet DG, Cole DE. G protein-coupled receptor mutations and human genetic disease. *Methods Mol Biol*. (2014) 1175:153–87. doi: 10.1007/978-1-4939-0956-8_8

14. Reh CM, Hendy GN, Cole DE, Jeandron DD. Neonatal hyperparathyroidism with a heterozygous calcium-sensing receptor (CASR) R185Q mutation: clinical benefit from cinacalcet. *J Clin Endocrinol Metab*. (2011) 96(4):E707–712. doi: 10.1210/jc.2010-1306

15. Grant MP, Stepanchick A, Breitwieser GE. Calcium signaling regulates trafficking of familial hypocalciuric hypercalcemia (FHH) mutants of the calcium sensing receptor. *Mol Endocrinol (Baltimore Md)*. (2012) 26(12):2081–91. doi: 10.1210/me.2012-1232

16. Tennakoon S, Aggarwal A, Kallay E. The calcium-sensing receptor and the hallmarks of cancer. *Biochim Biophys Acta*. (2016) 1863(6 Pt B):1398–407. doi: 10.1016/j.bbamcr.2015.11.017

17. Gorvin CM. Recent advances in calcium-sensing receptor structures and signaling pathways. *Prog Mol Biol Transl Sci*. (2023) 195:121–35. doi: 10.1016/bs.pmbts.2022.06.014

18. D'Souza-Li L, Yang B, Canaff L, Bai M, Hanley DA, Bastepe M, et al. Identification and functional characterization of novel calcium-sensing receptor mutations in familial hypocalciuric hypercalcemia and autosomal dominant hypocalcemia. *J Clin Endocrinol Metab*. (2002) 87(3):1309–18. doi: 10.1210/jc.87.3.1309

19. Liao J, Patel D, Zhao Q, Peng R, Guo H, Diwu Z. A novel Ca(2+) indicator for long-term tracking of intracellular calcium flux. *Biotechniques*. (2021) 70(5):271–7. doi: 10.2144/btn-2020-0161

20. Nemeth EF, Steffey ME, Hammerland LG, Hung BC, Van Wagenen BC, DelMar EG, et al. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *Proc Natl Acad Sci USA*. (1998) 95(7):4040–5. doi: 10.1073/pnas.95.7.4040

21. Liu H, Yi P, Zhao W, Wu Y, Acher F, Pin JP, et al. Illuminating the allosteric modulation of the calcium-sensing receptor. *Proc Natl Acad Sci USA*. (2020) 117 (35):21711–22. doi: 10.1073/pnas.1922231117

22. Schamber MR, Vafabakhsh R. Mechanism of sensitivity modulation in the calcium-sensing receptor via electrostatic tuning. *Nat Commun*. (2022) 13(1):2194. doi: 10.1038/s41467-022-29897-y

23. White E, McKenna J, Cavanaugh A, Breitwieser GE. Pharmacochaperone-mediated rescue of calcium-sensing receptor loss-of-function mutants. *Mol Endocrinol (Baltimore Md)*. (2009) 23(7):1115–23. doi: 10.1210/me.2009-0041

24. Huang Y, Zhou Y, Castiblanco A, Yang W, Brown EM, Yang JJ. Multiple Ca(2 +)-binding sites in the extracellular domain of the Ca(2+)-sensing receptor corresponding to cooperative Ca(2+) response. *Biochemistry*. (2009) 48(2):388–98. doi: 10.1021/bi8014604

25. He Y, Han L, Li W, Shu X, Zhao C, He Y, et al. Effects of the calcium-sensing receptor A986S polymorphism on serum calcium and parathyroid hormone levels in healthy individuals: a meta-analysis. *Gene*. (2012) 491(2):110–5. doi: 10.1016/j.gene.2011.10.017

26. O'Seaghdha CM, Yang Q, Glazer NL, Leak TS, Dehghan A, Smith AV, et al. Common variants in the calcium-sensing receptor gene are associated with total serum calcium levels. *Hum Mol Genet*. (2010) 19(21):4296–303. doi: 10.1093/hmg/ddq342

27. Zung A, Barash G, Banne E, Levine MA. Novel calcium-sensing receptor (CASR) mutation in a family with autosomal dominant hypocalcemia type 1 (ADH1): genetic study over three generations and clinical characteristics. *Horm Res Paediatr*. (2023) 96 (5):473-82. doi: 10.1159/000529833

28. Nemeth EF, Heaton WH, Miller M, Fox J, Balandrin MF, Van Wagenen BC, et al. et al: Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. *J Pharmacol Exp Ther*. (2004) 308(2):627–35. doi: 10.1124/jpet.103.057273

29. Leach K, Gregory KJ, Kufareva I, Khajehali E, Cook AE, Abagyan R, et al. Towards a structural understanding of allosteric drugs at the human calcium-sensing receptor. *Cell Res*. (2016) 26(5):574–92. doi: 10.1038/cr.2016.36

30. Rothe HM, Shapiro WB, Sun WY, Chou SY. Calcium-sensing receptor gene polymorphism Arg990Gly and its possible effect on response to cinacalcet HCl. *Pharmacogenetics Genomics*. (2005) 15(1):29–34. doi: 10.1097/01213011-200501000-00005

31. Capozza M, Chinellato I, Guarnieri V, Di Lorgi N, Accadia M, Traggiai C, et al. Case report: acute clinical presentation and neonatal management of primary hyperparathyroidism due to a novel CaSR mutation. *BMC Pediatr*. (2018) 18(1):340. doi: 10.1186/s12887-018-1319-0

32. Murphy H, Patrick J, Báez-Irizarry E, Lacassie Y, Gómez R, Vargas A, et al. Neonatal severe hyperparathyroidism caused by homozygous mutation in CASR: A rare cause of life-threatening hypercalcemia. *Eur J Med Genet*. (2016) 59(4):227–31. doi: 10.1016/j.ejmg.2016.02.001

33. Glaudo M, Letz S, Quinkler M, Bogner U, Elbelt U, Strasburger CJ, et al. Heterozygous inactivating CaSR mutations causing neonatal hyperparathyroidism: function, inheritance and phenotype. *Eur J Endocrinol*. (2016) 175(5):421–31. doi: 10.1530/EJE-16-0223

34. Cavanaugh A, Huang Y, Breitwieser GE. Behind the curtain: cellular mechanisms for allosteric modulation of calcium-sensing receptors. *Br J Pharmacol*. (2012) 165(6):1670–7. doi: 10.1111/j.1476-5381.2011.01403.x

35. Huang Y, Cavanaugh A, Breitwieser GE. Regulation of stability and trafficking of calcium-sensing receptors by pharmacologic chaperones. *Adv Pharmacol (San Diego Calif)*. (2011) 62:143–73. doi: 10.1016/B978-0-12-385952-5.00007-5

36. Goolam MA, Brown AP, Edwards KT, Gregory KJ, Leach K, Conigrave AD. Cell surface calcium-sensing receptor heterodimers: mutant gene dosage affects ca(2+) sensing but not G protein interaction. *J Bone Miner Res*. (2022) 37(9):1787–807. doi: 10.1002/jbmr.4651

37. Huang Y, Breitwieser GE. Rescue of calcium-sensing receptor mutants by allosteric modulators reveals a conformational checkpoint in receptor biogenesis. *J Biol Chem*. (2007) 282(13):9517–25. doi: 10.1074/jbc.M609045200

38. Rus R, Haag C, Bumke-Vogt C, Bahr V, Mayr B, Mohlig M, et al. Novel inactivating mutations of the calcium-sensing receptor: the calcimimetic NPS R-568 improves signal transduction of mutant receptors. *J Clin Endocrinol Metab*. (2008) 93 (12):4797–803. doi: 10.1210/jc.2008-1076

39. Lu JY, Yang Y, Gnacadja G, Christopoulos A, Reagan JD. Effect of the calcimimetic R-568 [3-(2-chlorophenyl)-N-((1R)-1-(3-methoxyphenyl)ethyl)-1-propanamine] on correcting inactivating mutations in the human calcium-sensing receptor. *J Pharmacol Exp Ther*. (2009) 331(3):775–86. doi: 10.1124/jpet.109.159228

40. Wilhelm-Bals A, Parvex P, Magdelaine C, Girardin E. Successful use of bisphosphonate and calcimimetic in neonatal severe primary hyperparathyroidism. *Pediatrics*. (2012) 129(3):e812–6. doi: 10.1542/peds.2011-0128

41. Leach K, Wen A, Cook AE, Sexton PM, Conigrave AD, Christopoulos A. Impact of clinically relevant mutations on the pharmacoregulation and signaling bias of the calcium-sensing receptor by positive and negative allosteric modulators. *Endocrinology*. (2013) 154(3):1105–16. doi: 10.1210/en.2012-1887

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Pradeep Kumar Dabla,
G B Pant Institute of Postgraduate Medical Education and Research (GIPMER), India
Benjamin Udoka Nwosu,
Hofstra University, United States

*CORRESPONDENCE
Tuanyu Fang
fangtuanyu_2010@163.com
Yangli He
1669413518@qq.com

†These authors share first authorship

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The impact of vitamin D on the etiopathogenesis and the progression of type 1 and type 2 diabetes in children and adults

Candong Li[1†], Jiaowen Fu[1†], Yipeng Ye[1†], Junsen Li[1], Yangli He[2*] and Tuanyu Fang[1*]

[1]Department of Endocrine, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China, [2]Department of Health Care Centre, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, China

Diabetes is a common chronic metabolic disease with complex causes and pathogenesis. As an immunomodulator, vitamin D has recently become a research hotspot in the occurrence and development of diabetes and its complications. Many studies have shown that vitamin D can reduce the occurrence of diabetes and delay the progression of diabetes complications, and vitamin D can reduce oxidative stress, inhibit iron apoptosis, promote Ca^{2+} influx, promote insulin secretion, and reduce insulin resistance. Therefore, the prevention and correction of vitamin D deficiency is very necessary for diabetic patients, but further research is needed to confirm what serum levels of vitamin D_3 are maintained in the body. This article provides a brief review of the relationship between vitamin D and diabetes, including its acute and chronic complications.

KEYWORDS

vitamin D, diabetes mellitus, diabetic complications, insulin resistance, pathogenesis

1 Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia, caused by defects in insulin secretion and/or utilization. Over the past 30 years, the prevalence of diabetes in China has significantly increased. A thyroid iodine nutrition status and diabetes epidemiological survey conducted by the Endocrinology Branch of the Chinese Medical Association from 2015 to 2017 showed that the prevalence of diabetes among the Chinese population aged 18 and above was 11.2% (1). Vitamin D, a fat-soluble vitamin, not only plays a role in calcium and phosphorus regulation but is also closely related to diabetes, cardiovascular diseases, immune regulation, tumors, muscle

strength enhancement, and fall prevention. Studies have shown that vitamin D is closely related to pancreatic function, immunity, genetic polymorphism, and the occurrence of diabetic complications. In recent years, the relationship between vitamin D and the development of diabetes has become a research focus in the field of diabetes. This article provides a brief overview of the effects of vitamin D on the pathogenesis and progression of complications in different types of diabetes.

2 Overview of vitamin D

Vitamin D, a derivative of steroids, belongs to the cyclopentane polyhydrophenyl compound class. It is chemically stable, except for being light-sensitive. There are two main sources of vitamin D: one is converted from 7-dehydrocholesterol in the skin under the influence of ultraviolet light; the other is from vitamin D_2 in mushrooms exposed to sunlight and vitamin D3 in foods such as liver, milk, and cod liver oil. The vitamin D_2 and D_3 obtained from these sources are inactive forms, and they cannot be converted into each other, collectively referred to as vitamin D. To obtain biologically active $1,25(OH)_2D_3$, it needs to undergo two hydroxylations in the body (Figure 1). Firstly, the inactive vitamin D is converted to $25(OH)D_3$ in the liver under the catalysis of the 25-hydroxylase enzyme. $25(OH)D_3$ is the main storage form in the body, and its level reflects the nutritional status of vitamin D. Then, $25(OH)D_3$ is further converted to $1,25(OH)_2D_3$ in the kidneys under the action of 1α-hydroxylase. $1,25(OH)_2D_3$ binds to vitamin D receptors(VDR) widely present in tissues and exerts its effects in the body (2).

3 Relationship between vitamin D levels and diabetes

3.1. Vitamin D and diabetes

A number of studies have been conducted in the population to explore the relationship between vitamin D_3 and glycemic control (Table 1). Serum $25(OH)D_3$ levels have been shown to have a negative dose-response correlation with the risk of type 2 diabetes mellitus (T2DM) (3), and vitamin D supplementation reduces the risk of T2DM (4), decreases the risk of T2DM in pre-diabetic patients, and increases the chances of restoring normal glucose tolerance (5). However, the benefits of vitamin D_3 for the prevention of T2DM may be limited to non-obese subjects (5) or patients with vitamin D deficiency (6).

Achieving blood glucose control is one of the goals of diabetes treatment. Serum $25(OH)D_3$ levels can be an independent risk factor for increased levels of glycated hemoglobin in T2DM, with women being at a higher risk of vitamin D deficiency (7). In addition to finding that vitamin D can be a risk factor for glycemic control, it is interesting to note that in patients with T2DM, the combined administration of metformin and vitamin D resulted in better glycemic and glycosylated hemoglobin control compared to metformin alone (8). The efficacy of vitamin D on

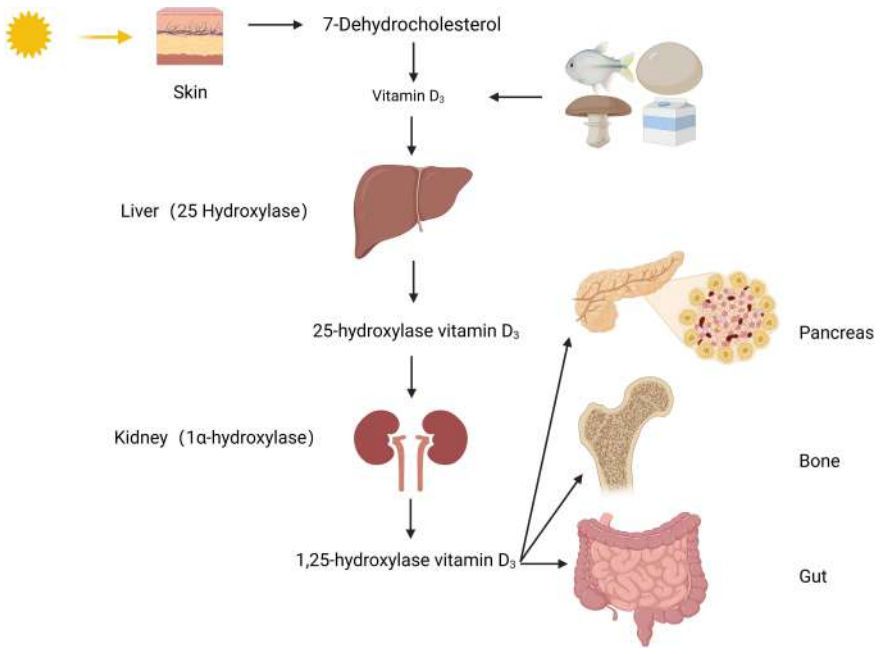

FIGURE 1
Metabolic pathways of vitamin D.

TABLE 1 The relationship between vitamin D and diabetes.

Reference	Year	Sample size	Research Design	Conclusion of the study
(3)	2020	6940	Cohort studies	In subjects with healthy sleep patterns, the higher the serum 25(OH)D_3 concentration, the lower the risk of developing T2DM.
(4)	2022	2423	Randomized clinical trials (RCT)	Supplementing with 4,000 IU of vitamin D per day can reduce the risk of diabetes. Vitamin D had a small beneficial effect on change in fasting plasma glucose.
(5)	2020	4896	Meta-analysis	Vitamin D supplementation reduces the risk of T2DM in participants with prediabetes. Reversion of prediabetes to normoglycemia was significantly increased by vitamin D supplementation. The benefit of the prevention of T2DM appears to be confined to nonobese subjects.
(6)	2019	2423	Clinical trial (CT)	Among persons at high risk for T2DM not selected for vitamin D insufficiency, vitamin D supplementation at a dose of 4000 IU per day did not result in a significantly lower risk of diabetes than placebo.
(7)	2023	1074	A cross-sectional study	In the T2DM patient cohort, the mean blood 25(OH)D_3 levels were 17.05 ng/ml. In comparison to the winter and spring, both males and females showed higher 25(OH)D_3 levels in the summer. HbA1c and vitamin D levels were negatively correlated.
(8)	2021	130	RCT	Oral daily doses of vitamin D improve HbA1c levels over the 3-month and 6-month period, followed by a significant decrease in advanced oxidation protein products levels over the 3-month period when higher vitamin D doses are given.
(9)	2021	1932	Meta-analysis	Vitamin D supplementation significantly improved fasting blood glucose, postprandial blood glucose, and quantitative insulin sensitivity check index in diabetes and prediabetes with baseline 25(OH)D_3<30 ng/ml. Higher percentages regressing from prediabetes to normal glucose status and lower percentage progressing from prediabetes to diabetes were found in the supplementation group. The positive effects of vitamin D supplementation on body mass index, waist, HDL-C, LDL-C, and CRP were also demonstrated.

T2DM, type 2 diabetes mellitus.

glycemic stability and insulin function was also found in patients with prediabetes (9). Similar results were obtained in animal experiments. Vitamin D supplementation reduced blood glucose, insulin levels, and improved insulin resistance (IR) in rats in a prediabetic model, and this efficacy was proportional to the dose of vitamin D supplementation (10). The above findings suggest that vitamin D helps in blood sugar control. However, there is still a need for larger studies in the future to examine the dose, duration, and most appropriate population for vitamin D supplementation to determine the relationship between vitamin D and glycemic control in diabetes. Serum 25(OH)D_3 insufficiency is closely related to the development of type 1 diabetes mellitus (T1DM) in children (11).

3.2 Vitamin D and diabetic complications

Vitamin D is not only related to the occurrence and development of diabetes, but numerous studies have also confirmed that vitamin D deficiency is closely associated with diabetic complications (Figure 2).

Although vitamin D does not reduce all-cause mortality in older adults (12). However, there are still a series of studies proving that vitamin D can prevent cardiovascular risk in people with diabetes. The incidence of cardiovascular diseases in T2DM patients increases by 2 to 3 times (13), and a follow-up study of T2DM patients confirmed that vitamin D deficiency is the strongest correlating factor for the occurrence of cardiovascular diseases in these patients (14). Vitamin D deficiency is also related to endothelial dysfunction and atherosclerosis (15, 16). The non-high-density lipoprotein cholesterol/high-density lipoprotein cholesterol ratio (NHHR) is a new comprehensive index of atherosclerotic lipids (17). NHHR is negatively correlated with vitamin D in T2DM patients (18). A higher NHHR index indicates a greater tendency for peripheral cholesterol deposition, and an increased distribution of cholesterol to the periphery may lead to IR or pancreatic β-cell dysfunction (19), resulting in vitamin D deficiency. Supplementing vitamin D can slow down the progression of myocardial dysfunction in T2DM patients without complications (20).

A prospective study on T2DM patients found that higher serum 25(OH)D_3 levels can reduce the risk of diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy, and serum 25 (OH)D_3 levels within a certain range (10-106 nmol/L) have a linear dose-response relationship with diabetic retinopathy and diabetic nephropathy (21). Inflammation has been shown to contribute to the occurrence and development of diabetic retinopathy (22–27). The pathogenesis of diabetic retinopathy is related to inflammation and fibrosis (23), and it has been reported that the production of pro-inflammatory cytokines such as IL-1, TNF-α, and VEGF increases in the vitreous body of patients with diabetic retinopathy and in animal models of the retina (22, 24). High glucose-induced upregulation of pro-inflammatory cytokines can lead to the destruction of the blood-retinal barrier (BRB), cell death, and angiogenesis (22, 25). Supplementing vitamin D can

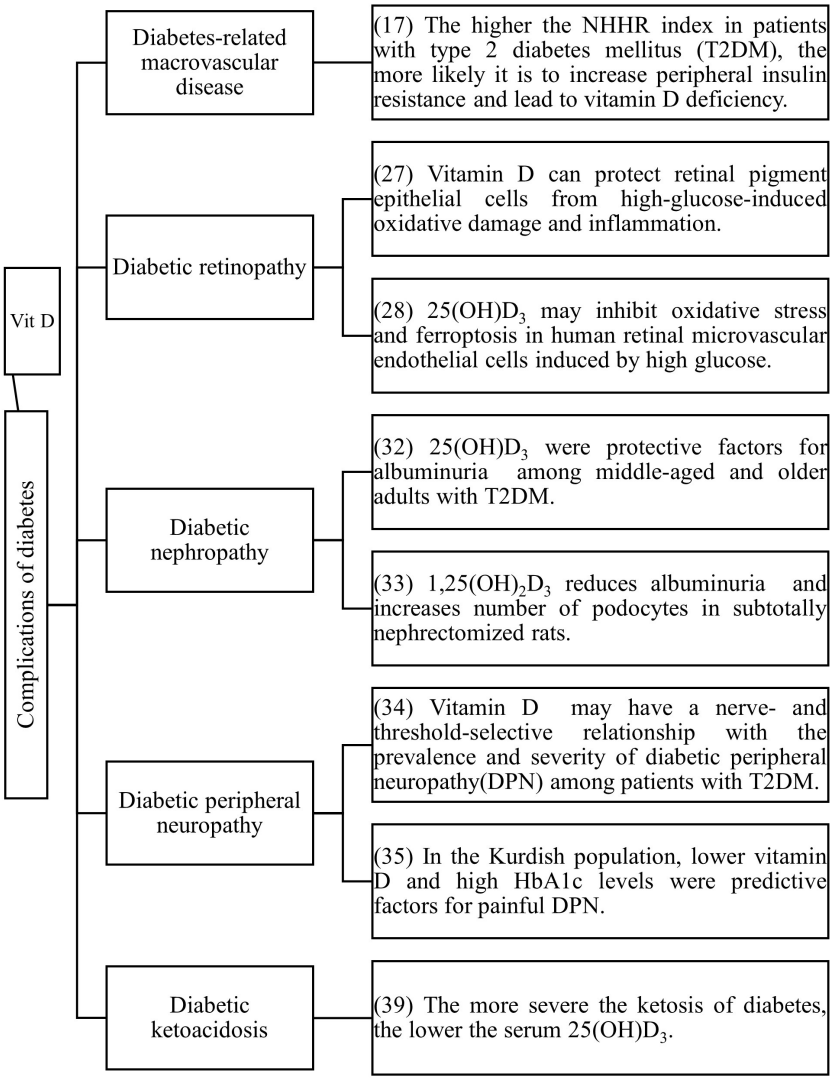

FIGURE 2
The relationship between vitamin D and diabetic complications.

protect retinal epithelial cells from high glucose-induced oxidative stress, inflammation and ferroptosis which may be one of the mechanisms by which vitamin D prevents the progression of diabetic retinopathy (28, 29).

Diabetic nephropathy is a major cause of disability and death in middle-aged and elderly patients with T2DM, significantly affecting their quality of life and safety. Progressive proteinuria and renal function deterioration are the main clinical symptoms of diabetic nephropathy (30–32). A study on elderly T2DM patients in China found that the incidence of vitamin D deficiency was significantly higher in patients with proteinuria than in those without (33). In animal experiments, vitamin D can prevent podocyte damage, thereby reducing proteinuria and glomerulosclerosis (34).

Peripheral nerve damage is also a common complication of diabetes mellitus. Patients lacking vitamin D are more likely to experience nerve function deficits associated with diabetic peripheral neuropathy than those with sufficient vitamin D (35). Among the Kurdish population, lower levels of vitamin D and higher levels of HbA1c are predictive risk factors for painful diabetic peripheral neuropathy (36). Controlling blood glucose alone cannot prevent the progression of peripheral neuropathy in T2DM. Chen T (37) found that monthly intramuscular injections of high-dose vitamin D improved peripheral neuropathy. This may provide new ideas for treating diabetic peripheral nerves. However, more research is needed to determine the exact course of treatment and dosage. In a study of diabetes mellitus in children (38), children with vitamin D deficiency did not complain of peripheral neuropathy, but sensory nerve action potential of sural nerve and motor peroneal nerve velocity were statistically significantly lower in diabetic patients with vitamin D deficiency compared to diabetic patients with normal vitamin D levels.

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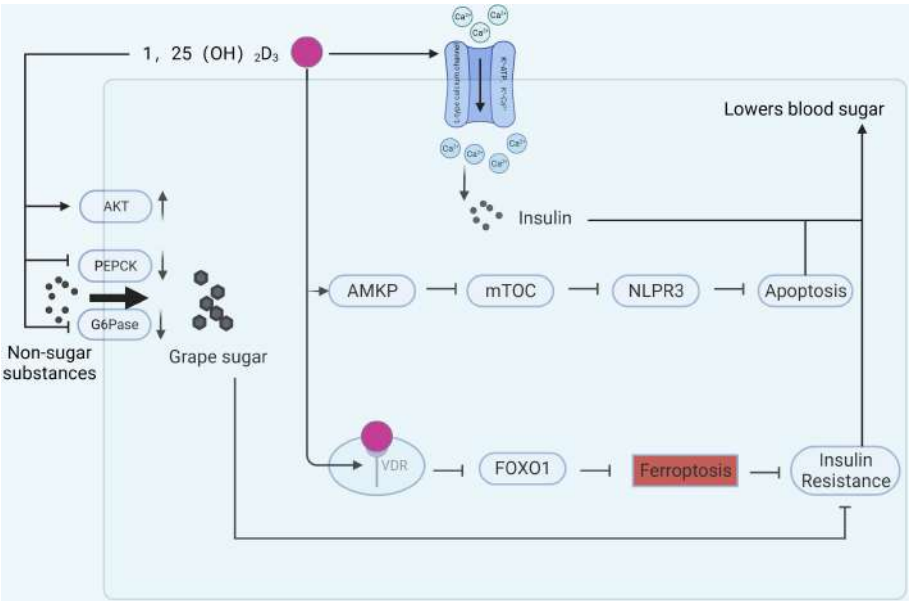

FIGURE 3
Mechanisms of vitamin D involvement in pancreatic function. (AKT, protein kinase B; PEACK, phosphoenolpyruvate carboxykinase; G6Pase, Glucose-6-phosphatase; AMPK, Adenosine 5'-monophosphate (AMP)-activated protein kinase; mTOR, mammalian target of rapamycin).

Diabetic ketosis is one of the acute complications of diabetes mellitus. Serum 25(OH)D_3 levels are lower in ketosis-prone T2DM compared to non-ketosis-prone T2DM (39, 40), and serum 25(OH)D_3 levels are related to the severity of pancreatitis concurrent with diabetic ketoacidosis (41).

4 Vitamin D and the pathogenesis of diabetes

4.1 Vitamin D and pancreatic function

Vitamin D may affect pancreatic function through several signaling pathways (Figure 3). Glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) are two key enzymes that convert non-carbohydrate substances into glucose (42, 43). Their abnormal expression is closely associated with enhanced gluconeogenesis and considered a marker for T2DM (44). Combined treatment with vitamin D and aerobic exercise can upregulate protein kinase B (AKT) in liver cells of T2DM rats, downregulate PEPCK and G6Pase expression, improve liver function, and alleviate IR (45). Vitamin D-binding protein (VDBP) is the primary plasma carrier maintaining vitamin D and its metabolites. Deficiency of VDBP may lead to pancreatic α-cell atrophy and proliferation, alters Na^+ channel conductance, reduces cellular activation by glucose, and decreases the rate of glucagon secretion *in vivo*, potentially increasing the incidence of late-onset T1DM (46). A double-blind, randomized, controlled clinical trial (47) showed that vitamin D supplementation improved β-cell function in patients with serum 25(OH)D_3 levels below 12 ng/mL compared to placebo.

Intraperitoneal injection of 1,25$(OH)_2D_3$ treats dexamethasone-induced IR in rats and improves islet function. The reason is related to the alteration of calcium ions after activation of L-type voltage-dependent calcium channel (VDCC), K^+-ATP, K^+-Ca^{2+}, and Kv channels by 1,25$(OH)_2D_3$, followed by activation of downstream PKC, PKA, and so on, which promotes insulin secretion (48). *In vitro* studies indicate that vitamin D can activate AMP-dependent protein kinase, inhibit the mammalian target of rapamycin (mTOR) pathway, thereby inhibiting the activation of the NLRP3 inflammasome and reducing β-cell apoptosis, promoting insulin release (49). Vitamin D may also reduce β-cell apoptosis in T2DM by inhibiting nuclear factor κB and downregulating the expression of divalent metal transporter 1 (DMT1) , alleviating pancreatic iron overload (50).

Ferroptosis, a newly discovered form of cell death, is considered a crucial factor in the pathogenesis of many inflammatory diseases (51). Ferroptosis is also considered a new target for diabetes (52). In the rat diabetes model, ferroptosis-related indicators such as GPX4 and SLC7A11 were downregulated (53) and ACSL4 was upregulated (54). 1,25$(OH)_2D_3$ treatment not only lowered blood glucose, but also reversed the changes in the above metrics. The mechanism is related to 1,25$(OH)_2D_3$/VDR/FOXO1. Binding of 1,25$(OH)_2D_3$ to the VDR inhibits ferroptosis in pancreatic β-cells and ameliorates IR by down-regulating FOXO1 expression (54). Vitamin D induces cellular autophagy while inhibiting streptozotocin-induced β-cell apoptosis, increasing insulin secretion and increasing β-cell resistance to cellular stresses encountered in hyperglycemic states (55). Excessive or

prolonged exposure to nitric oxide leads to β-cell dysfunction, whereas vitamin D induces and maintains high levels of the A20 gene protein and reduces nitric oxide levels, thus serving to protect β-cell function (56).

4.2 Vitamin D and immune function

Type 1 diabetes is an autoimmune disease. Vitamin D, by binding with its receptor, reduces pro-inflammatory cytokines in immune cells and has an immunomodulatory effect (57, 58). CD4+ T lymphocytes are the primary immune-mediated cells in the development of T1DM (59). Vitamin D supplementation can downregulate cathepsin G (Cat G) expression, hindering CD4+ T lymphocyte activation, thus enhancing pancreatic β-cell function (60). Systemic lupus erythematosus (SLE) is an immune disease. It has been found that IR is more prevalent in SLE patients than in controls (61), while serum vitamin D is negatively correlated with CD4+/CD8+ T cells (62), IFN-α levels (63), IL-17, IL-23 (64) in SLE patients. $25(OH)D_3$ is further converted into $1,25(OH)_2D_3$ in the kidneys by 1α-hydroxylase, which is expressed by antigen-presenting cells (65), indicating an association of the immune microenvironment with active vitamin D production, suggesting that vitamin D may mediate the disease process in T1DM. Complement can trigger the contraction of CD4+ type 1 helper T cell responses by inducing the intrinsic expression of VDR and vitamin D-activating enzyme CYP27B1, enabling T cells to both activate and respond to vitamin D (66). Vitamin D deficiency can epigenetically suppress Jarid2 expression in hepatic stellate cells (HSCs) and activate the Mef2/PGC1a pathway, leading to fat macrophage infiltration. Macrophages secrete MiR-106b-5p, inducing downregulation of the PIK3CA/PIK3R1/PDPK1/AKT signaling pathway, promoting fat IR (67). Vitamin D supplementation can reduce IR in diabetic rats by lowering phosphorylation levels of insulin receptor substrate 1 (IRS1), leading to impaired glucose transporter 4 and reduced glucose uptake, as well as increasing peroxisome proliferator-activated receptor γ expression and reducing nuclear factor κB phosphorylation levels (10). In addition, vitamin D increases insulin secretion and sensitivity by up-regulating mitogen-activated protein kinase phosphorylase-1 (MKP-1), inhibiting lipopolysaccharide-induced p38 phosphorylation, suppressing the production of IL-6 and TNF-α in human monocytes (68), and increasing the activity of the antioxidant system (69). Vitamin D has a strong protective effect on chronic kidney diseases (70, 71). The possible mechanism is that $1,25(OH)_2D_3$ reduces oxidative stress by increasing renal antioxidant capacity, inhibiting hyperglycemia-induced cell apoptosis, preventing podocyte damage, promoting anti-inflammatory action, and improving endothelial function (72–74) (Figure 4). In T1DM, some patients may experience a clinical remission period, also known as the "honeymoon phase", after receiving insulin therapy in the early stage of the disease, in which the islet function of the patient may partially or completely return to normal levels, and vitamin D may reduce the concentration of serum TNF-α through immunomodulatory effects, thereby reducing the inflammatory response during the honeymoon phase and prolonging the duration of the honeymoon phase (75), but the amount of vitamin D supplementation needs to be further studied.

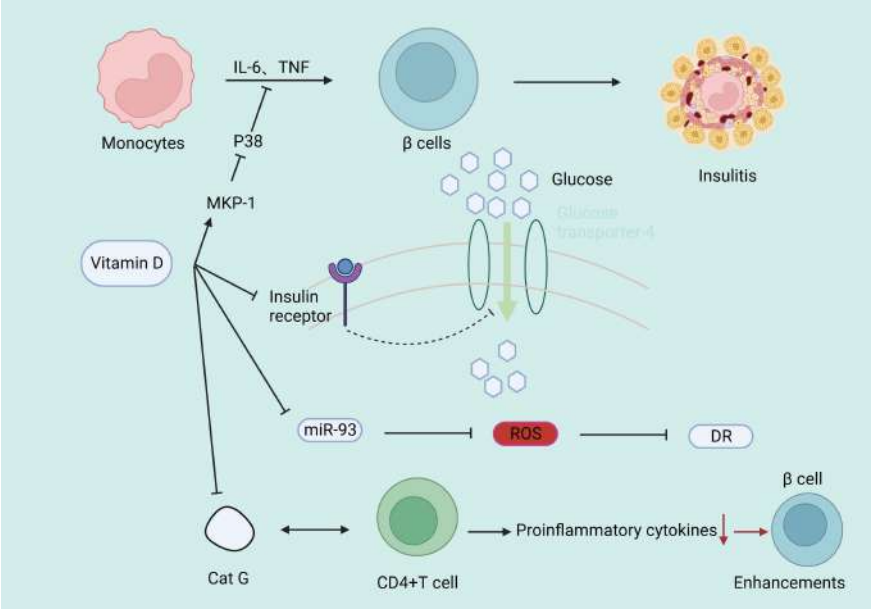

FIGURE 4
Vitamin D is involved in diabetes through immunization.

4.3 Vitamin D polymorphism and diabetes

Polymorphism in the VDR gene plays a role in the control and progression of T1DM, with higher levels of vitamin D providing protection for pancreatic cells (76). Polymorphisms in the 25-hydroxylase (CYP2R1) gene, rs12794714 and rs10766196, are associated with a higher risk of T1DM (77). It was found that CYP2R1 mRNA expression in the livers of mice fed a high-fat diet was significantly lower than in those fed a low-fat diet (78). As well, an activity analysis of the isolated liver showed that obese mice produced significantly less 25(OH)D_3 than lean mice, indicating that reduced circulating 25(OH)D_3 is partly due to the decreased expression of CYP2R1 in obese mice. The onset of T1DM might also be related to polymorphism in the CYP27B1 gene located on chromosome 12, where polymorphisms in the CYP27B1 gene could lead to reduced levels of 1α-hydroxylase, thereby affecting the conversion of vitamin D to 1,25$(OH)_2D_3$ and increasing the susceptibility to T1DM (79). Tangjittipokin (80) found that VDR gene-related variations of ApaI (rs7975232), TaqI (rs731236), and BsmI (rs1544410) were negatively associated with vitamin D and IL-10 levels in children with T1DM. Alleles of DHCR7, GC, CYP2R1, and CYP24A1 play a synergistic role in susceptibility to type 1 diabetes by functioning in the vitamin D pathway and serum vitamin D levels (81). A genome-wide association Meta-analysis study by Jiang X et al. (82) of 79,366 European individuals suggests that CYP24A1 (rs17216707) is negatively correlated with 25(OH)D_3 levels. The correlation between VDR gene rs739837 polymorphism and susceptibility to T2DM and gestational diabetes mellitus (GDM) (83) pointed out that the VDR gene rs739837 polymorphism is significantly correlated with susceptibility to T2DM. Studies in gestational diabetes mellitus (84, 85) confirmed that single nucleotide polymorphisms (SNPs) mutations at VDR-rs10783219 and MTNR1B-rs10830962 significantly increased the risk of GDM, ApaI-rs79785232, BsmI-rs1544410, FokI-rs2228570 and TaqI-rs731236 are associated with GDM occurrence in the Saudi Arabian region (Table 2).

TABLE 2 Effects of vitamin D and genes on diabetes.

Literature	Gene	SNPs
(78)	CYP2R1	rs12794714 rs10766196
(80)	CYP2R1 CYP27B1	- -
(81)	ApaI TaqI BsmI	rs7975232 rs731236 rs1544410
(82)	DHCR7 GC CYP2R1 CYP24A1	rs12785878 rs2282679 rs2060793 rs6013897
(83)	CYP24A1	rs17216707
(84)	VDR	rs739837
(85)	VDR MTNR1B	rs10783219 rs10830962
(79)	ApaI BsmI FokI TaqI	rs79785232 rs1544410 rs2228570 rs731236

5 Conclusion and prospects

In recent years, from D to D, the role of vitamin D in the occurrence and development of diabetes has gained attention. The relationship between vitamin D and the onset, progression of diabetes, the ideal daily dosage of vitamin D supplementation, and the optimal serum 25(OH)D_3 levels for maximum benefits in diabetes risk individuals, early-stage patients, blood sugar control, and diabetes-related complications still require more reliable clinical studies and basic experiments for confirmation. Current research suggests that moderate supplementation of vitamin D can improve the onset and progression of diabetes and its complications, but routine large-dose supplementation is not recommended. It is believed that the relationship between the two will be further verified in the near future.

Author contributions

CL: Conceptualization, Writing – review & editing. JF: Writing – original draft. YY: Writing – review & editing. JL: Writing – review & editing. YH: Conceptualization, Supervision, Writing – review & editing. TF: Conceptualization, Supervision, Writing – review & editing.

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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. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. *BMJ*. (2020) 369:m997. doi: 10.1136/bmj.m997

2. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev*. (2016) 96:365–408. doi: 10.1152/physrev.00014.2015. (1522-1210 (Electronic)).

3. Wang M, Zhou T, Li X, Ma H, Liang Z, Fonseca VA, et al. Baseline vitamin D status, sleep patterns, and the risk of incident type 2 diabetes in data from the UK biobank study. *Diabetes Care*. (2020) 43:2776–84. doi: 10.2337/dc20-1109

4. Hsia DS, Nelson J, Vickery EM, Rasouli N, LeBlanc ES, Kim S, et al. Effect of vitamin D on regression to normal glucose regulation and individual glycemic measures: A secondary analysis among participants adherent to the trial protocol in the randomized clinical trial vitamin D and type 2 diabetes (D2d) study. *Diabetes Res Clin Pract*. (2023) 202:110792. doi: 10.1016/j.diabres.2023.110792

5. Zhang Y, Tan H, Tang J, Li J, Chong W, Hai Y, et al. Effects of vitamin D supplementation on prevention of type 2 diabetes in patients with prediabetes: A systematic review and meta-analysis. *Diabetes Care*. (2020) 43:1650–8. doi: 10.2337/dc19-1708

6. Pittas AG, Dawson-Hughes B, Sheehan P, Ware JH, Knowler WC, Aroda VR, et al. Vitamin D supplementation and prevention of type 2 diabetes. *N Engl J Med*. (2019) 381:520–30. doi: 10.1056/NEJMoa1900906

7. Wang C, Li H, Huo L, Wang Q, Zhang T, He X, et al. Serum 25-hydroxyvitamin D levels in type 2 diabetes patients in north China: seasonality and the association between vitamin D status and glycosylated hemoglobin levels. *Int J Clin Pract*. (2023) 2023:4151224. doi: 10.1155/2023/4151224

8. Cojic M, Kocic R, Klisic A, Kocic G. The effects of vitamin D supplementation on metabolic and oxidative stress markers in patients with type 2 diabetes: A 6-month follow up randomized controlled study. *Front Endocrinol (Lausanne)*. (2021) 12:610893. doi: 10.3389/fendo.2021.610893

9. Zou Y, Guo B, Yu S, Wang D, Qiu L, Jiang Y. Effect of vitamin D supplementation on glycose homeostasis and islet function in vitamin D deficient or insufficient diabetes and prediabetes: a systematic review and meta-analysis. *J Clin Biochem Nutr*. (2021) 69:229–37. doi: 10.3164/jcbn.20-165

10. Krisnamurti DGB, Louisa M, Poerwaningsih EH, Tarigan TJE, Soetikno V, Wibowo H, et al. Vitamin D supplementation alleviates insulin resistance in prediabetic rats by modifying IRS-1 and PPARgamma/NF-kappaB expressions. *Front Endocrinol (Lausanne)*. (2023) 14:1089298. doi: 10.3389/fendo.2023.1089298

11. Liu C, Lu M, Xia X, Wang J, Wan Y, He L, et al. Correlation of serum vitamin D level with type 1 diabetes mellitus in children: a meta-analysis. *Nutr Hosp*. (2015) 32:1597–4. 1699-5198 (Electronic). doi: 10.3305/nh.2015.32.4.9198

12. Neale RE, Baxter C, Romero BD, McLeod DSA, English DR, Armstrong BK, et al. The D-Health Trial: a randomised controlled trial of the effect of vitamin D on mortality. *Lancet Diabetes Endocrinol*. (2022) 10:120–8. doi: 10.1016/S2213-8587(21)00345-4. 2213-8595 (Electronic).

13. Ma CX, Ma XN, Guan CH, Li YD, Mauricio D, Fu SB. Cardiovascular disease in type 2 diabetes mellitus: progress toward personalized management. *Cardiovasc Diabetol*. (2022) 21:74. doi: 10.1186/s12933-022-01516-6

14. de la Guia-Galipienso F, Martinez-Ferran M, Vallecillo N, Lavie CJ, Sanchis-Gomar F, Pareja-Galeano H. Vitamin D and cardiovascular health. *Clin Nutr*. (2021) 40:2946–57. doi: 10.1016/j.clnu.2020.12.025

15. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. *Nutrients*. (2020) 12. doi: 10.3390/nu12020575

16. Rodriguez AJ, Scott D, Srikanth V, Ebeling P. Effect of vitamin D supplementation on measures of arterial stiffness: a systematic review and meta-analysis of randomized controlled trials. *Clin Endocrinol (Oxf)*. (2016) 84:645–57. doi: 10.1111/cen.13031

17. Sheng GA-O, Liu D, Kuang M, Zhong Y, Zhang S, Zou YA-OX. Utility of non-high-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio in evaluating incident diabetes risk. *Diabetes Metab Syndr Obes*. (2022) 31:1677–86. doi: 10.2147/DMSO.S355980. 15(1178-7007 (Print).

18. Zhen R, Ban J, Jia Z, Liu Y, Li Z, Chen S. The relationship between non-HDL-C /HDL-C ratio (NHHR) and vitamin D in type 2 diabetes mellitus. *Diabetes Metab Syndr Obes*. (2023) 16:2661–73. doi: 10.2147/DMSO.S414673

19. Narvaez CJ, Simmons KM, Brunton J, Salinero A, Chittur SV, Welsh JE. Induction of STEAP4 correlates with 1,25-dihydroxyvitamin D3 stimulation of adipogenesis in mesenchymal progenitor cells derived from human adipose tissue. *J Cell Physiol*. (2013) 228:2024–36. doi: 10.1002/jcp.24371

20. Obert P, Nottin S, Philouze C, Aboukhoudir F. Major impact of vitamin D(3) deficiency and supplementation on left ventricular torsional mechanics during dobutamine stress in uncomplicated type 2 diabetes. *Nutr Metab Cardiovasc Dis*. (2023) 33(11):2269–79. doi: 10.1016/j.numecd.2023.06.017

21. Chen X, Wan Z, Geng T, Zhu K, Li R, Lu Q, et al. Vitamin D status, vitamin D receptor polymorphisms, and risk of microvascular complications among individuals with type 2 diabetes: A prospective study. *Diabetes Care*. (2023) 46:270–7. doi: 10.2337/dc22-0513

22. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, et al. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res*. (2016) 51:156–86. doi: 10.1016/j.preteyeres.2015.08.001

23. Platania CBM, Leggio GM, Drago F, Salomone S, Bucolo C. Computational systems biology approach to identify novel pharmacological targets for diabetic retinopathy. *Biochem Pharmacol*. (2018) 158:13–26. doi: 10.1016/j.bcp.2018.09.016

24. Lazzara F, Fidilio A, Platania CBM, Giurdanella G, Salomone S, Leggio GM, et al. Aflibercept regulates retinal inflammation elicited by high glucose via the PlGF/ERK pathway. *Biochem Pharmacol*. (2019) 168:341–51. doi: 10.1016/j.bcp.2019.07.021

25. Platania CBM, Lazzara F, Fidilio A, Fresta CG, Conti F, Giurdanella G, et al. Blood-retinal barrier protection against high glucose damage: The role of P2X7 receptor. *Biochem Pharmacol*. (2019) 168:249–58. doi: 10.1016/j.bcp.2019.07.010

26. D'Amico AG, Maugeri G, Reitano R, Bucolo C, Saccone S, Drago F, et al. PACAP modulates expression of hypoxia-inducible factors in streptozotocin-induced diabetic rat retina. *J Mol Neurosci*. (2015) 57:501–9. doi: 10.1007/s12031-015-0621-7

27. Platania CBM, Maisto R, Trotta MC, D'Amico M, Rossi S, Gesualdo C, et al. Retinal and circulating miRNA expression patterns in diabetic retinopathy: An in silico and in *vivo* approach. *Br J Pharmacol*. (2019) 176:2179–94. doi: 10.1111/bph.14665

28. Tohari AM, Almarhoun M, Alhasani RH, Biswas L, Zhou X, Reilly J, et al. Protection by vitamin D against high-glucose-induced damage in retinal pigment epithelial cells. *Exp Cell Res*. (2020) 392:112023. doi: 10.1016/j.yexcr.2020.112023

29. Zhan D, Zhao J, Shi Q, Lou J, Wang W. 25-hydroxyvitamin D3 inhibits oxidative stress and ferroptosis in retinal microvascular endothelial cells induced by high glucose through down-regulation of miR-93. *BMC Ophthalmol*. (2023) 13:1471–2415. doi: 10.1186/s12886-022-02762-8

30. Khatib ST, Hemadneh MK, Hasan SA, Khazneh E, Zyoud SH. Quality of life in hemodialysis diabetic patients: a multicenter cross-sectional study from Palestine. *BMC Nephrol*. (2018) 19:49. doi: 10.1186/s12882-018-0849-x

31. Foundation NK. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. *Am J Kidney Dis*. (2012) 60:850–86. doi: 10.1053/j.ajkd.2012.07.005. (1523-6838 (Electronic)).

32. Bonner R, Albajrami O, Hudspeth J, Upadhyay A. Diabetic kidney disease. *Prim Care*. (2020) 47:645–59. doi: 10.1016/j.pop.2020.08.004

33. Zhang L, Guo Q, Xu Y, Wei W, Wang Y. Association between serum 25-hydroxyvitamin D and albuminuiria in middle-aged and older Chinese patients with type 2 diabetes. *BMC Endocr Disord*. (2023) 23:189. doi: 10.1186/s12902-023-01440-0

34. Kuhlmann A, Haas Cs Fau - Gross M-L, Gross Ml Fau - Reulbach U, Reulbach U Fau - Holzinger M, Holzinger M Fau - Schwarz U, Schwarz U Fau - Ritz E, et al. 1,25-Dihydroxyvitamin D3 decreases podocyte loss and podocyte hypertrophy in the subtotally nephrectomized rat. *Am J Physiol Renal Physiol*. (2003) 286:F526–533. doi: 10.1152/ajprenal.00316.2003. (1931-857X (Print)).

35. Pang C, Yu H, Cai Y, Song M, Feng F, Gao L, et al. Vitamin D and diabetic peripheral neuropathy: A multi-centre nerve conduction study among Chinese patients with type 2 diabetes. *Diabetes Metab Res Rev*. (2023) 39:e3679. doi: 10.1002/dmrr.3679

36. Hassan AB, Al-Dosky AHA. Vitamin D status and its association with inflammatory markers among Kurdish type 2 diabetic patients with painful diabetic peripheral neuropathy. *Steroids*. (2023) 199:109289. doi: 10.1016/j.steroids.2023.109289

37. Chen T, Xing X, Huang L, Tu M, Lai X, Wen S, et al. Efficacy and safety of high-dose intramuscular vitamin D(2) injection in type 2 diabetes mellitus with distal symmetric polyneuropathy combined with vitamin D insufficiency: study protocol for a

multicenter, randomized, double-blinded, and placebo-controlled trial. *Front Endocrinol (Lausanne)*. (2023) 14:1202917. doi: 10.3389/fendo.2023.1202917

38. Polat İ, Can Yılmaz G, Dedeoğlu Ö. Vitamin D and nerve conduction in pediatric type-1 diabetes mellitus. *Brain Dev*. (2022) 44:336–42. doi: 10.1016/j.braindev.2022.01.001. (1872-7131 (Electronic)).

39. He X, Luo Y, Hao J, Wang C, Gan K, Zhen Y, et al. Association between serum vitamin D levels and ketosis episodes in hospitalized patients with newly diagnosed ketosis-prone type 2 diabetes. *Diabetes Metab Syndr Obes*. (2022) 15:3821–9. doi: 10.2147/DMSO.S389609

40. Nóvoa-Medina YA-O, Barreiro-Bautista M, Perdomo-Quinteiro M, González-Martín JA-O, Quinteiro-González S, Domínguez Á, et al. 25OHVitamin D levels in a canarian pediatric population with and without type 1 diabetes: the role of acidosis. *Nutrients*. (2023) 15:3067. doi: 10.3390/nu15133067. (2072-6643 (Electronic)).

41. Lu S, Wei D, Yin C, Xiong J, Zhu L, Yan S, et al. Correlation between PCT, 25 (OH)D, PTX-3, AMS levels and the severity of diabetic ketoacidosis complicated by pancreatitis. *BMC Endocr Disord*. (2021) 21:136. doi: 10.1186/s12902-021-00792-9

42. Barthel A, Schmoll D, Kruger KD, Bahrenberg G, Walther R, Roth RA, et al. Differential regulation of endogenous glucose-6-phosphatase and phosphoenolpyruvate carboxykinase gene expression by the forkhead transcription factor FKHR in H4IIE-hepatoma cells. *Biochem Biophys Res Commun*. (2001) 285:897–902. doi: 10.1006/bbrc.2001.5261

43. Zhu YX, Hu HQ, Zuo ML, Mao L, Song GL, Li TM, et al. Effect of oxymatrine on liver gluconeogenesis is associated with the regulation of PEPCK and G6Pase expression and AKT phosphorylation. *BioMed Rep*. (2021) 15:56. doi: 10.3892/br.2021.1432

44. Sharma R, Kumari M, Prakash P, Gupta S, Tiwari SA-O. Phosphoenolpyruvate carboxykinase in urine exosomes reflect impairment in renal gluconeogenesis in early insulin resistance and diabetes. *Am J Physiol Renal Physiol*. (2020) 318:F720–31. doi: 10.1152/ajprenal.00507.2019. (1522-1466 (Electronic)).

45. Hoseini Z, Behpour N, Hoseini R. Vitamin D improves the antidiabetic effectiveness of aerobic training via modulation of Akt, PEPCK, and G6Pase expression. *Diabetol Metab Syndr*. (2023) 15:184. doi: 10.1186/s13098-023-01158-y

46. Viloria K, Nasteska D, Briant LJB, Heising S, Larner DP, Fine NHF, et al. Vitamin-D-binding protein contributes to the maintenance of alpha cell function and glucagon secretion. *Cell Rep*. (2020) 31:107761. doi: 10.1016/j.celrep.2020.107761

47. Rasouli N, Brodsky IG, Chatterjee R, Kim SH, Pratley RE, Staten MA, et al. Effects of vitamin D supplementation on insulin sensitivity and secretion in prediabetes. *J Clin Endocrinol Metab*. (2022) 107:230–40. doi: 10.1210/clinem/dgab649

48. Mendes AKB, Sulis PM, Cavalari FC, Padilla DPR, Aragon M, Gaspar JM, et al. 1alpha,25-(OH)(2) vitamin D(3) prevents insulin resistance and regulates coordinated exocytosis and insulin secretion. *J Nutr Biochem*. (2022) 99:108864. doi: 10.1016/j.jnutbio.2021.108864

49. Wu M, Lu L, Guo K, Lu J, Chen H. Vitamin D protects against high glucose-induced pancreatic beta-cell dysfunction via AMPK-NLRP3 inflammasome pathway. *Mol Cell Endocrinol*. (2022) 547:111596. doi: 10.1016/j.mce.2022.111596

50. Zhao Y, Mei G, Zhou F, Kong B, Chen L, Chen H, et al. Vitamin D decreases pancreatic iron overload in type 2 diabetes through the NF-κB-DMT1 pathway. *J Nutr Biochem*. (2021) 99:108870. doi: 10.1016/j.jnutbio.2021.108870. (1873-4847 (Electronic)).

51. Li JY, Yao YM, Tian YP. Ferroptosis: A trigger of proinflammatory state progression to immunogenicity in necroinflammatory disease. *Front Immunol*. (2021) 12:701163. doi: 10.3389/fimmu.2021.701163. (1664-3224 (Electronic)).

52. Yang XD, Yang YY. Ferroptosis as a novel therapeutic target for diabetes and its complications. *Front Endocrinol (Lausanne)*. (2022) 29:853822. doi: 10.3389/fendo.2022.853822. (1664-2392 (Print)).

53. Zhou Y. The Protective Effects of Cryptochlorogenic Acid on β-Cells Function in Diabetes in vivo and vitro via Inhibition of Ferroptosis. *Diabetes Metab Syndr Obes*. (2020) 8:1921–31. doi: 10.2147/DMSO.S249382. (1178-7007 (Print)).

54. Ding Y, Wu Q. 1,25D/VDR inhibits pancreatic β cell ferroptosis by downregulating FOXO1 expression in diabetes mellitus. *Cell Signal*. (2023) 105. doi: 10.1016/j.cellsig.2022.110564. (1873-3913 (Electronic)).

55. Wang Y, He D, Ni C, Zhou H, Wu S, Xue Z, et al. Vitamin D induces autophagy of pancreatic β-cells and enhances insulin secretion. *Mol Med Rep*. (2016) 14:44–50. doi: 10.3892/mmr.2016.5531. (1791-3004 (Electronic)).

56. Nord JA, Wynia-Smith SL, Gehant AL, Jones Lipinski RA, Naatz A, Rioja I, et al. N-terminal BET bromodomain inhibitors disrupt a BRD4-p65 interaction and reduce inducible nitric oxide synthase transcription in pancreatic beta-cells. *Front Endocrinol (Lausanne)*. (2022) 13:923925. doi: 10.3389/fendo.2022.923925

57. LB E, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. *JBMR Plus*. (2021) 5:e10405. doi: 10.1002/jbm4.10405

58. Zughaier SM, Lubberts E, Bener A. Editorial: immune-modulatory effects of vitamin D. *Front Immunol*. (2020) 11:596611. doi: 10.3389/fimmu.2020.596611

59. Atkinson MA, Roep BO, Posgai A, Wheeler DCS, Peakman M. The challenge of modulating beta-cell autoimmunity in type 1 diabetes. *Lancet Diabetes Endocrinol*. (2019) 7:52–64. doi: 10.1016/S2213-8587(18)30112-8

60. Lai X, Liu X, Cai X, Zou FA-O. Vitamin D supplementation induces CatG-mediated CD4(+) T cell inactivation and restores pancreatic β-cell function in mice with type 1 diabetes. *Am J Physiol Endocrinol Metab*. (2021) 322:E74–84. doi: 10.1152/ajpendo.00066.2021. (1522-1555 (Electronic)).

61. García-Carrasco M, Mendoza-Pinto C, Munguía-Realpozo P, Etchegaray-Morales I, Vélez-Pelcastre SK, Méndez-Martínez S, et al. Insulin resistance and diabetes mellitus in patients with systemic lupus erythematosus. *Endocr Metab Immune Disord Drug Targets*. (2023) 23:503–14. doi: 10.2174/1871530322666220908154253. (2212-38736).

62. Zhao M, Duan XH, Wu ZZ, Gao CC, Wang N, Zheng ZH. Severe vitamin D deficiency affects the expression of autophagy related genes in PBMCs and T-cell subsets in active systemic lupus erythematosus. *Am J Clin Exp Immunol*. (2017) 6:43–51. (2164-7712 (Print)).

63. Abdel Galil SM, El-Shafey AM, Abdul-Maksoud RS, El-Boshy M. Interferon alpha gene expression and serum level association with low vitamin D levels in Egyptian female patients with systemic lupus erythematosus. *Lupus*. (2017) 27:199–209. doi: 10.1177/0961203317716321. (1477-0962 (Electronic)).

64. Shahin D, El-Farahaty RM, Houssen ME, Machaly SA, Sallam M, ElSaid TO, et al. Serum 25-OH vitamin D level in treatment-naïve systemic lupus erythematosus patients: Relation to disease activity, IL-23 and IL-17. *Lupus*. (2017) 26:917–26. doi: 10.1177/0961203316682095. (1477-0962 (Electronic)).

65. Harrison SR, Li D, Jeffery LE, Raza K, Hewison M. Vitamin D, autoimmune disease and rheumatoid arthritis. *Calcif Tissue Int*. (2020) 106:58–75. doi: 10.1007/s00223-019-00577-2

66. Chauss D, Freiwald T, McGregor R, Yan B, Wang L, Nova-Lamperti E, et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of T(H)1 cells. *Nat Immunol*. (2022) 23:62–74. doi: 10.1038/s41590-021-01080-3

67. Oh J, Riek AE, Bauerle KT, Dusso A, McNerney KP, Barve RA, et al. Embryonic vitamin D deficiency programs hematopoietic stem cells to induce type 2 diabetes. *Nat Commun*. (2023) 14:3278. doi: 10.1038/s41467-023-38849-z

68. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol*. (2012) 188:2127–35. doi: 10.4049/jimmunol.1102412

69. Fathi F, Sadek KM, Khafaga AF, Al Senosy AW, Ghoniem HA, Fayez S, et al. Vitamin D regulates insulin and ameliorates apoptosis and oxidative stress in pancreatic tissues of rats with streptozotocin-induced diabetes. *Environ Sci pollut Res Int*. (2022) 29:90219–29. doi: 10.1007/s11356-022-22064-2

70. Nakhoul NA-O, Thawko T, Farber E, Dahan I, Tadmor H, Nakhoul R, et al. The therapeutic effect of active vitamin D supplementation in preventing the progression of diabetic nephropathy in a diabetic mouse model. *J Diabetes Res*. (2020) 2020:7907605. doi: 10.1155/2020/7907605. (2314-6753 (Electronic)).

71. Hong SH, Kim YB, Choi HS, Jeong TD, Kim JT, Sung YA. Association of vitamin D deficiency with diabetic nephropathy. *Endocrinol Metab*. (2020) 36(1):106–13. doi: 10.3803/EnM.2020.826. (2093-5978 (Electronic)).

72. Deng X, Cheng J, Shen M. Vitamin D improves diabetic nephropathy in rats by inhibiting renin and relieving oxidative stress. *J Endocrinol Invest*. (2016) 39:657–66. doi: 10.1007/s40618-015-0414-4

73. Li YC. Podocytes as target of vitamin D. *Curr Diabetes Rep*. (2011) 7:35–40. doi: 10.2174/157339911794273964. (1875-6417 (Electronic)).

74. Doorenbos CR, van den Born J, Navis G, de Borst MH. Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism. *Nat Rev Nephrol*. (2009) 5:691–700. doi: 10.1038/nrneph.2009.185

75. Nwosu BA-O, Parajuli S, Jasmin G, Fleshman J, Sharma RB, Alonso LA-O, et al. Ergocalciferol in new-onset type 1 diabetes: A randomized controlled trial. *J Endocr Soc*. (2021) 6:bvab179. doi: 10.1210/jendso/bvab179. (2472-1972 (Electronic)).

76. Habibian N, Amoli MM, Abbasi F, Rabbani A, Alipour A, Sayarifard F, et al. Role of vitamin D and vitamin D receptor gene polymorphisms on residual beta cell function in children with type 1 diabetes mellitus. *Pharmacol Rep*. (2019) 71:282–8. doi: 10.1016/j.pharep.2018.12.012

77. Nam HK, Rhie YJ, Lee KH. Vitamin D level and gene polymorphisms in Korean children with type 1 diabetes. *Pediatr Diabetes*. (2019) 20:750–8. doi: 10.1111/pedi.12878

78. Roizen JD, Long C, Casella A, O'Lear L, Caplan I, Lai M, et al. Obesity decreases hepatic 25-hydroxylase activity causing low serum 25-hydroxyvitamin D. *J Bone Miner Res*. (2019) 34:1068–73. doi: 10.1002/jbmr.3686

79. Voutsadakis IA-O. Vitamin D receptor (VDR) and metabolizing enzymes CYP27B1 and CYP24A1 in breast cancer. *Mol Biol Rep*. (2020) 47:9821–30. doi: 10.1007/s11033-020-05780-1. (1573-4978 (Electronic)).

80. Tangjittipokin W, Umjai P, Khemaprasit K, Charoentawornpanich P, Chanprasert C, Teerawattanapong N, et al. Vitamin D pathway gene polymorphisms, vitamin D level, and cytokines in children with type 1 diabetes. *Gene*. (2021) 791:145691. doi: 10.1016/j.gene.2021.145691. (1879-0038 (Electronic)).

81. Almeida JA-O, Rodrigues D, Guimarães J, Lemos MA-O. Vitamin D pathway genetic variation and type 1 diabetes: A case-control association study. *Genes (Basel)*. (2020) 11:897. doi: 10.3390/genes11080897. (2073-4425 (Electronic)).

82. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun*. (2018) 9:260. doi: 10.1038/s41467-017-02662-2

83. Zeng Q, Zou D, Wei Y, Ouyang Y, Lao Z, Guo R. Association of vitamin D receptor gene rs739837 polymorphism with type 2 diabetes and gestational diabetes mellitus susceptibility: a systematic review and meta-analysis. *Eur J Med Res*. (2022) 27:65. doi: 10.1186/s40001-022-00688-x

84. Mo M, Shao B, Xin X, Luo W, Si S, Jiang W, et al. The association of gene variants in the vitamin D metabolic pathway and its interaction with vitamin D on gestational diabetes mellitus: A prospective cohort study. *Nutrients*. (2021) 13:4220. doi: 10.3390/nu13124220. (2072-6643 (Electronic)).

85. Ali Khan IA-O, Alhaizan MA-O, Neyazi SM, Al-Hakeem MM, Alshammary AA-O. Relevance of serum levels and functional genetic variants in vitamin D receptor gene among Saudi women with gestational diabetes mellitus. *Nutrients*. (2023) 15:4288. doi: 10.3390/nu15194288. (2072-6643 (Electronic)).

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EDITED BY
Giacomina Brunetti,
University of Bari Aldo Moro, Italy

REVIEWED BY
Khashayar Sakhaee,
University of Texas Southwestern Medical Centre, United States
Ejigayehu Gigi Abate,
Mayo Clinic, United States

*CORRESPONDENCE
Dorota Leszczyńska
d.leszczynska@tlen.pl
Alicja Szatko
alicja.szatko@gmail.com

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Persistent hypercalcaemia associated with two pathogenic variants in the *CYP24A1* gene and a parathyroid adenoma—a case report and review

Dorota Leszczyńska[1*], Alicja Szatko[1,2*], Julia Latocha[3], Magdalena Kochman[1], Maria Duchnowska[1], Anna Wójcicka[4,5], Waldemar Misiorowski[1], Wojciech Zgliczyński[1] and Piotr Glinicki[1,2]

[1]Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland, [2]EndoLab Laboratory, Centre of Postgraduate Medical Education, Warsaw, Poland, [3]Students' Scientific Group Affiliated with the Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland, [4]Warsaw Genomics, Warsaw, Poland, [5]Fundacja Wiedzieć Więcej, Warsaw, Poland

Introduction: 24-Hydroxylase, encoded by the *CYP24A1* gene, is a crucial enzyme involved in the catabolism of vitamin D. Loss-of-function mutations in *CYP24A1* result in PTH-independent hypercalcaemia with high levels of 1,25(OH)$_2$D$_3$. The variety of clinical manifestations depends on age, and underlying genetic predisposition mutations can lead to fatal infantile hypercalcaemia among neonates, whereas adult symptoms are usually mild.

Aim of the study: We report a rare case of an adult with primary hyperparathyroidism and loss-of-function mutations in the *CYP24A1* gene and a review of similar cases.

Case presentation: We report the case of a 58-year-old woman diagnosed initially with primary hyperparathyroidism. Preoperatively, the suspected mass adjoining the upper pole of the left lobe of the thyroid gland was found via ultrasonography and confirmed by 99mTc scintigraphy and biopsy as the parathyroid gland. The patient underwent parathyroidectomy (a histopathology report revealed parathyroid adenoma), which led to normocalcaemia. After 10 months, vitamin D supplementation was introduced due to deficiency, and the calcium level remained within the reference range. Two years later, biochemical tests showed recurrence of hypercalcaemia with suppressed parathyroid hormone levels and elevated 1,25(OH)$_2$D$_3$ concentrations. Further investigation excluded the most common causes of PTH-independent hypercalcaemia, such as granulomatous disease, malignancy, and vitamin D intoxication. Subsequently, vitamin D metabolites were measured using LC–MS/MS, which revealed high levels of 25(OH)D$_3$, low levels of 24,25(OH)$_2$D$_3$ and elevated 25(OH)$_2$D$_3$/24,25(OH)$_2$D$_3$ ratios, suggesting a defect in vitamin D catabolism. Molecular analysis of the *CYP24A1* gene using the NGS technique revealed two pathogenic variants: p.(Arg396Trp) and p.(Glu143del) (rs114368325 and rs777676129, respectively).

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Conclusions: The diagnostic process for hypercalcaemia becomes complicated when multiple causes of hypercalcaemia coexist. The measurement of vitamin D metabolites using LC–MS/MS may help to identify carriers of *CYP24A1* mutations. Subsequent molecular testing may contribute to establishing the exact frequency of pathogenic variants of the *CYP24A1* gene and introducing personalized treatment.

KEYWORDS

hypercalcaemia, *CYP24A1* gene, vitamin D, primary hyperparathyroidism, 25(OH)D_3, 1,25$(OH)_2D_3$, 24,25$(OH)_2D_3$, 24-hydroxylase deficiency

Introduction

The active form of vitamin D (1,25$(OH)_2D_3$ or calcitriol), in addition to parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), is critical for maintaining calcium haemostasis (1). Primary hyperparathyroidism (PHPT) and malignancy are the most common causes of hypercalcaemia. Among the less frequent aetiologies, vitamin D-mediated hypercalcaemia should be considered.

The main source of vitamin D is skin synthesis from 7-dehydrocholestrol (7-DHC); however, it can also be obtained from the diet. For full hormonal activity, vitamin D_3 requires two-stage hydroxylation. First, 25 hydroxylase generates 25(OH)D_3 in the liver. The second hydroxylation by 1-alpha-hydroxylase (CYP27B1, Cytochrome P450 Family 27 Subfamily B Member 1) leads to the conversion of 25(OH)D_3 to the biologically active metabolite of vitamin D (1,25$(OH)_2D_3$) (2). This process occurs mainly in the kidneys, but *CYP27B1* is also expressed at several external sites (3). 1,25$(OH)_2D_3$ and 25(OH)D_3 are inactivated by 24-hydroxylase (24HD) (cytochrome P450 family 24 subfamily a member 1 (CYP24A1)), the key enzymes involved in vitamin D catabolism. The direct products of the CYP24A1 reaction are 24,25$(OH)_2D_3$ and 1,24,25$(OH)_2D_3$, which are further converted to calcitroic acid destined for biliary excretion (4) (Figure 1). The activity of CYP24A1 is regulated by numerous biochemical factors. Hypocalcaemia and PTH downregulate *CYP24A1* expression and enzymatic activity (5). The opposite stimulating effect is caused by 1,25$(OH)_2D_3$ and FGF23, together with its coreceptor α-Klotho protein (5).

The concentration of 25(OH)D, the best indicator of vitamin D status, can be measured by numerous diagnostic methods. The following immunoassays were used: chemiluminescence immunoassay (CLIA), electrochemiluminescence immunoassay (ECLIA), vitamin D binding protein (DBP-based assays), high-performance liquid chromatography (HPLC) and liquid chromatography with tandem mass spectrometry (LC–MS/MS). However, the 'gold standard' and reference technique that allows for the most reliable assessment of many vitamin D metabolites simultaneously is LC–MS/MS (6).

Excessive vitamin D or calcitriol ingestion, ectopic production of 1,25$(OH)_2D_3$ in granulomatous or lymphoproliferative disease and genetically determined dysregulation of vitamin D metabolism

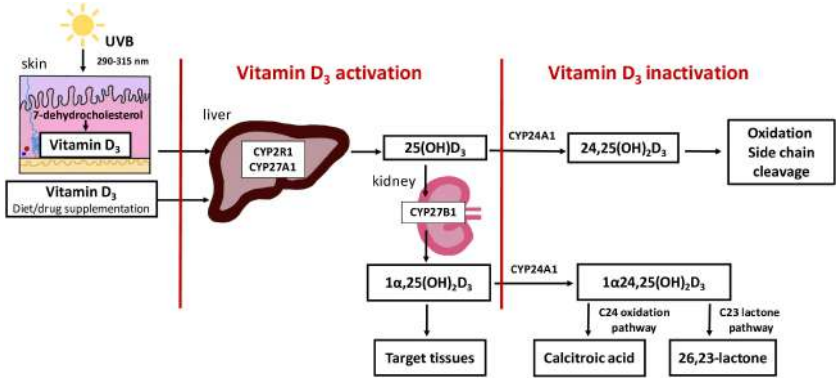

FIGURE 1
Overview of vitamin D synthesis, activation and inactivation. The main source of vitamin D is skin synthesis from 7-dehydrocholestrol (7-DHC), however, it can also be obtained from the diet. For full hormonal activity, vitamin D_3, requires two-stage hydroxylation. First, 25 hydroxylase generates 25(OH)D_3 in the liver. The second hydroxylation by 1-alpha-hydroxylase (CYP2781, Cytochrome P450 Family 27 Subfamily B Member 1) leads to the conversion of 25(OH)D_3 to the biologically active metabolite of vitamin D (1,25$(OH)_2D_3$). This process occurs mainly in the kidneys. 1,25$(OH)_2$ D_3 and 25(OH)D_3 are inactivated by 24-hydroxylase (CYP24A1, cytochrome P450 family 24 subfamily a member 1) the key enzymes involved in vitamin D catabolism. The direct products of the CYP24A1 reaction are 24,25$(OH)_2D_3$ and 1,24,25$(OH)_2D_3$, which are further converted to calcitroic acid or a 26,23- lactone derivative.

may lead to PTH-independent hypercalcaemia (7). There are two known underlying genetic mechanisms resulting from either mutations in *CYP24A1*encoding 24HD leading to an inability to deactivate $25(OH)D_3$ and $1,25(OH)_2D_3$ or in the sodium phosphate cotransporter IIa gene (SLC34A1, Solute Carrier Family 27 Subfamily B Member 1), resulting in excessive $1,25(OH)_2D_3$ biosynthesis secondary to chronic phosphate wasting (8). Both genetic disorders cause idiopathic infantile hypercalcaemia (HCINF), which is associated with hypersensitivity to vitamin D, especially in patients receiving vitamin D supplementation. In patients with *CYP24A1* mutations, laboratory tests revealed an increased $25(OH)D_3/24,25(OH)_2D_3$ ratio. This investigation has been recommended as a screening tool whenever genetic causes of hypercalcaemia are considered (9).

Aim of the study

The aim of this study was to determine the importance of determining vitamin D metabolites in the differential diagnosis of hypercalcaemia and the possibility of co-occurring various causes of hypercalcaemia. In this study, we present a rare case of an adult patient with PHPT and hypercalcaemia caused by two heterozygous pathogenic *CYP24A1* variants (confirmed by next-generation sequencing [NGS]) with a review of reported cases of patients with hypercalcaemia associated with pathogenic variants of *CYP24A1* and concomitant PHPT.

Case presentation

Clinical data

A 58-year-old woman was referred to the Endocrinology Unit in 2019 due to incidental findings of hypercalcaemia. Her medical history included hypertension, stage 3a chronic kidney disease (CKD), and carpal tunnel syndrome treated surgically. She had no family history of endocrine diseases or nephrolithiasis.

Biochemical tests

A laboratory test revealed hypercalcaemia, hypercalciuria, and unsuppressed PTH levels (27 pg/mL, reference range, 15-65 pg/mL) (Table 1; Figure 2).

Imaging

Computed tomography (CT) scans of the chest, abdomen and pelvis were conducted and showed no signs of malignancy or granulomatous disease.

Neck ultrasonography and technetium 99m sestamibi (MIBI) scintigraphy revealed a mass in the upper pole of the left thyroid gland lobe, which was confirmed to be the parathyroid gland after biopsy via immunohistochemical staining (Figure 2).

There were no abnormalities in the densitometry results (T score of the lumbar spine 1.1; T score of the proximal femur -0.2; T score of the radius 0.5). However, an X-ray revealed advanced subperiosteal bone resorption in the fingers and bone loss in the thoracic spine and clavicles. There were no renal stones or fractures.

Treatment and outcomes

The patient underwent effective parathyroidectomy, after which the calcium level was normalized (2.45 mmol/L, reference range, 2.2-2.55 mmol/L). Histopathology confirmed parathyroid adenoma.

Ten months after the operation, postoperative assessment revealed a normal calcium concentration (2.48 mmol/L), but vitamin D deficiency (16.4 ng/mL, reference range, 30-50 ng/mL); thus, supplementation with cholecalciferol was administered (4000 IU/daily) (Table 1; Figure 2). The patient did not attend her follow-up appointments for two years. In 2022, a laboratory test showed hypercalcaemia and hypercalciuria recurrence, which was initially identified as recurrent primary hyperparathyroidism. However, blood tests revealed PTH suppression with elevated $1,25(OH)_2D_3$ concentrations (Table 1; Figure 2).

TABLE 1 Laboratory results presented in chronological order.

Analysed parameter, units	2018[a]	2020[b]	2022[c]	2023[d]	Reference range
Corrected calcium, mmol/L	3.0	2.48	2.88	2.51	2.2-2.55
Phosphate, mg/dL	0.81	1.1	0.93	1.06	0.81-1.45
$25(OH)D_3$, ng/mL	34.8	16.4	34.2	27.4	30-50
$1,25(OH)_2D_3$, pg/mL	72.0	48.0	90.6	64.4	19.9 – 79.3
Parathormone, pg/mL	27.0	19.0	7.7	15.0	15-65
Creatinine, mg/dL GFR, ml/min/1,73 m^2	1.05 54.6	1.08 52.5	1.38 39.3	1.26 43.4	0.5-0.9 >90
Calcium in 24h urine collection, mmol/24hr	11.97	5.13	7.81	N/A	2.5-7.5

a. baseline results before parathyroidectomy; b. 10 months after parathyroidectomy without vitamin D supplementation; c. 3 years after parathyroidectomy with vitamin D 4000 IU daily; d. 4 years after parathyroidectomy without vitamin D supplementation; GFR- glomerular filtration rate; N/A - not available.

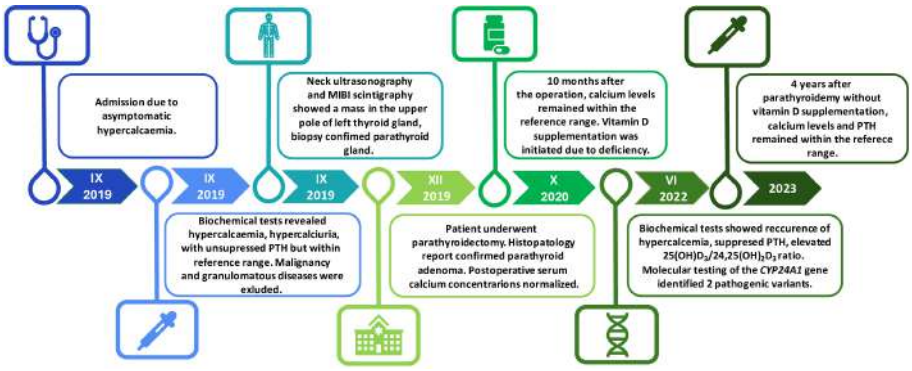

FIGURE 2
Timeline summarization of the patient's clinical course. Asymptomatic hypercalcaemia was incidentally found in 2019. The same year, primary hyperparathyroidism (PHPT) was diagnosed, and the presence of suspected lesion was confirmed in the imaging studies. The patient underwent parathyroidectomy which led to normocaicaemia. After 10 months, vitamin D supplementation was initiated due to deficiency. In 2022, biochemical tests revealed recurrence of hypercalcaemia with suppressed PTH concentration. After the exclusion of the common causes of PTH-independent hypercalceamia, the presence of two heterozygous loss-of-function mutations in the *CYP24A1* gene was confirmed in Next-Generation Sequencing (NGS). After discontinuation of vitamin D supplementation, calcium and PTH concentrations normalized. PTH-parathormone; MIBI -technetium-99m-sestamibi scintigraphy; *CYP24A1*- cytochrome P450 family 24 subfamily a member 1.

The patient was screened for granulomatous disease and hypercalcaemia of malignancy again, and the results were negative.

Vitamin D metabolites were measured using LC–MS/MS, which revealed high 25(OH)D_3 (72.62 ng/mL) and low 24,25 $(OH)_2D_3$ (0.09 ng/mL) concentrations and an elevated 25(OH) D_3/24,25$(OH)_2D_3$ ratio 806,9 (reference range, 7.0-23.6), suggesting a defect in vitamin D catabolism.

Molecular testing

The genetic testing of the *CYP24A1* gene was conducted using the NGS technique, and two pathogenic variants were identified NM_000782.5:c; 1186C>T(p.Arg396Trp, rs114368325) and NM_000782.5:c; 428_430del (p.Glu143del, rs777676129). Both variants are classified as pathogenic/likely pathogenic, and associated with hypercalcemia in the available databases, including ClinVar NIH (ClinVar archives, National Institutes of Health). Due to the unavailability of family members, it was impossible to assess whether the variants were located in the same of different alleles of *CYP24A1*, what would be a direct proof of their dominant or recessive involvement in development of the observed symptoms.

The recommendation was to discontinue vitamin D supplementation, maintain adequate hydration and avoid excessive sunlight exposure. Follow-up evaluation showed normalization of calcium and PTH concentrations (Table 1).

Discussion

Hypercalcaemia is a common clinical abnormality. PTH measurement is the key initial test for differentiating between PTH-dependent and PTH-independent hypercalcaemia.

A high or inappropriately normal PTH level in relation to a high calcium concentration is typical for PTH-dependent causes. Among them, the most common is PHPT, whose reported incidence varies between 0.2% and 1.3% across the population (10). In PTH-independent hypercalcaemia, the PTH level is appropriately suppressed for hypercalcaemia status. Malignancy is the most common disorder in this population. Among other causes of PTH-independent hypercalcaemia, vitamin D-mediated hypercalcaemia should be considered.

Currently, in many countries where the evaluation of serum calcium levels has become routine, PHPT appears to be the most common cause of hypercalcaemia and is identified most frequently in the initial asymptomatic stage (11). A growing prevalence of PHPT results in the identification of unusual cases of overlapping PTH-dependent and PTH-independent causes of hypercalcaemia. We described a woman with asymptomatic mild hypercalcaemia and ambiguous low-normal PTH, which made the initial classification of hypercalcaemia difficult. For this reason, in the differentiation, we considered both PTH-dependent and PTH-independent causes of hypercalcaemia. After excluding malignancy and granulomatous diseases, PHTP was diagnosed based on imaging studies and parathyroid biopsy. The patient underwent successful parathyroidectomy. Only the recurrence of PTH-independent hypercalcaemia after vitamin D supplementation revealed the other cause of hypercalcaemia, which turned out to be a genetic disorder of vitamin D catabolism.

Pathogenic mutations in the *CYP24A1* gene clinically manifest as infantile hypercalcaemia-1 (HCINF1, OMIM 143880), a rare disorder linked with a disturbance in vitamin D degradation (12). HCINF1 is classically characterized by severe symptoms such as vomiting, polysomnia, hypotonia, constipation, failure to thrive, and renal stone disease (13). The first case reports of HCINF1 were published in the 1950s, when approximately 200 cases of PTH-independent hypercalcaemia in infants were noted in the United Kingdom because of the wide use of formula milk enriched with increased doses of

vitamin D (up to 4000 International Units (IU)) (14, 15). Although the majority of affected children developed mild hypercalcaemia not associated with other syndromic features (HCINF1), the percentage of fatal cases was remarkable, and some affected children had multisystem disorders, which were later described as Willams–Beuren syndrome (OMIM 194050) (12, 16). At that time, vitamin D dietary intake was identified as a precipitating factor of HCINF1, yet the exact pathogenesis has remained unknown (12, 14).

Only recently, the structure of the *CYP24A1* gene and the pathways involved in vitamin D metabolism leading to HCINF1 were revealed. In 1991, Ohyama et al. described the isolation of complementary deoxyribonucleic acid (cDNA) from a rat kidney cDNA library utilizing antibodies specific for the enzyme (17). Two years later, Chen et al. published a paper on the isolation, sequencing and expression of cDNA encoding the human 24HD (18). In the same year, Hahn et al. independently isolated human kidney cDNA encoding vitamin D 24HD (19). In 2011, Schlingmann et al. confirmed that HCINF1 is mutated in *CYP24A1* based on 8 cases of infants receiving vitamin D supplementation (either daily or bolus doses) (12). The affected children were homozygotes or compound heterozygotes for nonsense or missense mutations of *CYP24A1* (inherited as autosomal recessive trait), leading to complete loss of function of 24HD confirmed in the transfected eukaryotic cell line (12). Since then, multiple pathogenic variants (PVs) in the *CYP24A1* gene have been identified, revealing that the spectrum of biallelic variants (either homozygous or compound heterozygous) ranges from severe hypercalcaemia in infants to mild hypercalcaemia in adults (20–23). Heterozygotes usually present with milder or asymptomatic phenotypes (24). However, in the case of a 44-year-old patient with intermittent hypercalcaemia and two intron–exon splice junction mutations (IVS5 + 1G>A and IVS6–2A>G) of *CYP24A1*, an analysis of family members suggested autosomal dominant inheritance with partial penetrance (25). Furthermore, the clinical phenotype is dependent not only on the PV but also on vitamin D intake, sunlight exposure or pregnancy (12, 26, 27). It is known that $1,25(OH)_2D_3$ is elevated during normal pregnancy, which additionally promotes hypercalcaemia in women with disturbed calcitriol catabolism associated with *CYP24A1* mutation (5, 28).

Hypercalcaemia related to the presence of PVs in the *CYP24A1* gene is often overlooked. Although epidemiological data concerning the prevalence of PVs of the *CYP24A1* gene are scarce, the estimated frequency of deleterious minor alleles is 0.140 (29). Thus, the calculated frequency (using the Hardy–Weinberg equilibrium) of recessive disorders is 1960 per 100.000 people (29). By adding patients harbouring monoallelic mutations who may present with a clinical phenotype, the number of affected individuals is significant, and this should be considered more often in the differential diagnosis of hypercalcaemia (30, 31).

In 2016, an autosomal recessive loss-of-function mutation in the *SLC34A1* gene was identified as the second cause of infantile hypercalcaemia-2 (HCINF2, OMIM 616963) (32). Like in patients with *CYP24A1* mutations, hypercalcaemia, suppressed PTH and inappropriately high $1,25(OH)_2D_3$ are observed. The distinguishing feature is hypophosphatemia due to renal phosphate wasting. (32)

Low PTH and high $1,25(OH)_2D_3$ levels are specific to the whole group of endogenous causes of vitamin D related to hypercalcaemia (including HCINF, granulomatous disease and some lymphomas); therefore, $24,25(OH)_2D_3$ assessment is crucial for differentiation. The distinguishing feature of HCINF1 is the very low concentration of $24,25(OH)_2D_3$. Therefore, the measurement of this metabolite is the first screening tool for HCINF1, allowing to select subjects for further genetic tests. However, in healthy individuals, there is a positive linear correlation between the concentration of $25(OH)D_3$ and $24,25(OH)_2D_3$, which results in physiological inhibition of the production of $24,25(OH)_2D_3$ when the serum $25OHD_3$ concentration falls into the vitamin D deficiency range (33). For this reason, a more accurate parameter for expressing the absence of $24,25(OH)_2D_3$ in HCINF1 patients is the ratio of $25(OH)D_3$ to $24,25(OH)_2D_3$, especially because some of these patients have a low vitamin D status (34). The technique allowing a reliable assessment of $24,25(OH)_2D_3$ is LC–MS/MS. Chromatographic separation is the reference method that allows for the precise resolution of $24,25(OH)_2D_3$ from other vitamin D metabolites. Using the LC–MS/MS method involving derivatization with DMEQ-TAD {4-[2-(6,7-dimethoxy-4-methyl-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione}, a ratio greater than 80 (normal ratio, 5 to 25) allows for the identification of HCINF1 due to the *CYP24A1* mutation (34). Using extended chromatography, which resolves $24,25(OH)_2D_3$, $25,26\text{-}(OH)_2D_3$ and $1,25(OH)_2D_3$, a ratio > 140 was used as the *cut-off* value (35).

To date, 6 cases of primary hyperparathyroidism coexisting with a *CYP24A1* mutation have been reported in 5 publications (Table 2) (36–40). The diagnosis of PHPT was made due to elevated serum calcium and inadequate nonsuppressed PTH. In most of these patients, parathyroidectomy was initially performed following the diagnosis of primary hyperparathyroidism, and after surgery, due to the persistence of hypercalcaemia, a diagnosis of 24-hydroxylase deficiency was established. Pathological examination revealed hyperplasia in the parathyroid glands in 2 patients after total or partial parathyroidectomy (37, 38). For the other subjects, adenomas were found; in one patient, a complete histopathology report was unavailable (36, 37, 39, 40). In all patients, removal of autonomous parathyroid tissue resulted in a significant decrease in the serum calcium concentration. PTH reduction may have additional value for individuals with a *CYP24A1* mutation because it eliminates the continuous stimulation of $1,25(OH)_2D_3$ synthesis, whose catabolism is disrupted. In the present case, the relatively low concentration of PTH (27 pg/mL) before parathyroidectomy was noteworthy. Primary hyperparathyroidism is characterized by elevated calcium concentrations and a lack of portal feedback between calcium concentration and PTH secretion. Usually, the PTH level is significantly elevated but can also be within the normal range. In both situations, PTH levels are clearly inadequate for elevating serum calcium levels. However, well-documented PHPT has been reported with PTH levels as low as 20 pg/mL to 25 pg/mL, given a reference range of 10 pg/mL to 65 pg/mL (11).

Vitamin D and PTH are conjugated: $1,25(OH)_2D_3$ reduces PTH directly by inhibiting its transcription and indirectly by increasing calcium absorption from the gastrointestinal tract (41). Furthermore, calcitriol induces the expression of FGF23 in bones, and FGF23 decreases PTH secretion and PTH mRNA levels (42). (42). On the other hand, PTH is the main stimulator of $1,25(OH)_2D_3$ synthesis in the kidneys (43).

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TABLE 2 The summary of reported cases of concomitant primary hyperparathyroidism and mutations in the *CYP24A1* (human cytochrome P450 24 subfamily A member 1) gene leading to impaired function of 24-hydroxylas.

Authors	Year	Country	Sex	Age of diagnosis	Type of mutation	Biallelic mutation	Protein change	Serum calcium, mmol/L	PTH, pg/ml
Helmuth et al. (36)	2014	Switzerland	M	31	missense	Yes, HH	p. Arg396Trp	3.50	49
Loyer et al. (37)	2016	France	F	39	missense/ missense	Yes, CH	p.Cys380Arg /p.Leu409Ser	3.47	92
			M	51	frameshift/ missense	Yes, CH	p.Leu335Profs11 /.p.Arg396Gln	2.62 to 2.87	9, 22 then 27
David et al. (38)	2020	Belgium	M	44	missense	Yes, HH	p.Arg396Trp	2.90	36.1
Collins et al. (39)	2023	Australia	M	53	missense	Yes, HH	p.Arg396Trp	3.08	23.6
Liu et al. (40)	2023	USA	F	23	nonsense	No, Hh	p.Pro392Argfs*9	3.45	62

PTH, parathormone; M, male; F, female; HH, homozygous; CH, compound heterozygous; Hh, heterozygous.

As shown in a systematic review and meta-analysis by Song et al., vitamin D supplementation in patients with PHPT and vitamin D deficiency significantly reduces PTH without causing hypercalcaemia or hypercalciuria (44). The coexistence of 24-hydroxylase insufficiency and the consequent extraordinarily high $1,25(OH)_2D_3$ level may intensify this process. The observed concentrations of PTH in earlier described patients with HPT coexisting with *CYP24A1* mutations differed greatly from each other. Loyer et al. described 2 patients with inappropriate/high PTH levels (22 pg/mL to 92 pg/mL) (37). In the case described by Helmuth et al., the preoperative PTH concentration was 58 pg/mL (36). Another patient had PTH ranging between 36.6 pg/mL and 80.3 pg/mL during the year preceding parathyroid surgery (38). In a recent case, low-normal PTH concentrations (23.6 pg/mL) before and after the first partial parathyroidectomy were observed, and after the second parathyroid surgery, PTH became undetectable (39). The only known patient with a heterozygous pathogenic variant in *CYP24A1* had a preoperative PTH level of 63 pg/mL (40).

It is unclear whether the co-occurrence of PHPT with a *CYP24A1* mutation in these patients is a unique coincidence or is a new phenotype of *CYP24A1* mutation combined with hyperparathyroidism. The biochemical profile of patients with 24-hydroxylase deficiency-hypercalcaemia and especially high $1,25(OH)_2D_3$ decreases not only PTH secretion but also parathyroid cell proliferation. Calcitriol reduces parathyroid volume through the suppression of the cell cycle regulator c-Myc, the suppression of transforming growth factor-α (TGF-α), and the induction of p21 (p21 protein), which is an inhibitor of the cell cycle (45). The opposite condition in which parathyroid hyperplasia occurs as a result of hyperphosphatemia, calcitriol deficiency, or hypocalcaemia is renal failure. In our patient, as well as in the other 5 described patients with the *CYP24A1* mutation and PHPT, mild renal failure was noted; however, the phosphate concentration remained low (36–39).

In most of the discussed cases, the $25(OH)D_3$:$24.25(OH)_2D_3$ ratio was determined before genetic testing (37, 38, 40). Except for one patient with a heterozygous pathogenic variant in *CYP24A1* ($25(OH)D_3$:$24.25(OH)_2D_3$ ratio of 25.18), the ratios were above 100 (37, 38, 40).

In the first reported case, a homozygous loss-of-function mutation (p.Arg396Trp) in the gene encoding vitamin D 24-hydroxylase was identified; second, the most prevalent mutation in the *CYP24A1* gene was found in patients with HCINF1 (5, 30, 36). In patients with the p.Arg396Trp mutation, the arginine-to-tryptophane substitution leads to complete loss of catabolic activity of 24-hydroxylase due to destruction of the hydrogen bonds between arginine and the haem propionate group, thus blocking transient but crucial 24-hydroxylase substrate binding to haem (46, 47). Interestingly, the homozygous p.Arg396Trp mutation was also found in the cases described by David et al. and Collins et al. (38, 39). In most of the reported cases, the genetic panel was extended by the analysis of the genes associated with hyperparathyroidism, i.e., MEN1 (encoding the tumour suppressor protein menin; MEN1, Multiple Endocrine Neoplasia type 1), CaSR (encoding the G protein-coupled extracellular calcium-sensing receptor), HRPT2 (encoding the tumour suppressor parafibromin) and CDK (encoding the tumour suppressor cyclin-dependent kinase) (37, 48, 49). In the case reported by David et al., CaSR and MEN1 mutations, which are underlying causes of hyperparathyroidism, were excluded (38). In the case described by Collins et al., genetic testing confirmed that the patient was homozygous for the pathogenic variant c.1186C>T, p.Arg396Trp in the *CYP24A1* gene (39). (39) Documented cases of primary hyperparathyroidism and missense p.Arg396Trp mutation raise the question of whether the described genetic alteration predisposes patients to parathyroid autonomy. The link between loss-of-function *CYP24A1* mutations and the development of primary hyperparathyroidism has not been confirmed (50). In the present case, a heterozygous missense variant (rs114368325; p.Arg396Trp) was also identified, together with the second

pathogenic variant. In this study, we were unable to confirm the heterozygosity or compound heterozygosity of both variants; therefore, we cannot discuss the recessive or dominant inheritance of the disease.

The coexistence of parathyroid autonomy and pathogenic variants in the *CYP24A1* gene is not associated only with p.Arg396Trp mutations. Loyer et al. reported two cases of concomitant PTH-dependent hypercalcaemia and deleterious compound heterozygous mutations in the gene encoding vitamin D 24-hydroxylase (37). In the first case, a 39-year-old female with persistent hypercalcaemia after the removal of hyperplastic parathyroid fluid, p.Cys380Arg/p. Leu409Ser compound heterozygous mutations were identified (37). Interestingly, in the same patient, an intronic HRPT2 gene polymorphism (c.1418-17C>G) was found (37). In the second case, genetic testing confirmed compound heterozygous (p.Leu335Profs11/.p.Arg396Gln) mutations in the *CYP24A1* gene, intronic polymorphisms (c.237 + 28 T>C) in the HRPT2 gene and the p.Gln1011Glu variant in the CaSR gene (37). Hypercalcaemia due to a novel heterozygous pathogenic variant (p.Pro392Argfs*9) in the CYP24A1 gene and concomitant primary hyperparathyroidism were documented in the case of a 23-year-old female by Liu et al. (40).

In PHPT, the only curative treatment is parathyroidectomy. In patients with proven *CYP*24A1 mutations, management should concentrate on eliminating or reducing hypercalcaemia and hypercalciuria. Thus, the basis of long-term strategies is a low vitamin D and calcium diet, in which vitamin D supplementation is omitted and sunlight exposure is avoided. In patients for whom this approach is not sufficient, various pharmacologic therapies have been described. Glucocorticoids that reduce enteral calcium absorption and inhibit the conversion of serum $25(OH)D_3$ to active $1,25(OH)_2D_3$ were found to be ineffective in patients with *CYP24A1* defects (7, 51). However, effective therapeutic outcomes have been described in patients treated with imidazoles, such as ketoconazole or less toxic fluconazole, which reduce $1,25(OH)_2D_3$ synthesis by inhibiting CYP enzymes (cytochrome enzymes) (25, 52).

The limitation of this study was the unavailability of patient family members, which allowed us to clearly determine whether the variants were located cis or trans within the *CYP24A1* gene and therefore how their concurrence affected the clinical outcome of the patient.

Conclusions

The present case underlines the importance of accurate clinical evaluation of hypercalcaemia. Rarely may multiple causes of hypercalcaemia coexist, which complicates the diagnostic process. Identification of such conditions often requires a wider range of diagnostic techniques. In patients with PTH-independent hypercalcaemia, the measurement of vitamin D metabolites using LC–MS/MS analytical technique, followed by genetic testing (e.g., NGS technique), may help to identify carriers of *CYP24A1* mutations.

Patient perspective

Currently, the patient reports an improvement in her wellbeing. The patient is pleased that the present treatment was limited by adequate hydration, the avoidance of sun exposure and the lack of vitamin D supplementation.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding authors.

Ethics statement

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

DL: Project administration, Methodology, Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. AS: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. JL: Writing – review & editing, Writing – original draft, Investigation, Data curation. MK: Writing – review & editing, Writing – original draft, Investigation, Data curation. MD: Writing – review & editing, Writing – original draft, Investigation, Data curation. AW: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. WM: Writing – review & editing, Writing – original draft, Supervision. WZ: Validation, Writing – review & editing, Writing – original draft, Supervision, Conceptualization. PG: Validation, Project administration, Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

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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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References

1. Goltzman D. Pathophysiology of hypercalcemia. *Endocrinol Metab Clinics North America*. (2021) 50:591–607. doi: 10.1016/j.ecl.2021.07.008

2. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinol Metab Clinics North America*. (2010) 39:243–53. doi: 10.1016/j.ecl.2010.02.002

3. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab*. (2009) 94:26–34. doi: 10.1210/jc.2008-1454

4. Prosser DE, Jones G. Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem Sci*. (2004) 29:664–73. doi: 10.1016/j.tibs.2004.10.005

5. De Paolis E, Scaglione GL, De Bonis M, Minucci A, Capoluongo E. CYP24A1 and SLC34A1 genetic defects associated with idiopathic infantile hypercalcemia: From genotype to phenotype. *Clin Chem Lab Med (CCLM)*. (2019) 57:1650–67. doi: 10.1515/cclm-2018-1208

6. Saponaro F, Saba A, Frascarelli S, Prontera C, Clerico A, Scalese M, et al. Vitamin D measurement and effect on outcome in a cohort of patients with heart failure. *Endocrine Connections*. (2018) 7:957–64. doi: 10.1530/ec-18-0207

7. Schlingmann KP. Vitamin D–dependent hypercalcemia. *Endocrinol Metab Clinics North America*. (2021) 50:729–42. doi: 10.1016/j.ecl.2021.08.005

8. Kaufmann M, Schlingmann KP, Berezin L, Molin A, Sheftel J, Vig M, et al. Differential diagnosis of vitamin D related hypercalcemia using serum vitamin D metabolite profiling. *J Bone Mineral Res*. (2021) 36(7):1340–50. doi: 10.1002/jbmr.4306

9. Dirks NF, Ackermans MT, de Jonge R, Heijboer AC. Reference values for 24,25-dihydroxyvitamin D and the 25-hydroxyvitamin D/24,25-dihydroxyvitamin D ratio. *Clin Chem Lab Med*. (2019) 57:e259–61. doi: 10.1515/cclm-2018-1096

10. Soto-Pedre E, Newey PJ, Leese GP. Stable incidence and increasing prevalence of primary hyperparathyroidism in a population-based study in scotland. *J Clin Endocrinol Metab*. (2023) 108(10):e1117–e1124. doi: 10.1210/clinem/dgad201

11. Bilezikian JP. Primary hyperparathyroidism. *J Clin Endocrinol Metab*. (2018) 103:3993–4004. doi: 10.1210/jc.2018-01225

12. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *New Engl J Med*. (2011) 365:410–21. doi: 10.1056/NEJMoa1103864

13. Marks BE, Doyle DA. Idiopathic infantile hypercalcemia: Case report and review of the literature. *J Pediatr Endocrinol Metab*. (2016) 29. doi: 10.1515/jpem-2015-0133

14. Group BMJP. Hypercalcaemia in infants and vitamin D. *Br Med J*. (1956) 2:149–9. doi: 10.1136/bmj.2.4985.149

15. Lightwood R, Stapleton T. Idiopathic hypercalcæmia in infants. *Lancet*. (1953) 262:255–6. doi: 10.1016/s0140-6736(53)90187-1

16. Pober BR. Williams–beuren syndrome. *New Engl J Med*. (2010) 362:239–52. doi: 10.1056/nejmra0903074

17. Ohyama Y, Noshiro M, Okuda K. Cloning and expression of cDNA encoding 25-hydroxyvitamin D3 24-hydroxylase. *FEBS Lett*. (1991) 278:195–8. doi: 10.1016/0014-5793(91)80115-J

18. Chen K-S, Prahl JM, DeLuca HF. Isolation and expression of human 1,25-dihydroxyvitamin D3 24-hydroxylase cDNA. *Proc Natl Acad Sci United States America*. (1993) 90:4543–7. doi: 10.1073/pnas.90.10.4543

19. Hahn C, Baker E, Laslo P, May BK, Omdahl J, Sutherland GR. Localization of the human vitamin D 24-hydroxylase gene (CYP24) to chromosome 20q13.2→q13.3. *Cytogenetic Genome Res*. (1993) 62:192–3. doi: 10.1159/000133473

20. Jones G, Kottler ML, Schlingmann KP. Genetic diseases of vitamin D metabolizing enzymes. *Endocrinol Metab Clinics North America*. (2017) 46:1095–117. doi: 10.1016/j.ecl.2017.07.011

21. Carpenter TO. CYP24A1 loss of function: Clinical phenotype of monoallelic and biallelic mutations. *J Steroid Biochem Mol Biol*. (2017) 173:337–40. doi: 10.1016/j.jsbmb.2017.01.006

22. Cappellani D, Brancatella A, Kaufmann M, Minucci A, Vignali E, Canale D, et al. Hereditary hypercalcemia caused by a homozygous pathogenic variant in the CYP24A1 gene: a case report and review of the literature. *Case Rep Endocrinol*. (2019) 2019:4982621. doi: 10.1155/2019/4982621

23. Tebben PJ, Singh RJ, Kumar R. Vitamin D-mediated hypercalcemia: Mechanisms, diagnosis, and treatment. *Endocrine Rev*. (2016) 37:521–47. doi: 10.1210/er.2016-1070

24. Cools M, Goemaere S, Baetens D, Raes A, Desloovere A, Kaufman JM. Calcium and Bone Homeostasis in Heterozygous Carriers of CYP24A1 mutations: a cross-sectional Study. *Bone*. (2015) 81:89–96. doi: 10.1016/j.bone.2015.06.018

25. Tebben PJ, Milliner DS, Horst RL, Harris PC, Singh RJ, Wu Y. Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to*CYP24A1*Mutations: Effects of ketoconazole therapy. *J Clin Endocrinol Metab*. (2012) 97:E423–7. doi: 10.1210/jc.2011-1935

26. Figueres M-L, Linglart A, Bienaime F, Allain-Launay E, Roussey-Kessler G, Ryckewaert A. Kidney function and influence of sunlight exposure in patients with impaired 24-hydroxylation of vitamin D due to CYP24A1 mutations. *Am J Kidney Diseases: Off J Natl Kidney Foundation*. (2015) 65:122–6. doi: 10.1053/j.ajkd.2014.06.037

27. Shah A, Hsiao EC, O'Donnell B, Salmeen K, Nussbaum RL, Krebs M. Maternal hypercalcemia due to failure of 1,25-dihydroxyvitamin-D_3Catabolism in a patient with*CYP24A1*Mutations. *J Clin Endocrinol Metab*. (2015) 100:2832–6. doi: 10.1210/jc.2015-1973

28. Kumar R, Cohen WR, Lisbôa C, Epstein FH. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J Clin Invest*. (1979) 63:342–4. doi: 10.1172/jci109308

29. Nesterova G, Malicdan MC, Yasuda K, Sakaki T, Vilboux T, Ciccone C. 1,25-(OH)2D-24 hydroxylase (CYP24A1) deficiency as a cause of nephrolithiasis. *Clin J Am Soc Nephrol CJASN*. (2013) 8:649–57. doi: 10.2215/CJN.05360512

30. Cappellani D, Brancatella A, Morganti R, Borsari S, Baldinotti F, Caligo MA. Hypercalcemia due to CYP24A1 mutations: A systematic descriptive review. *Eur J Endocrinol*. (2021) 186:137–49. doi: 10.1530/eje-21-0713

31. Brancatella A, Cappellani D, Kaufmann M, Borsari S, Piaggi P, Baldinotti F. Do the heterozygous carriers of a *CYP24A1* mutation display a different biochemical phenotype than wild types? *J Clin Endocrinol Metab*. (2020) 106:708–17. doi: 10.1210/clinem/dgaa876

32. Schlingmann KP, Ruminska J, Kaufmann M, Dursun I, Patti M, Kranz B. Autosomal-recessive mutations in SLC34A1 encoding sodium-phosphate cotransporter 2A cause idiopathic infantile hypercalcemia. *J Am Soc Nephrol*. (2016) 27:604–14. doi: 10.1681/asn.2014101025

33. Leszczyńska D, Szatko A, Raczkiewicz D, Ostrowska M, Misiorowski W, Zgliczyński W. The effect of a single high dose of vitamin D on serum levels of its metabolites in the elderly. *Front Bioscience*. (2022) 27:289–9. doi: 10.31083/j.fbl2710289

34. Kaufmann M, Gallagher JC, Peacock M, Schlingmann K-P, Konrad M, DeLuca HF. Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD. *J Clin Endocrinol Metab*. (2014) 99:2567–74. doi: 10.1210/jc.2013-4388

35. Kaufmann M, Morse N, Molloy BJ, Cooper DP, Schlingmann KP, Molin A. Improved screening test for idiopathic infantile hypercalcemia confirms residual levels of serum 24,25-$(OH)_2D_3$ in affected patients. *J Bone Mineral Res*. (2017) 32:1589–96. doi: 10.1002/jbmr.3135

36. Helmuth A, Konrad M, Schlingmann KP, Pasch A. The case | Hypercalcemia in a 60-year-old male. *Kidney Int*. (2014) 85:219–21. doi: 10.1038/ki.2013.184

37. Loyer C, Leroy C, Molin A, Odou MF, Huglo D, Lion G. Hyperparathyroidism complicating CYP 24A1 mutations. *Annales d'Endocrinologie*. (2016) 77:615–9. doi: 10.1016/j.ando.2016.03.002

38. David K, Khalil R, Hannon H, Evenepoel P, Decallonne B. Therapy-resistant hypercalcemia in a patient with inactivating CYP24A1 mutation and recurrent nephrolithiasis: Beware of concomitant hyperparathyroidism. *Calcified Tissue Int*. (2020) 107:524–8. doi: 10.1007/s00223-020-00738-8

39. Collins L, Boehm E, Luxford C, Clifton-Bligh R, Grill V. Hypervitaminosis D secondary to a *CYP24A1* loss-of-function mutation: An unusual cause of hypercalcemia in two siblings. *JBMR plus*. (2023) 7(9):e10788. doi: 10.1002/jbm4.10788

40. Liu J, Angelos P, Barhoum M, Jain R. Severe hypercalcemia due to primary hyperparathyroidism and heterozygous pathogenic variant of CYP24A1. *JCEM Case Rep*. (2023) 1(4):luad071. doi: 10.1210/jcemcr/luad071

41. Bienaimé F, Prié D, Friedlander G, Souberbielle JC. Vitamin D metabolism and activity in the parathyroid gland. *Mol Cell Endocrinol*. (2011) 347:30–41. doi: 10.1016/j.mce.2011.05.031

42. Martin A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathways. *Physiol Rev*. (2012) 92:131–55. doi: 10.1152/physrev.00002.2011

43. Saliba W, Barnett O, Rennert HS, Lavi I, Rennert G. The relationship between serum 25(OH)D and parathyroid hormone levels. *Am J Med*. (2011) 124:1165–70. doi: 10.1016/j.amjmed.2011.07.009

44. Song A, Zhao H, Yang Y, Liu S, Nie M, Wang O, et al. Safety and efficacy of common vitamin D supplementation in primary hyperparathyroidism and coexistent vitamin D deficiency and insufficiency: a systematic review and meta-analysis. *J Endocrinological Invest*. (2021) 44(8):1667–77. doi: 10.1007/s40618-020-01473-5

45. Mizobuchi M, Ogata H, Koiwa F. Secondary hyperparathyroidism: pathogenesis and latest treatment. *Ther Apheresis Dialysis*. (2018) 23:309–18. doi: 10.1111/1744-9987.12772

46. Hong-Fang J, Shen L. *CYP24A1*Mutations in idiopathic infantile hypercalcemia. *New Engl J Med*. (2011) 365:1741–3. doi: 10.1056/nejmc1110226

47. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *J Lipid Res*. (2013) 55:13–31. doi: 10.1194/jlr.r031534

48. Falchetti A, Marini F, Luzi E, Giusti F, Cavalli L, Cavalli T. Multiple endocrine neoplasia type 1 (MEN1): Not only inherited endocrine tumors. *Genet Med*. (2009) 11:825–35. doi: 10.1097/gim.0b013e3181be5c97

49. Hannan FM, Thakker RV. Calcium-sensing receptor (CaSR) mutations and disorders of calcium, electrolyte and water metabolism. *Best Pract Res Clin Endocrinol Metab*. (2013) 27:359–71. doi: 10.1016/j.beem.2013.04.007

50. Chorti A, Cheva A, Chatzikyriakidou A, Achilla C, Boulogeorgou K, Despoina K. Sporadic parathyroid adenoma: an updated review of molecular genetics. *Front Endocrinol*. (2023) 14:2023.1180211. doi: 10.3389/fendo.2023.1180211

51. Colussi G, Ganon L, Penco S, De Ferrari ME, Ravera F, Querques M. Chronic hypercalcaemia from inactivating mutations of vitamin D 24-hydroxylase (CYP24A1): implications for mineral metabolism changes in chronic renal failure. *Nephrology Dialysis Transplantation: Off Publ Eur Dialysis Transplant Assoc - Eur Renal Assoc*. (2014) 29:636–43. doi: 10.1093/ndt/gft460

52. Sayers J, Hynes AM, Srivastava S, Dowen F, Quinton R, Datta HK. Successful treatment of hypercalcaemia associated with a CYP24A1 mutation with fluconazole. *Clin Kidney J*. (2015) 8:453–5. doi: 10.1093/ckj/sfv028

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Dalila Scaturro,
University of Palermo, Italy
Ziyi Wang,
Johns Hopkins University, United States
Hao Zhang,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Peng Xu
xupeng369@mail.xjtu.edu.cn

†These authors have contributed equally to this work

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The role of magnesium in the pathogenesis of osteoporosis

Lin Liu[1†], Pan Luo[2†], Pengfei Wen[1] and Peng Xu[1*]

[1]Department of Joint Surgery, HongHui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, China, [2]Department of Auricular Reconstruction, Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Magnesium (Mg), a nutritional element which is essential for bone development and mineralization, has a role in the progression of osteoporosis. Osteoporosis is a multifactorial disease characterized by significant deterioration of bone microstructure and bone loss. Mg deficiency can affect bone structure in an indirect way through the two main regulators of calcium homeostasis (parathyroid hormone and vitamin D). In human osteoblasts (OBs), parathyroid hormone regulates the expression of receptor activator of nuclear factor-κ B ligand (RANKL) and osteoprotegerin (OPG) to affect osteoclast (OC) formation. In addition, Mg may also affect the vitamin D3 -mediated bone remodeling activity. vitamin D3 usually coordinates the activation of the OB and OC. The unbalanced activation OC leads to bone resorption. The RANK/RANKL/OPG axis is considered to be a key factor in the molecular mechanism of osteoporosis. Mg participates in the pathogenesis of osteoporosis by affecting the regulation of parathyroid hormone and vitamin D levels to affect the RANK/RANKL/OPG axis. Different factors affecting the axis and enhancing OC function led to bone loss and bone tissue microstructure damage, which leads to the occurrence of osteoporosis. Clinical research has shown that Mg supplementation can alleviate the symptoms of osteoporosis to some extent.

KEYWORDS

magnesium, osteoporosis, parathyroid hormone, vitamin D, nutrition

1 Introduction

Bone is continuously remodeled through the coordination and interaction between osteoclasts (OCs) and osteoblasts (OBs) to achieve bone homeostasis (1). Individuals with osteoporosis (OP), a systemic bone disease associated with ageing, are prone to fractures because of decreased bone density and quality caused by bone homeostasis imbalance and destruction of the bone microstructure (2, 3). In addition, patients with OP are more prone to micronutrient deficiency. An increase in micronutrient intake may have an osteoprotective effect on patients with OP (4). A meta-analysis by Feng et al. showed that dietary patterns were related to the incidence of OP (5). Magnesium (Mg), along with calcium (Ca) and vitamin D (VD), are key regulators of bone health and have an obvious influence on OP risk (6).

Approximately 99% of Mg is found in bones, muscles and soft tissues (7). Approximately 50–60% of Mg resides as surface substituents of the hydroxyapatite mineral component of bone (8). A considerable portion of skeletal Mg is mainly distributed on cortical bones (9). A large part of the Mg in bone may be deposited as apatite crystals. In addition to its structural function, Mg is a key element for all living cells, including OBs and OCs. In cells, Mg is essential for many physiological functions. First, Mg is the basis of ATP, and ATP is an essential energy source for cells (10). In addition, Mg is a cofactor of different enzymes associated with the synthesis of lipids and proteins. Moreover, Mg antagonizes Ca and acts as a signal sensor (11, 12). Therefore, changes in Mg homeostasis can affect cell and tissue function.

Many studies have suggested that Mg deficiency is a risk factor for OP (6, 13). In a study of rats, researchers have shown that controlling Mg intake in the daily diet can alleviate the symptoms of OP (13). If animals consume less Mg, it can lead to fragile bones and further cause microfractures of the trabeculae, resulting in extremely significant harm (14). Cohort research involving 73684 postmenopausal women revealed that lower Mg intake was related to reduced hip bone density (15). In addition, cross-sectional studies from the UK have found that dietary Mg may play a role in musculoskeletal health and is associated with population prevention strategies for myopenia, osteoporosis and fractures (16). Therefore, the main aim of this article is to summarize the effect of Mg on the pathogenesis of OP.

2 Pathogenesis of OP

Bone remodeling is regulated by osteocytes, OCs, OBs, bone lining cells, and endothelial cells in the bone microenvironment (17). These cells play a dynamic role in the formation and maintenance of bone integrity. Osteocytes exist in the voids of the matrix and are the mechanical receptors of bone tissue. Osteocytes maintain the physiological function of bone by interacting with various signals to transmit mechanical force to chemical signaling pathways (18). The OB plays an important role in body tissues and is synthesized by undifferentiated mesenchymal cells. Medical research has shown that OBs are involved in bone formation and growth. OCs are a type of multinucleated giant cell whose function is to promote bone resorption. Their main function is to absorb bone and prepare a matrix for bone generation (18, 19).

A variety of proteins and signaling molecules are involved in the regulation of bone homeostasis. It is wildly believed that the RANK/RANKL/OPG axis is a key factor in the molecular mechanism of OP (20–22). (Figure 1) Various factors affect the axis and enhance the formation of OCs to a state of decompensation, resulting in reduced bone mass and damage to the bone tissue microstructure, which leads to the occurrence of OP (23). In the process of OC differentiation and activation, OBs participate in the regulation of OC differentiation by expressing RANKL and OPG (24). RANKL binds to RANK and activates OC differentiation through the

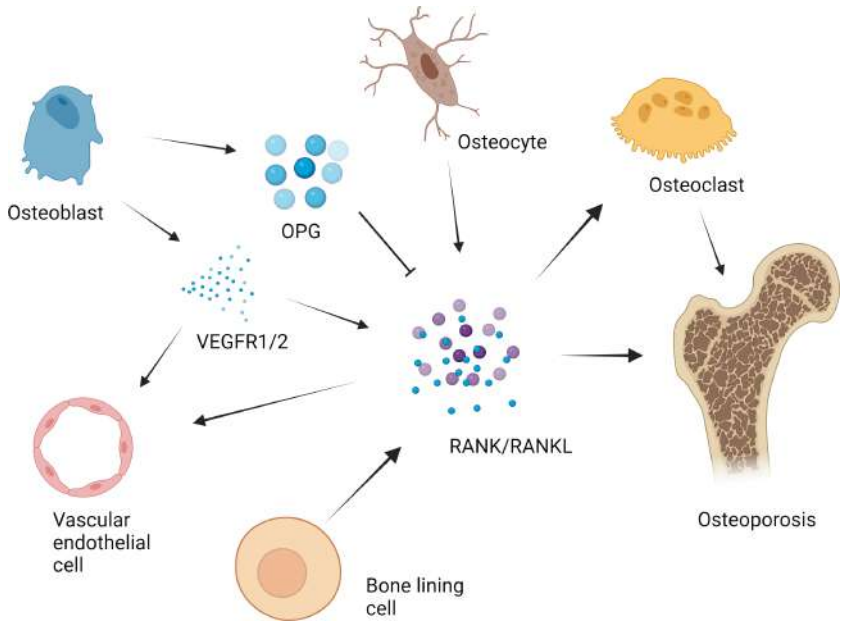

FIGURE 1
RANK/RANKL/OPG axis in the pathogenesis of osteoporosis. The RANK/RANKL/OPG axis plays a key role in the molecular mechanism of osteoporosis. Various factors affect the axis and cause osteoclast formation to a state of bone remodeling decompensation, resulting in a reduction in bone mass and damage to the bone tissue microstructure, which leads to the occurrence of osteoporosis. For example, in the process of osteoclast differentiation and activation, osteoblasts express RANKL and OPG, which participate in the regulation of osteoclast differentiation. RANKL binds to RANK and activates the downstream signaling pathway, leading to the activation of osteoclast differentiation. OPG suppresses the above effects by inhibiting the RANKL-RANK interaction. The RANKL secreted by osteocytes plays an important role in the formation of osteoclasts in bone. The expression of RANK in vascular endothelial cells in the bone microenvironment is upregulated by vascular endothelial growth factor (VEGF), which enhances the angiogenic response to RANKL. Previous studies have shown that bone lining cells express RANKL and other osteoblast markers during active remodeling intervals in the lining, which is responsible for the interaction between RANKL and the osteoclast precursor receptor RANK.

activation of downstream signaling pathways, while OPG inhibits these effects by inhibiting the RANKL-RANK interaction.

Osteocytes have been shown to be regulators of mineral metabolism and periluminal matrix remodeling as well as the function of mechanosensory cells (25). It has been determined that osteocytes express RANKL and that RANKL secreted by osteocytes is most important for the formation of physiologically supported OCs in developing bones (26). After long-term research, some scholars have shown that bone lining cells can synthesize factors such as RANKL (27). Further research revealed that these cells can also regulate the ability of RANKL to bind to RANK receptors (28). Long-term studies have shown that vascular endothelial growth factor (VEGF) is involved in bone remodeling (29). OBs can express VEGF receptor 1 (VEGFR1) and VEGFR2 and release VEGF upon stimulation with VD3 (30). The expression of RANK on endothelial cells in the bone microenvironment was upregulated by VEGF, which subsequently enhanced the angiogenic response to RANKL (29).

The most common cause of OP is a lack of estrogen in postmenopausal women, which can lead to increased OC activity and bone mass loss, resulting in OP and osteoporotic fractures (31). Ca is the most basic mineral component in bone, and insufficient Ca intake will lead to decreases in bone mass. Therefore, VD deficiency can cause OP. Parathyroid hormone (PTH) is a hormone secreted by the parathyroid gland that is mainly responsible for the metabolism of Ca and phosphorus and regulating the levels of these two elements in the body. PTH plays a key role in maintaining Ca and phosphorus levels. A high or low level of secreted PTH may lead to abnormal metabolism of the two elements in the body, leading to OP (32).

3 The role of Mg in the pathogenesis of OP

Mg can strongly promote bone development and mineralization, by increasing the activity of phosphatase (33). Insufficient intake of magnesium in daily diet can lead to a decrease in bone mineral density. According to the results of animal studies, insufficient dietary Mg intake promotes the occurrence of OP (6). That not only reduces bone density but also alters the levels of PTH and 1,25(OH)2-VD in tissues, thereby inhibiting the body's absorption of Ca and ultimately inducing hypocalcemia. Scholars have conducted long-term studies on humans, and the results show that hypomagnesemia can significantly inhibit the synthesis of PTH in the body, leading to damage to related organs (34). For example, clinical studies by Ohya et al. have shown a significant correlation between serum Mg and intact PTH (iPTH) levels (35). The clinical evidence of Cheung et al. shows that combined Mg and VD therapy may increase serum 25-hydroxyvitamin D concentration more effectively than supplementation of VD alone (36). Due to the increase in PTH levels, the activity of adenylate cyclase can be enhanced, thereby promoting the secretion of cyclic adenosine monophosphate (AMP) (13, 37). And these enzymes need sufficient participation of Mg to function, which means that resistance to PTH can lead to not fully stimulated enzyme activity. Notably, hypomagnesemia can also induce an inflammatory response (38) and further lead to bone loss (39). Scholars have analyzed the effects of Mg on blood vessels by constructing animal models and concluded that Mg can improve endothelial function and lower blood pressure. However, a decrease in the volume of blood vessels within the bone may trigger nerve injury-induced OP (40) and OP in elderly individuals (41).

3.1 Effect of Mg on OBs in the pathogenesis of OP

Several researchers have investigated the influence of Mg on the differentiation or function of OBs (42, 43). Bed et al. indicated that extracellular Mg (2+) and melastatin-like transient receptor potential 7 (TRPM7) are important for platelet-derived growth factor (PDGF)-induced proliferation and migration of human osteoblasts (44).

In vivo experiments and clinical studies have shown that high concentrations of Mg can inhibit the secretion of PTH (45–47). Under normal physiological conditions, Mg affects the secretion of PTH in a manner similar to that of Ca. (Figure 2) Specifically, increased serum Mg binds to Ca sensor receptors on parathyroid cells, resulting in increased levels of intracellular Ca and decreased PTH secretion. In contrast, the level of serum PTH increased with decreasing serum Mg. PTH enhances bone formation via different mechanisms, including direct activation on OBs, induction of insulin-like growth factor (IGF)-1 and possible inhibition of sclerostin (SOST) (48). PTH mainly promotes OB division through the involvement of enzymes such as protein kinase A (PKA) (49). Scholars have explored the effects of PTH on bone tissue by constructing animal models and conducting intergroup control experiments in humans (50). The results showed that PTH administration can significantly promote the division of OBs, thereby accelerating the formation of bone tissue stimulated. Moreover, PTH administration can also promote the deposition of mineralized matrix by regulating the proliferation of osteoblast precursors and other pathways (51).

Nevertheless, the main direct effect of iPTH on OBs *in vivo* is to reduce the apoptosis of OBs but not to promote the proliferation of preosteoblasts. Moreover, PTH signal transduction in OBs affects Runx2, which is a transcription factor associated with OB differentiation and function (52). In addition to Runx2, many studies have suggested that multiple additional target genes (liver ligand protein B2, IGF-1, FGF2, PTH-related protein and MMP13) in OBs contribute to the antiapoptotic effect of iPTH therapy (53–57). In addition to apoptosis, iPTH can play a vital role in OB differentiation and function through the WNT signaling pathway (58). However, WNT signaling has different effects on OBs depending on their differentiation stage (59). Research has shown that in preosteoblasts, WNT signaling can stimulate and accelerate the division of OBs, playing a very important role in the development of bone tissue (60). In mature OBs, these signals can increase the level of OPG, which is the bait receptor for RANKL, thereby slowing the absorption of bone (61).

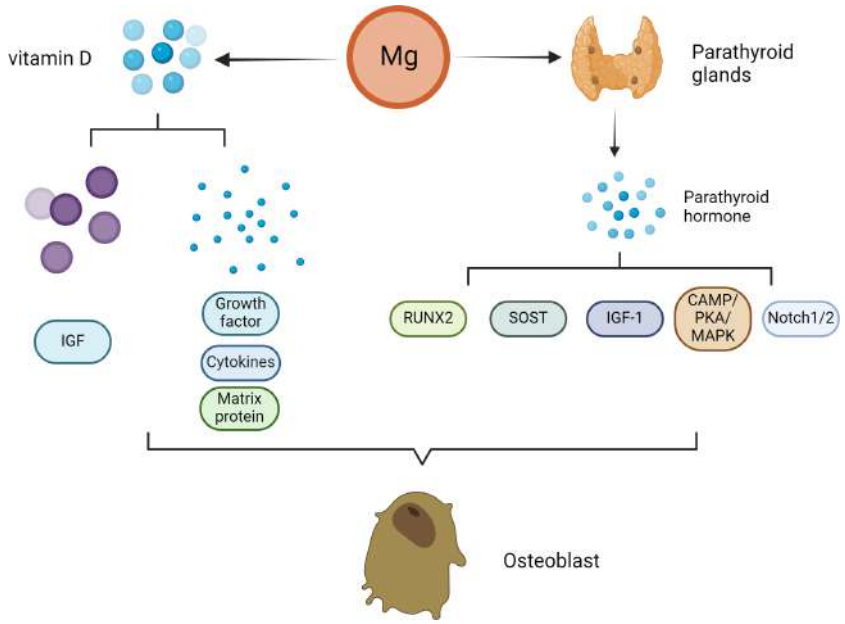

FIGURE 2
Effect of Mg on osteoclasts in the pathogenesis of osteoporosis. With decreasing serum Mg, the serum parathyroid hormone level increased. PTH enhances bone formation through a variety of mechanisms, including direct action on osteoblasts, induction of insulin-like growth factor (IGF)-1 and possible inhibition of sclerostin (SOST). The direct stimulation of osteoblast function by PTH is mainly mediated by the activation of cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) and mitogen activated protein kinase (MAPK). PTH signal transduction in osteoblasts affects Runx2. Runx2 is a transcription factor that plays an important role in osteoblast differentiation and function. Notch signal regulation is another mechanism by which PTH promotes osteogenesis. Notch1 inhibits the differentiation of mesenchymal progenitor cells and mature osteoblasts and bone trabecular formation, and Notch2 has a similar inhibitory effect on the function of osteoblasts. In addition to patients with parathyroid hormone secretion disorders, patients with Mg deficiency usually have low serum vitamin D concentrations. Vitamin D can affect not only the proliferation of osteoblasts but also the differentiation of osteoblasts. The extracellular environment (growth factors, cytokines, matrix proteins, calcium/phosphorus and other signaling molecules) and the intracellular environment (e.g., insulin-like growth factor binding protein-6) influence the final effect of vitamin D.

PTH can also promote bone tissue growth through Notch signaling. Previous studies have shown that PTH upregulates Jag1 expression in OBs (62). In addition to *Jag1*, PTH can up-regulate the expression of other Notch components in bone, especially *Notch2* and *Dll1* (63). Researchers have shown that Notch1 can prevent the proliferation and division of mature OBs and interfere with the formation of bone trabeculae. Moreover, Notch2 has a similar inverse effect on OB function (64). Studies by Canalis et al. have shown that gene activation of Notch2 signal transduction stimulates OC differentiation and absorption (65). In addition, Jesus et al. shown that the increases in Notch ligands and receptors lead to Notch activation, as PTH also elevated the expression of several Notch target genes in bone, particularly Hes1 that was elevated across all the experiments (63). In addition, the activation of WNT signal in OBs or osteocytes increases the Notch signal in bone, indicating that there is crosstalk between the two pathways (66, 67).

In addition to PTH secretion disorders, the serum concentrations of the VD active metabolite 1,25(OH) 2 D 3 are often low in patients with Mg deficiency. This may be due explained by low serum PTH levels or renal are resistant to PTH because PTH is the main physiological regulator of $1,25(OH)_2$-VD synthesis. Mg deficiency is harmful to this process because the synthesis of $1,25(OH)_2$-VD depends on the presence of Mg. Previous studies have demonstrated the direct influence of 1α,25(OH) (2)D(3) on the survival of OBs, which varies with the treatment time, dose, source and environment of the OBs (68–70).

Additionally, 1α,25(OH) (2)D(3) can affect not only the proliferation of OBs but also the differentiation of OBs (71, 72). The extracellular environment (VEGF, cytokines, Ca/phosphorus ions et al.) and the intracellular environment (IGF binding protein-6) affect the final effect of 1α,25(OH) (2)D(3) (73). These factors can regulate the role of 1α,25(OH) (2)D(3) and impact the final reaction. A typical intracellular pathway is WNT signaling. Standard WNT signals are essential for bone formation. The lipoprotein-related receptors 5 and 6 (LRP5/6) promote the secretion of members of the WNT-related protein family and binds to the membrane receptor on OBs. The absence of LRP5 leads to a decrease in the number of OBs, delayed mineralization and a reduction in peak bone mineral density. However, 1α,25(OH) (2)D(3) can cause the binding of vitamin D receptor (VDR) to the LRP5 locus (74). Therefore, VD3 is a key factor in OB differentiation and bone generation because it affects WNT signaling (74, 75).

3.2 Effect of Mg on OC in the pathogenesis of OP

Mg deficiency promotes OC formation and bone loss (6, 76). Mg deficiency in animal models has been shown to stimulate the generation of cytokines, which can promote bone resorption by OCs. For example, an increase in RANKL and a reduction in OPG can lead to increased bone resorption (77).

As previously described, high levels of Mg inhibit PTH secretion (45). In body tissue, PTH mainly enhances bone resorption by enhancing OC activity (78). In recent years, some scholars have conducted *in vitro* experiments and found that if OCs are cocultured with conditioned medium from stromal cells and other similar cells, they are more likely to interact with PTH (79). Therefore, PTH may indirectly activate OCs via its effect on OBs, thus inducing bone resorption. In OBs, RANKL and OPG play different roles by coordinating with each other to maintain the stability of bone tissue (80, 81). The combination of RANKL and RANK can accelerate the generation of OCs. The above functions of RANKL are inhibited by OPG, thereby reducing the activity of OCs (80, 81). (Figure 3) Specifically, with the participation of OPG, RANKL binding can inhibit its entry into the corresponding receptor (82). After PTH administration, OPG mRNA (83) can be detected in rat bones. Currently, Fu et al. (84) conducted a series of *in vitro* studies to explore the mechanism of action of PTH. These results indicate that PTH mainly enhances RANKL levels by activating cAMP/PKA-CREB. and during this process, it can also downregulate OPG levels by regulating PKA-CREB-AP-1.

Mg may affect the bone remodeling activity of VD3. For example, VD3 usually participate the activation of OBs and OCs. Mg deficiency leads to an imbalance in OC activation, which in turn leads to bone resorption (85). A Mg-deficient diet may be associated with abnormal bone remodeling, and increased OC activity as well as the risk of OP in animals. In addition, an Mg-integrated diet results in Ca deposition in bones through interactions with VD3, thus increasing bone mass to block or limit OP (86). In addition, 1α,25(OH) (2)D(3) can indirectly promote OC formation by enhancing the level of RANKL. The formation of OCs is also associated with 1α,25(OH) (2)D (3). In addition, 1α,25(OH) (2)D (3) prevents the expression of OC-associated proteins, such as *Ctsk*, *MMP-9* and *NFATc1* (87). Sakai reported that 1α,25(OH) (2)D (3) inhibits OC generation by preventing the expression of *c-Fos* and *NFATc1* (88). Kikuta et al. reported that 1α,25(OH) (2)D(3) promoted OC migration by regulating the sphingosine 1-phosphate (S1P) receptor system (89). Therefore, 1α,25(OH) (2)D (3) can promote the genealogical nature of OCs and inhibit the formation of OCs in different environments, thus achieving bone health balance.

3.3 Effect of Mg on osteocytes in the pathogenesis of OP

Recent evidence suggests that osteocytes are important cellular targets for PTH (90). PTH can prevent the synthesis of sclerotin in osteocytes to accelerate the development of bone tissue. Research has shown that SOST is a secreted protein that can directly act on the WNT–β-catenin signaling pathway to delay the development of bone tissue (91). Scholars have also pointed out that the substances formed by the binding of WNT ligands to frizzled (Fzd) receptors can act on the WNT–β-catenin signaling pathway to accelerate the development of bone tissue (50). The relevant research results indicate that RANKL can also be produced by osteocytes (26). In osteocytes, this protein is upregulated by PTH, which in turn plays a role in osteoclastogenesis (92). Therefore, Mg can regulate PTH signals through paracrine mediators (such as RANKL) in osteocytes to affect bone remodeling.

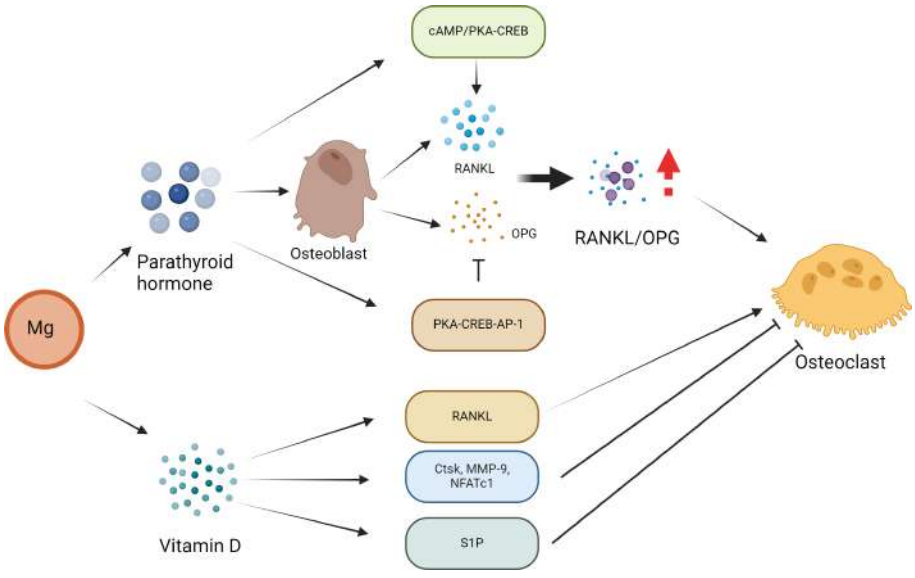

FIGURE 3
Effect of Mg on osteoclasts in the pathogenesis of osteoporosis. PTH may activate osteoclasts indirectly through its effect on osteoblasts, thus inducing bone resorption. In osteoblasts, parathyroid hormone regulates the expression of RANKL and OPG, and these two receptors play a leading role in osteoclast formation. PTH directly increases the expression of RANKL by activating the cAMP/PKA-CREB pathway and inhibits the expression of OPG via the PKA-CREB-AP-1 pathway. These PTH actions lead to an increase in the RANKL/OPG ratio, which is considered to be the main mechanism by which PTH affects osteoclast formation and bone resorption. The formation of OCs can also be regulated directly by vitamin D. In addition, vitamin D inhibits the expression of OC-related genes and proteins (Ctsk, MMP-9 and NFATc1) to inhibit osteoclast formation. In addition, vitamin D and its analogue decalcified alcohol inhibit OC formation by regulating the sphingosine-1-phosphate (S1P) receptor system.

Mg is the second most abundant cation in cells and is an essential factor in the synthesis and metabolism of PTH and VD (93, 94). Previous studies have shown that the activities of the three main enzymes that determine 25(OH)D levels and VD-binding proteins are dependent on Mg (95). In addition, a previous study showed that people with a combined 25(OH)D and Mg deficiency were more likely to have OP than those with a single 25(OH)D deficiency (96). These findings suggest a potential interaction between VD and Mg. The osteocytes are closely related to increased blood phosphate and 1,25(OH)2 VD (97, 98). Pereira et al. reported that the overall influence of active VD sterols on bone in patients with chronic kidney disease is to promote the generation of osteocytes in the early stage of maturation, increase the number of late osteocytes, and increase osteocyte turnover (97). In addition, VD may be a key factor in regulating OB/OC/osteocyte coupling by promoting RANKL/OPG expression (97).

3.4 Effect of Mg on vascular endothelial cells in the pathogenesis of OP

Bone angiogenesis is closely associated with bone metabolism, remodeling and repair. There is evidence that the influence of PTH is mediated by the BMP/Smad1 pathway and is associated with the regulation of angiogenesis (99). VEGF can increase bone vascular invasion and regulate the morphology of growth plates (100). Fu et al. reported that the zinc-finger E-box-binding homeobox-1 (ZEB1)/Notch signaling pathway controls the recruitment/differentiation of perivascular bone progenitor cells, thus promoting osteogenesis by regulating the expression of many vascular secretory factors (such as TGF-β 1, 2, BMP2, BMP4, FGF1 and Noggin) (101). All these vascular secretory factors are thought to be actively involved in osteogenesis.

As described in previous studies, Mg deficiency is associated with decreased PTH levels and induced PTH resistance in terminal organs (102). Currently, VEGF is the most well-studied angiogenic factor in the skeletal system of mammals. As the most important HIF1α target gene after translocation to the nucleus, VEGF is considered to be a key link between angiogenesis and osteogenesis (100, 103, 104). Many scholars have shown that PTH may increase the expression of VEGF in OBs, and VEGF can increase the activity of endothelial cells and promote angiogenesis (99, 105–107). In addition, Ding et al. showed that PTH may affect the expression of VEGF through the PKA/pAKT/HIF1α pathway, thus affecting angiogenesis (108). In addition, Previous studies have suggested that PTH can induce oxidative stress, but current studies have shown that its effect depends on the level of PTHR in endothelial cells (109).

3.5 Effect of Mg on osteosarcopenia

Mg plays an important metabolic and physiological role in musculoskeletal system (110, 111). For example, the results of Welch et al. show that dietary Mg may have a clinical effect on skeletal muscle and bone health in the middle-aged and elderly (16). In addition, in a nationwide cross-sectional survey in 10,279 participants with hypertension based on NHANES, a positive association was observed between dietary Mg intake and appendicular skeletal muscle mass index (ASMI), but not between Mg supplements and ASMI, implying the importance and uniqueness of dietary source of Mg (112). The positive correlation between dietary Mg intake and ASMI can be explained by several possibilities. Animal studies have shown that Mg may improve exercise performance by increasing glucose availability in muscles and blood (113). Mg may also affect muscle performance by maintaining protein synthesis and circulation in muscle through energy metabolism (114). In addition, recent studies have shown that Mg deficiency may increase inflammation and be associated with muscle damage. Inflammation is one of the important factors leading to the occurrence and development of myopenia (115). Experimental animals with Mg deficiency showed systemic inflammation and increased levels of inflammatory markers. Dietary Mg supplements reduced the production of pro-inflammatory cytokines and oxidative stress (116). Several studies have shown that higher Mg intake is associated with lower serum CRP (117).

Previous studies showed that a decrease in muscle mass may be the result of a decrease in protein synthesis or an increase in protein degradation, particularly the ATP-dependent ubiquitin–proteasome proteolytic pathway (118–120). Mg may also affect muscle performance through energy metabolism (production of ATP), transmembrane transport, and muscle contraction and relaxation (121). Skeletal muscle aging is strongly affected by the loss of balance between molecular and muscle cell injury and repair processes, and is characterized by immune activation (122). Chronic systemic inflammation during aging is associated with muscle decrease and weakness, and involves the increase of resident macrophages in aging muscles (123). Cui et al. found that oral Mg supplements could modulate macrophage phenotype by decreasing the M2 population and reduce inflammation during sarcopenia (124). In addition, they found that oral Mg supplements can reduce the deterioration of muscle function in the later stage of muscular dystrophy (124).

4 Mg supplements and clinical treatment of osteoporosis

An increasing number of clinical studies are exploring the influence of Mg supplementation on bone mass and OP. Consistent with the results of a meta-analysis by Farsinejad-Marj et al. (125), Groenendijk et al. reported a significant positive correlation between Mg intake and hip joint BMD (126). Moreover, Groenendijk et al. found a significant positive correlation between Mg intake and the bone density of the femoral neck but no significant association between Mg intake and the bone density of the lumbar spine. (Table 1) In addition, Aydin et al. analyzed the influence of various nutrients on OP (127). Researchers have studied the effects of bone Mg supplementation on postmenopausal women. The experimental group received daily oral administration of Mg citrate, while the control group did not receive any form of intervention. Afterwards, blood was collected for testing, and the results showed a significant decrease in PTH

TABLE 1 Clinical evaluation of magnesium in the treatment of osteoporosis.

First Author, year	Country	Participants	Case	Mean Mg intake	Sources	Relevant Outcomes	Reference
Inge Groenendijk, 2022	Netherlands	Men and woman≥60 y	988	Men:350mg/day Woman:300mg/day	Food and Supplement	BMC/TB BMD/Hip BMD/FN BMD/LS BMD/ BTM/Fracture risk	(126)
Hasan Aydin, 2010	Turkey	PM women	20	1830 mg/day	Supplement	FN BMD/LS BMD	(127)
G Stendig-Lindberg, 1993	Israel	PM women 61.2 ± 6.2 y	31	250–750 mg/day	Supplement	TB BMD	(128)
Liam E.Fouhy, 2023	United States	Men and woman 47–79 y	955	704 ± 153 mg/day	Food	BMD	(129)
Nicola Veronese, 2017	United States	Men and woman 60.6 ± 9.1 y	3765	Men: 205/269/323/398mg/day Woman: 190/ 251/306/373mg/day	Food and Supplement	Fracture risk	(130)
Kathryn M Ryder, 2005	United States	Black/White men and woman 70–79 y	238	Black Women 279.2 ± 115.6; Black Men 304.7 ± 127.5; White Women 307.6 ± 121.9; White Men 330.8 ± 111.9;	Food and Supplement	TH BMD	(131)

Mg, magnesium; BMC, bone mineral content; BMD, bone mineral density; FN, femoral neck; TB, total body; LS, lumbar spine; BTM, bone turnover markers; PM, postmenopausal.

content in the experimental group; moreover, the content of osteocalcin increased (127).

A previous Israeli clinical case–control study showed that Mg therapy significantly increased bone mineral density in 71% of women and prevented bone loss in 16% of women (128). In addition, Mg supplements in menopausal women have been shown to be more effective in combination with other elements. For example, patients who consumed a complete supplement of 500 mg of Ca citrate and 200 mg of Mg oxide exhibited an increase in the average bone mineral density of 11% compared with that of patients who received 500 mg of Ca citrate alone (132). Ca: Mg intake in the range of 2.2–3.2 seems to have the most protective effect, which indicates that the balance of these nutrients can be included in the recommendations for patients with OP (129). Veronese et al. reported that Mg had a greater effect on fracture risk in women than in men (62% and 53%, respectively) (130). Ryder et al. also reported that the correlation between Mg intake and whole-body BMD and hip BMD in men was lower than that in women (131).

A large number of studies have indicated that moderate supplementation of Ca and VD is an effective intervention for preventing bone loss (133). Some scholars believe that supplementing 1000 mg/d Ca and VD appropriately is necessary to prevent bone loss in individuals in the elderly population (134, 135). In addition, the intake of Mg should also be strictly controlled, with adult men consuming approximately 350 milligrams per day and women consuming 300 milligrams per day (136). Not all older people can meet this intake recommendation. In Western countries, the average Mg intake of healthy elderly people ranges from 274 to 421 mg/day for men and from 227 to 373 mg/day for women. This proportion is lower among the weaker elderly. Dietary sources rich in Mg include leafy vegetables, legumes, nuts, and seeds (137, 138). In addition, nuts and seeds contain abundant protein and Ca, which has a positive impact on bone homeostasis. These phenomena indicate that the effect of nutrients on OP cannot be considered in isolation.

5 Conclusion and prospects

There is an important relationship between the occurrence and development of OP and the imbalance of bone homeostasis, in which the interaction among OBs, OCs and osteocytes is a key factor in maintaining bone stability. Although the effects of various trace elements on bone health and OP have been widely studied, there are no related articles summarizing the role of Mg in maintaining bone homeostasis. Therefore, in the present article, we explore the influence of Mg on various cells involved in the maintenance of bone homeostasis during the pathogenesis of OP and summarize the current clinical studies on the use of Mg in the treatment of OP. The findings are expected to fully clarify the relationship between Mg and OP from a basic to a clinical perspective.

Author contributions

LL: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. PL: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Visualization,

Writing – original draft, Writing – review & editing. PW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. PX: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Writing – original draft, Writing – review & editing.

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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. Nieves JW. Osteoporosis: the role of micronutrients. *Am J Clin Nutr*. (2005) 81:1232s–9s. doi: 10.1093/ajcn/81.5.1232

2. Adejuyigbe B, Kallini J, Chiou D, Kallini JR. Osteoporosis: molecular pathology, diagnostics, and therapeutics. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms241914583

3. Ström O, Borgström F, Kanis JA, Compston J, Cooper C, McCloskey EV, et al. Osteoporosis: burden, health care provision and opportunities in the eu: A report prepared in collaboration with the international osteoporosis foundation (Iof) and the european federation of pharmaceutical industry associations (Efpia). *Arch osteoporosis*. (2011) 6:59–155. doi: 10.1007/s11657–011-0060–1

4. Stazi AV. [Micronutrient deficiencies in osteoporosis]. *Minerva Med*. (2013) 104:455–70.

5. Feng W, Wang X, Huang D, Lu A. Role of diet in osteoporosis incidence: umbrella review of meta-analyses of prospective observational studies. *Crit Rev Food Sci Nutr*. (2023) 63:3420–9. doi: 10.1080/10408398.2021.1989374

6. Castiglioni S, Cazzaniga A, Albisetti W, Maier JA. Magnesium and osteoporosis: current state of knowledge and future research directions. *Nutrients*. (2013) 5:3022–33. doi: 10.3390/nu5083022

7. Jahnen-Dechent W, Ketteler M. Magnesium basics. *Clin Kidney J*. (2012) 5:i3–i14. doi: 10.1093/ndtplus/sfr163

8. Fiorentini D, Cappadone C, Farruggia G, Prata C. Magnesium: biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. *Nutrients*. (2021) 13. doi: 10.3390/nu13041136

9. Alfrey AC, Miller NL. Bone magnesium pools in uremia. *J Clin Invest*. (1973) 52:3019–27. doi: 10.1172/jci107500

10. Kleczkowski LA, Igamberdiev AU. Magnesium and cell energetics: at the junction of metabolism of adenylate and non-adenylate nucleotides. *J Plant Physiol*. (2023) 280:153901. doi: 10.1016/j.jplph.2022.153901

11. Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. *Am Heart J*. (1984) 108:188–93. doi: 10.1016/0002–8703(84)90572–6

12. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for mg2+ Revealed by human T-cell immunodeficiency. *Nature*. (2011) 475:471–6. doi: 10.1038/nature10246

13. Rude RK, Singer FR, Gruber HE. Skeletal and hormonal effects of magnesium deficiency. *J Am Coll Nutr*. (2009) 28:131–41. doi: 10.1080/07315724.2009.10719764

14. Boskey AL, Rimnac CM, Bansal M, Federman M, Lian J, Boyan BD. Effect of short-term hypomagnesemia on the chemical and mechanical properties of rat bone. *J orthopaedic research: Off Publ Orthopaedic Res Soc*. (1992) 10:774–83. doi: 10.1002/jor.1100100605

15. Orchard TS, Larson JC, Alghothani N, Bout-Tabaku S, Cauley JA, Chen Z, et al. Magnesium intake, bone mineral density, and fractures: results from the women's health initiative observational study. *Am J Clin Nutr*. (2014) 99:926–33. doi: 10.3945/ajcn.113.067488

16. Welch AA, Skinner J, Hickson M. Dietary magnesium may be protective for aging of bone and skeletal muscle in middle and younger older age men and women: cross-sectional findings from the uk biobank cohort. *Nutrients*. (2017) 9:1189. doi: 10.3390/nu9111189

17. Ansari N, Sims NA. The cells of bone and their interactions. *Handb Exp Pharmacol*. (2020) 262:1–25. doi: 10.1007/164_2019_343

18. Gao Y, Patil S, Jia J. The development of molecular biology of osteoporosis. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22158182

19. Aibar-Almazán A, Voltes-Martínez A, Castellote-Caballero Y, Afanador-Restrepo DF, Carcelén-Fraile MDC, López-Ruiz E. Current status of the diagnosis and management of osteoporosis. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms23169465

20. Ding Z, Shi H, Yang W. Osteoprotective effect of cimiracemate in glucocorticoid-induced osteoporosis by osteoprotegerin/receptor activator of nuclear factor K B/receptor activator of nuclear factor kappa-B Ligand signaling. *Pharmacology*. (2019) 103:163–72. doi: 10.1159/000495509

21. Lee SJ, Jang SA, Kim SC, Ryuk JA, Ha H. Lophatherum gracile bronghiart suppresses receptor activator of nuclear factor kappa-B ligand-stimulated osteoclastogenesis and prevents ovariectomy-induced osteoporosis. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms232213942

22. Ferrari S, Langdahl B. Mechanisms underlying the long-term and withdrawal effects of denosumab therapy on bone. *Nat Rev Rheumatol*. (2023) 19:307–17. doi: 10.1038/s41584–023-00935–3

23. Boyce BF, Li J, Yao Z, Xing L. Nuclear factor-kappa B regulation of osteoclastogenesis and osteoblastogenesis. *Endocrinol Metab (Seoul Korea)*. (2023) 38:504–21. doi: 10.3803/EnM.2023.501

24. Zhivodernikov IV, Kirichenko TV, Markina YV, Postnov AY, Markin AM. Molecular and cellular mechanisms of osteoporosis. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms242115772

25. Bonewald LF. The amazing osteocyte. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2011) 26:229–38. doi: 10.1002/jbmr.320

26. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, et al. Evidence for osteocyte regulation of bone homeostasis through rankl expression. *Nat Med*. (2011) 17:1231–4. doi: 10.1038/nm.2452

27. Streicher C, Heyny A, Andrukhova O, Haigl B, Slavic S, Schüler C, et al. Estrogen regulates bone turnover by targeting rankl expression in bone lining cells. *Sci Rep*. (2017) 7:6460. doi: 10.1038/s41598–017-06614–0

28. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2001) 16:1575–82. doi: 10.1359/jbmr.2001.16.9.1575

29. Deckers MM, Karperien M, van der Bent C, Yamashita T, Papapoulos SE, Löwik CW. Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. *Endocrinology*. (2000) 141:1667–74. doi: 10.1210/endo.141.5.7458

30. Engsig MT, Chen QJ, Vu TH, Pedersen AC, Therkidsen B, Lund LR, et al. Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. *J Cell Biol*. (2000) 151:879–89. doi: 10.1083/jcb.151.4.879

31. Cheng CH, Chen LR, Chen KH. Osteoporosis due to hormone imbalance: an overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms23031376

32. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, et al. Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *New Engl J Med*. (2001) 344:1434–41. doi: 10.1056/nejm200105103441904

33. Leidi M, Dellera F, Mariotti M, Maier JA. High magnesium inhibits human osteoblast differentiation *in vitro*. *Magnesium Res*. (2011) 24:1–6. doi: 10.1684/mrh.2011.0271

34. Pironi L, Malucelli E, Guidetti M, Lanzoni E, Farruggia G, Pinna AD, et al. The complex relationship between magnesium and serum parathyroid hormone: A study in patients with chronic intestinal failure. *Magnesium Res*. (2009) 22:37–43.

35. Ohya M, Negi S, Sakaguchi T, Koiwa F, Ando R, Komatsu Y, et al. Significance of serum magnesium as an independent correlative factor on the parathyroid hormone level in uremic patients. *J Clin Endocrinol Metab*. (2014) 99:3873–8. doi: 10.1210/jc.2013–4396

36. Cheung MM, Dall RD, Shewokis PA, Altasan A, Volpe SL, Amori R, et al. The effect of combined magnesium and vitamin D supplementation on vitamin D status, systemic inflammation, and blood pressure: A randomized double-blinded controlled trial. *Nutr (Burbank Los Angeles County Calif)*. (2022) 99–100:111674. doi: 10.1016/j.nut.2022.111674

37. Rude RK, Gruber HE, Wei LY, Frausto A, Mills BG. Magnesium deficiency: effect on bone and mineral metabolism in the mouse. *Calcified Tissue Int*. (2003) 72:32–41. doi: 10.1007/s00223–001-1091–1

38. Mazur A, Maier JA, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: potential physiopathological implications. *Arch Biochem biophysics*. (2007) 458:48–56. doi: 10.1016/j.abb.2006.03.031

39. Baker-LePain JC, Nakamura MC, Lane NE. Effects of inflammation on bone: an update. *Curr Opin Rheumatol*. (2011) 23:389–95. doi: 10.1097/BOR.0b013e3283474dbe

40. Ding WG, Yan WH, Wei ZX, Liu JB. Difference in intraosseous blood vessel volume and number in osteoporotic model mice induced by spinal cord injury and sciatic nerve resection. *J Bone mineral Metab*. (2012) 30:400–7. doi: 10.1007/s00774-011-0328-y

41. Prisby RD, Ramsey MW, Behnke BJ, Dominguez JM 2nd, Donato AJ, Allen MR, et al. Aging reduces skeletal blood flow, endothelium-dependent vasodilation, and no bioavailability in rats. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2007) 22:1280–8. doi: 10.1359/jbmr.070415

42. Yang C, Yuan G, Zhang J, Tang Z, Zhang X, Dai K. Effects of magnesium alloys extracts on adult human bone marrow-derived stromal cell viability and osteogenic differentiation. *Biomed materials (Bristol England)*. (2010) 5:45005. doi: 10.1088/1748–6041/5/4/045005

43. He LY, Zhang XM, Liu B, Tian Y, Ma WH. Effect of magnesium ion on human osteoblast activity. *Braz J Med Biol Res = Rev Bras pesquisas medicas e biologicas*. (2016) 49. doi: 10.1590/1414–431x20165257

44. Abed E, Moreau R. Importance of melastatin-like transient receptor potential 7 and magnesium in the stimulation of osteoblast proliferation and migration by platelet-derived growth factor. *Am J Physiol Cell Physiol*. (2009) 297:C360–8. doi: 10.1152/ajpcell.00614.2008

45. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. (2001) 81:239–97. doi: 10.1152/physrev.2001.81.1.239

46. Ferment O, Garnier PE, Touitou Y. Comparison of the feedback effect of magnesium and calcium on parathyroid hormone secretion in man. *J Endocrinol*. (1987) 113:117–22. doi: 10.1677/joe.0.1130117

47. Brown AJ, Zhong M, Ritter C, Brown EM, Slatopolsky E. Loss of calcium responsiveness in cultured bovine parathyroid cells is associated with decreased calcium receptor expression. *Biochem Biophys Res Commun*. (1995) 212:861–7. doi: 10.1006/bbrc.1995.2048

48. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: A novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology*. (2005) 146:4577–83. doi: 10.1210/en.2005–0239

49. Mahalingam CD, Sampathi BR, Sharma S, Datta T, Das V, Abou-Samra AB, et al. Mkp1-dependent pth modulation of bone matrix mineralization in female mice is osteoblast maturation stage specific and involves P-erk and P-P38 mapks. *J Endocrinol*. (2013) 216:315–29. doi: 10.1530/joe-12–0372

50. Chen T, Wang Y, Hao Z, Hu Y, Li J. Parathyroid hormone and its related peptides in bone metabolism. *Biochem Pharmacol*. (2021) 192:114669. doi: 10.1016/j.bcp.2021.114669

51. Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent pth. *Bone*. (2007) 40:1434–46. doi: 10.1016/j.bone.2007.03.017

52. Hu G, Yu Y, Sharma D, Pruett-Miller SM, Ren Y, Zhang GF, et al. Glutathione limits runx2 oxidation and degradation to regulate bone formation. *JCI Insight*. (2023) 8. doi: 10.1172/jci.insight.166888

53. Allan EH, Häusler KD, Wei T, Gooi JH, Quinn JM, Crimeen-Irwin B, et al. Ephrinb2 regulation by pth and pthrp revealed by molecular profiling in differentiating osteoblasts. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2008) 23:1170–81. doi: 10.1359/jbmr.080324

54. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell*. (2008) 133:38–52. doi: 10.1016/j.cell.2008.03.011

55. Takyar FM, Tonna S, Ho PW, Crimeen-Irwin B, Baker EK, Martin TJ, et al. Ephrinb2/ephb4 inhibition in the osteoblast lineage modifies the anabolic response to parathyroid hormone. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2013) 28:912–25. doi: 10.1002/jbmr.1820

56. Bikle DD, Sakata T, Leary C, Elalieh H, Ginzinger D, Rosen CJ, et al. Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2002) 17:1570–8. doi: 10.1359/jbmr.2002.17.9.1570

57. Shimizu E, Selvamurugan N, Westendorf JJ, Olson EN, Partridge NC. Hdac4 represses matrix metalloproteinase-13 transcription in osteoblastic cells, and parathyroid hormone controls this repression. *J Biol Chem*. (2010) 285:9616–26. doi: 10.1074/jbc.M109.094862

58. Baron R, Kneissel M. Wnt signaling in bone homeostasis and disease: from human mutations to treatments. *Nat Med*. (2013) 19:179–92. doi: 10.1038/nm.3074

59. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by wnt signaling. *J Clin Invest*. (2006) 116:1202–9. doi: 10.1172/jci28551

60. Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass DA 2nd, et al. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in lrp5, a wnt coreceptor. *J Cell Biol*. (2002) 157:303–14. doi: 10.1083/jcb.200201089

61. Glass DA 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, et al. Canonical wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell*. (2005) 8:751–64. doi: 10.1016/j.devcel.2005.02.017

62. Lawal RA, Zhou X, Batey K, Hoffman CM, Georger MA, Radtke F, et al. The notch ligand jagged1 regulates the osteoblastic lineage by maintaining the osteoprogenitor pool. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2017) 32:1320–31. doi: 10.1002/jbmr.3106

63. Delgado-Calle J, McAndrews K, Wu G, Orr AL, Ferrari A, Tu X, et al. The notch pathway regulates the bone gain induced by pth anabolic signaling. *FASEB journal: Off Publ Fed Am Societies Exp Biol*. (2022) 36:e22196. doi: 10.1096/fj.202101807R

64. Canalis E, Schilling L, Yee SP, Lee SK, Zanotti S. Hajdu cheney mouse mutants exhibit osteopenia, increased osteoclastogenesis, and bone resorption. *J Biol Chem*. (2016) 291:1538–51. doi: 10.1074/jbc.M115.685453

65. Yu J, Schilling L, Eller T, Canalis E. Hairy and enhancer of split 1 is a primary effector of notch2 signaling and induces osteoclast differentiation and function. *J Biol Chem*. (2021) 297:101376. doi: 10.1016/j.jbc.2021.101376

66. Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, et al. Leukaemogenesis induced by an activating B-catenin mutation in osteoblasts. *Nature*. (2014) 506:240–4. doi: 10.1038/nature12883

67. Tu X, Delgado-Calle J, Condon KW, Maycas M, Zhang H, Carlesso N, et al. Osteocytes mediate the anabolic actions of canonical wnt/B-catenin signaling in bone. *Proc Natl Acad Sci United States America*. (2015) 112:E478–86. doi: 10.1073/pnas.1409857112

68. Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino AC, et al. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D3. *Bone*. (2007) 40:1517–28. doi: 10.1016/j.bone.2007.02.024

69. Döhla J, Kuuluvainen E, Gebert N, Amaral A, Englund JI, Gopalakrishnan S, et al. Metabolic determination of cell fate through selective inheritance of mitochondria. *Nat Cell Biol*. (2022) 24:148–54. doi: 10.1038/s41556–021-00837–0

70. Maehata Y, Takamizawa S, Ozawa S, Kato Y, Sato S, Kubota E, et al. Both direct and collagen-mediated signals are required for active vitamin D3-elicited differentiation of human osteoblastic cells: roles of osterix, an osteoblast-related transcription factor. *Matrix biology: J Int Soc Matrix Biol*. (2006) 25:47–58. doi: 10.1016/j.matbio.2005.09.001

71. Wang D, Song J, Ma H. An *in vitro* experimental insight into the osteoblast responses to vitamin D3 and its metabolites. *Pharmacology*. (2018) 101:225–35. doi: 10.1159/000486446

72. van Leeuwen JP, van Driel M, van den Bemd GJ, Pols HA. Vitamin D control of osteoblast function and bone extracellular matrix mineralization. *Crit Rev eukaryotic Gene Expression*. (2001) 11:199–226. doi: 10.1615/CritRevEukarGeneExpr.v11.i1-3

73. Cui J, Ma C, Qiu J, Ma X, Wang X, Chen H, et al. A novel interaction between insulin-like growth factor binding protein-6 and the vitamin D receptor inhibits the role of vitamin D3 in osteoblast differentiation. *Mol Cell Endocrinol*. (2011) 338:84–92. doi: 10.1016/j.mce.2011.03.011

74. Fretz JA, Zella LA, Kim S, Shevde NK, Pike JW. 1,25-dihydroxyvitamin D3 induces expression of the wnt signaling co-regulator lrp5 via regulatory elements located significantly downstream of the gene's transcriptional start site. *J Steroid Biochem Mol Biol*. (2007) 103:440–5. doi: 10.1016/j.jsbmb.2006.11.018

75. Guler E, Baripoglu YE, Alenezi H, Arikan A, Babazade R, Unal S, et al. Vitamin D(3)/vitamin K(2)/magnesium-loaded polylactic acid/tricalcium phosphate/polycaprolactone composite nanofibers demonstrated osteoinductive effect by increasing runx2 via wnt/B-catenin pathway. *Int J Biol macromolecules*. (2021) 190:244–58. doi: 10.1016/j.ijbiomac.2021.08.196

76. Swaminathan R. Magnesium metabolism and its disorders. *Clin biochemist Rev*. (2003) 24:47–66.

77. Kunutsor SK, Whitehouse MR, Blom AW, Laukkanen JA. Low serum magnesium levels are associated with increased risk of fractures: A long-term prospective cohort study. *Eur J Epidemiol*. (2017) 32:593–603. doi: 10.1007/s10654–017-0242–2

78. Chambers TJ, Fuller K, McSheehy PM, Pringle JA. The effects of calcium regulating hormones on bone resorption by isolated human osteoclastoma cells. *J Pathol*. (1985) 145:297–305. doi: 10.1002/path.1711450403

79. Kaji H, Sugimoto T, Kanatani M, Fukase M, Chihara K. Involvement of dual signal transduction systems in the stimulation of osteoclast-like cell formation by

parathyroid hormone and parathyroid hormone-related peptide. *Biochem Biophys Res Commun*. (1993) 194:157–62. doi: 10.1006/bbrc.1993.1798

80. Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR, et al. 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*. (2001) 142:4047–54. doi: 10.1210/endo.142.9.8356

81. Kanzawa M, Sugimoto T, Kanatani M, Chihara K. Involvement of osteoprotegerin/osteoclastogenesis inhibitory factor in the stimulation of osteoclast formation by parathyroid hormone in mouse bone cells. *Eur J Endocrinol*. (2000) 142:661–4. doi: 10.1530/eje.0.1420661

82. Kearns AE, Khosla S, Kostenuik PJ. Receptor activator of nuclear factor kappab ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocrine Rev*. (2008) 29:155–92. doi: 10.1210/er.2007–0014

83. Huang JC, Sakata T, Pfleger LL, Bencsik M, Halloran BP, Bikle DD, et al. Pth differentially regulates expression of rankl and opg. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2004) 19:235–44. doi: 10.1359/jbmr.0301226

84. Fu Q, Jilka RL, Manolagas SC, O'Brien CA. 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*. (2002) 277:48868–75. doi: 10.1074/jbc.M208494200

85. Mammoli F, Castiglioni S, Parenti S, Cappadone C, Farruggia G, Iotti S, et al. Magnesium is a key regulator of the balance between osteoclast and osteoblast differentiation in the presence of vitamin D_3. *Int J Mol Sci*. (2019) 20. doi: 10.3390/ijms20020385

86. Nieves JW. Bone. Maximizing bone health–magnesium, bmd and fractures. . *Nat Rev Endocrinol*. (2014) 10:255–6. doi: 10.1038/nrendo.2014.39

87. Gu J, Zhang X, Zhang C, Li Y, Bian J, Liu X, et al. Galectin-3 contributes to the inhibitory effect of Lα,25-(Oh)(2)D(3) on osteoclastogenesis. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms222413334

88. Sakai S, Takaishi H, Matsuzaki K, Kaneko H, Furukawa M, Miyauchi Y, et al. 1-alpha, 25-dihydroxy vitamin D3 inhibits osteoclastogenesis through ifn-beta-dependent nfatc1 suppression. *J Bone mineral Metab*. (2009) 27:643–52. doi: 10.1007/s00774–009-0084–4

89. Kikuta J, Kawamura S, Okiji F, Shirazaki M, Sakai S, Saito H, et al. Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. *Proc Natl Acad Sci United States America*. (2013) 110:7009–13. doi: 10.1073/pnas.1218799110

90. Wein MN. Parathyroid hormone signaling in osteocytes. *JBMR plus*. (2018) 2:22–30. doi: 10.1002/jbm4.10021

91. Wein MN, Kronenberg HM. Regulation of bone remodeling by parathyroid hormone. *Cold Spring Harbor Perspect Med*. (2018) 8. doi: 10.1101/cshperspect.a031237

92. Ben-awadh AN, Delgado-Calle J, Tu X, Kuhlenschmidt K, Allen MR, Plotkin LI, et al. Parathyroid hormone receptor signaling induces bone resorption in the adult skeleton by directly regulating the rankl gene in osteocytes. *Endocrinology*. (2014) 155:2797–809. doi: 10.1210/en.2014–1046

93. Deng X, Song Y, Manson JE, Signorello LB, Zhang SM, Shrubsole MJ, et al. Magnesium, vitamin D status and mortality: results from us national health and nutrition examination survey (Nhanes) 2001 to 2006 and nhanes iii. *BMC Med*. (2013) 11:187. doi: 10.1186/1741–7015-11–187

94. Dai Q, Zhu X, Manson JE, Song Y, Li X, Franke AA, et al. Magnesium status and supplementation influence vitamin D status and metabolism: results from a randomized trial. *Am J Clin Nutr*. (2018) 108:1249–58. doi: 10.1093/ajcn/nqy274

95. Rude RK, Adams JS, Ryzen E, Endres DB, Niimi H, Horst RL, et al. Low serum concentrations of 1,25-dihydroxyvitamin D in human magnesium deficiency. *J Clin Endocrinol Metab*. (1985) 61:933–40. doi: 10.1210/jcem-61–5-933

96. Sahota O, Mundey MK, San P, Godber IM, Hosking DJ. Vitamin D insufficiency and the blunted pth response in established osteoporosis: the role of magnesium deficiency. *Osteoporosis international: J established as result cooperation between Eur Foundation Osteoporosis Natl Osteoporosis Foundation USA*. (2006) 17:1013–21. doi: 10.1007/s00198–006-0084–3

97. Pereira RC, Salusky IB, Bowen RE, Freymiller EG, Wesseling-Perry K. Vitamin D sterols increase fgf23 expression by stimulating osteoblast and osteocyte maturation in ckd bone. *Bone*. (2019) 127:626–34. doi: 10.1016/j.bone.2019.07.026

98. Bergwitz C, Jüppner H. Regulation of phosphate homeostasis by pth, vitamin D, and fgf23. *Annu Rev Med*. (2010) 61:91–104. doi: 10.1146/annurev.med.051308.111339

99. Prisby R, Guignandon A, Vanden-Bossche A, Mac-Way F, Linossier MT, Thomas M, et al. Intermittent pth(1–84) is osteoanabolic but not osteoangiogenic and relocates bone marrow blood vessels closer to bone-forming sites. *J Bone mineral research: Off J Am Soc Bone Mineral Res*. (2011) 26:2583–96. doi: 10.1002/jbmr.459

100. 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

101. Fu R, Lv WC, Xu Y, Gong MY, Chen XJ, Jiang N, et al. Endothelial zeb1 promotes angiogenesis-dependent bone formation and reverses osteoporosis. *Nat Commun*. (2020) 11:460. doi: 10.1038/s41467–019-14076–3

102. Mutnuri S, Fernandez I, Kochar T. Suppression of parathyroid hormone in a patient with severe magnesium depletion. *Case Rep Nephrol*. (2016) 2016:2608538. doi: 10.1155/2016/2608538

103. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, et al. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the cbp/P300 coactivator by the hypoxia-inducible factor-1alpha. *EMBO J*. (1998) 17:6573–86. doi: 10.1093/emboj/17.22.6573

104. Liu Y, Berendsen AD, Jia S, Lotinun S, Baron R, Ferrara N, et al. Intracellular vegf regulates the balance between osteoblast and adipocyte differentiation. *J Clin Invest*. (2012) 122:3101–13. doi: 10.1172/jci61209

105. Esbrit P, Alvarez-Arroyo MV, DE Miguel F, Martin O, Martinez ME, Caramelo C. C-terminal parathyroid hormone-related protein increases vascular endothelial growth factor in human osteoblastic cells. *J Am Soc Nephrology: JASN*. (2000) 11:1085–92. doi: 10.1681/asn.V1161085

106. Mayr-Wohlfart U, Waltenberger J, Hausser H, Kessler S, Günther KP, Dehio C, et al. Vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. *Bone*. (2002) 30:472–7. doi: 10.1016/s8756–3282(01)00690–1

107. Street J, Bao M, deGuzman L, Bunting S, Peale FV Jr., Ferrara N, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci United States America*. (2002) 99:9656–61. doi: 10.1073/pnas.152324099

108. Ding Q, Sun P, Zhou H, Wan B, Yin J, Huang Y, et al. Lack of endogenous parathyroid hormone delays fracture healing by inhibiting vascular endothelial growth factor–Mediated angiogenesis. *Int J Mol Med*. (2018) 42:171–81. doi: 10.3892/ijmm.2018.3614

109. Gambardella J, De Rosa M, Sorriento D, Prevete N, Fiordelisi A, Ciccarelli M, et al. Parathyroid hormone causes endothelial dysfunction by inducing mitochondrial ros and specific oxidative signal transduction modifications. *Oxid Med Cell Longevity*. (2018) 2018:9582319. doi: 10.1155/2018/9582319

110. Capozzi A, Scambia G, Lello S. Calcium, vitamin D, vitamin K2, and magnesium supplementation and skeletal health. *Maturitas*. (2020) 140:55–63. doi: 10.1016/j.maturitas.2020.05.020

111. Scaturro D, Vitagliani F, Terrana P, Tomasello S, Camarda L, Letizia Mauro G. Does the association of therapeutic exercise and supplementation with sucrosomial magnesium improve posture and balance and prevent the risk of new falls? *Aging Clin Exp Res*. (2022) 34:545–53. doi: 10.1007/s40520-021-01977-x

112. Wang Q, Si K, Xing X, Ye X, Liu Z, Chen J, et al. Association between dietary magnesium intake and muscle mass among hypertensive population: evidence from the national health and nutrition examination survey. *Nutr J*. (2024) 23:37. doi: 10.1186/s12937–024-00940–6

113. Zhang Y, Xun P, Wang R, Mao L, He K. Can magnesium enhance exercise performance? *Nutrients*. (2017) 9:946. doi: 10.3390/nu9090946

114. Dørup I, Clausen T. Effects of magnesium and zinc deficiencies on growth and protein synthesis in skeletal muscle and the heart. *Br J Nutr*. (1991) 66:493–504. doi: 10.1079/bjn19910050

115. Bano G, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, et al. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas*. (2017) 96:10–5. doi: 10.1016/j.maturitas.2016.11.006

116. López-Baltanás R, Encarnación Rodríguez-Ortiz M, Canalejo A, Díaz-Tocados JM, Herencia C, Leiva-Cepas F, et al. Magnesium supplementation reduces inflammation in rats with induced chronic kidney disease. *Eur J Clin Invest*. (2021) 51:e13561. doi: 10.1111/eci.13561

117. Simental-Mendia LE, Sahebkar A, Rodriguez-Moran M, Zambrano-Galvan G, Guerrero-Romero F. Effect of magnesium supplementation on plasma C-reactive protein concentrations: A systematic review and meta-analysis of randomized controlled trials. *Curr Pharm design*. (2017) 23:4678–86. doi: 10.2174/1381612823666170525153605

118. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, et al. Akt/mtor pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy *in vivo*. *Nat Cell Biol*. (2001) 3:1014–9. doi: 10.1038/ncb1101–1014

119. Jagoe RT, Goldberg AL. What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? *Curr Opin Clin Nutr Metab Care*. (2001) 4:183–90. doi: 10.1097/00075197–200105000–00003

120. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB journal: Off Publ Fed Am Societies Exp Biol*. (2004) 18:39–51. doi: 10.1096/fj.03-0610com

121. Dominguez LJ, Barbagallo M, Lauretani F, Bandinelli S, Bos A, Corsi AM, et al. Magnesium and muscle performance in older persons: the inchianti study. *Am J Clin Nutr*. (2006) 84:419–26. doi: 10.1093/ajcn/84.1.419

122. Cui CY, Driscoll RK, Piao Y, Chia CW, Gorospe M, Ferrucci L. Skewed macrophage polarization in aging skeletal muscle. *Aging Cell*. (2019) 18:e13032. doi: 10.1111/acel.13032

123. Wang Y, Wehling-Henricks M, Welc SS, Fisher AL, Zuo Q, Tidball JG. Aging of the immune system causes reductions in muscle stem cell populations, promotes their shift to a fibrogenic phenotype, and modulates sarcopenia. *FASEB journal: Off Publ Fed Am Societies Exp Biol*. (2019) 33:1415–27. doi: 10.1096/fj.201800973R

124. Cui C, Bao Z, Chow SK, Wong RMY, Welch A, Qin L, et al. Coapplication of magnesium supplementation and vibration modulate macrophage polarization to attenuate sarcopenic muscle atrophy through pi3k/akt/mtor signaling pathway. *Int J Mol Sci*. (2022) 23:12944. doi: 10.3390/ijms232112944

125. Farsinejad-Marj M, Saneei P, Esmaillzadeh A. Dietary magnesium intake, bone mineral density and risk of fracture: A systematic review and meta-analysis. *Osteoporosis international: J established as result cooperation between Eur Foundation Osteoporosis Natl Osteoporosis Foundation USA*. (2016) 27:1389–99. doi: 10.1007/s00198-015-3400-y

126. Groenendijk I, van Delft M, Versloot P, van Loon LJC, de Groot LCPGM. Impact of magnesium on bone health in older adults: A systematic review and meta-analysis. *Bone*. (2022) 154:116233. doi: 10.1016/j.bone.2021.116233

127. Aydin H, Deyneli O, Yavuz D, Gözü H, Mutlu N, Kaygusuz I, et al. Short-term oral magnesium supplementation suppresses bone turnover in postmenopausal osteoporotic women. *Biol Trace element Res*. (2010) 133:136–43. doi: 10.1007/s12011–009-8416–8

128. Stendig-Lindberg G, Tepper R, Leichter I. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. *Magnesium Res*. (1993) 6:155–63.

129. Fouhy LE, Mangano KM, Zhang X, Hughes BD, Tucker KL, Noel SE. Association between a calcium-to-magnesium ratio and osteoporosis among Puerto Rican adults. *J Nutr*. (2023) 153:2642–50. doi: 10.1016/j.tjnut.2023.05.009

130. Veronese N, Stubbs B, Solmi M, Noale M, Vaona A, Demurtas J, et al. Dietary magnesium intake and fracture risk: data from a large prospective study. *Br J Nutr*. (2017) 117:1570–6. doi: 10.1017/s0007114517001350

131. Ryder KM, Shorr RI, Bush AJ, Kritchevsky SB, Harris T, Stone K, et al. Magnesium intake from food and supplements is associated with bone mineral density in healthy older white subjects. *J Am Geriatrics Soc*. (2005) 53:1875–80. doi: 10.1111/j.1532-5415.2005.53561.x

132. Abraham GE, Grewal H. A total dietary program emphasizing magnesium instead of calcium. Effect on the mineral density of calcaneous bone in postmenopausal women on hormonal therapy. *J Reprod Med*. (1990) 35:503–7.

133. Groenendijk I, den Boeft L, van Loon LJC, de Groot L. High versus low dietary protein intake and bone health in older adults: A systematic review and meta-analysis. *Comput Struct Biotechnol J*. (2019) 17:1101–12. doi: 10.1016/j.csbj.2019.07.005

134. Rizzoli R, Stevenson JC, Bauer JM, van Loon LJ, Walrand S, Kanis JA, et al. The role of dietary protein and vitamin D in maintaining musculoskeletal health in postmenopausal women: A consensus statement from the European society for clinical and economic aspects of osteoporosis and osteoarthritis (Esceo). *Maturitas*. (2014) 79:122–32. doi: 10.1016/j.maturitas.2014.07.005

135. 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) 17:58. doi: 10.1007/s11657–022-01061–5

136. Kristjansdottir AG, Johannsson E, Thorsdottir I. Effects of a school-based intervention on adherence of 7–9-year-olds to food-based dietary guidelines and intake of nutrients. *Public Health Nutr*. (2010) 13:1151–61. doi: 10.1017/s1368980010000716

137. Groenendijk I, Kramer CS, den Boeft LM, Hobbelen HSM, van der Putten GJ, de Groot L. Hip fracture patients in geriatric rehabilitation show poor nutritional status, dietary intake and muscle health. *Nutrients*. (2020) 12. doi: 10.3390/nu12092528

138. Verlaan S, Aspray TJ, Bauer JM, Cederholm T, Hemsworth J, Hill TR, et al. Nutritional status, body composition, and quality of life in community-dwelling sarcopenic and non-sarcopenic older adults: A case-control study. *Clin Nutr (Edinburgh Scotland)*. (2017) 36:267–74. doi: 10.1016/j.clnu.2015.11.013

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EDITED BY
Mohammed S. Razzaque,
Lake Erie College of Osteopathic Medicine, United States

REVIEWED BY
Kelli George,
West Virginia University, United States
Olga Czerwińska-Ledwig,
University of Physical Education in Kraków, Poland
Colby Vorland,
Indiana University, United States
Joško Osredkar,
University Medical Centre Ljubljana, Slovenia

*CORRESPONDENCE
Xiaoyu Tian
✉ janetian136@sina.com

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Correlation and consistency between two detection methods for serum 25 hydroxyvitamin D levels in human venous blood and capillary blood

Yutong Xing, Kaixi Wang, Xinyu Ma, Huifeng Zhang and Xiaoyu Tian*

Department of Paediatrics, The Second Hospital Affiliated to Hebei Medical University, Shijiazhuang, China

Introduction: The study assessed the correlation and concordance of 25-hydroxyvitamin D [25(OH)D] levels in capillary and venous plasma collected simultaneously after vitamin D3 supplementation in 42 healthy adults. They were randomly divided into three groups by random number table method. Group A took 1,000 IU vitamin D3 daily, group B took 10,000 IU vitamin D3 every 10 days, and group C took 30,000 IU vitamin D3 every 30 days until the end of the 12th month. Venous blood serum 25(OH)D level was detected by chemiluminescence immunoassay (CLIA) and mass spectrometry (LC-MS) at day 1, day 14, day 28, month 6, and month 12 respectively, the capillary blood serum 25(OH)D level was detected by chemiluminescence immunoassay (CLIA) at the same time. Pearson correlation analysis and linear regression analysis were employed to investigate the relationship and transformation equation between the findings of the two samples and the results obtained from different detection methods within the same sample. The Bland-Altman method, Kappa analysis, and receiver operating characteristic (ROC) curve were utilized for assessing consistency, sensitivity, and specificity.

Results: The three groups all reached a stable peak at 6 months, and the average levels of the three groups were 49.21, 42.50 and 43.025 nmol/L, respectively. The average levels of group A were higher than those of group B and group C ($P < 0.001$).The mean values of serum 25(OH)D measured by LC-MS and CLIA in 42 healthy adults were 45.32 nmol/L and 49.88 nmol/L, respectively, and the mean values of 25(OH)D measured by LC-MS in capillary blood were 52.03 nmol/L, and the difference was statistically significant ($P < 0.001$). Pearson correlation analysis showed that the linear fitting formula of scatter data was as follows: venous 25(OH)D concentration (nmol/L) = 1.105 * capillary 25(OH)D concentration −7.532 nmol/L, $R^2 = 0.625$. Good agreement was observed between venous and corrected capillary 25(OH)D levels in clinical diagnosis (Kappa value 0.75). The adjusted serum 25(OH)D in capillary blood had a high clinical predictive value.

Conclusions: The agreement between the two methods is good when the measured 25(OH)D level is higher. Standardized capillary blood chemiluminescence method can be used for 25(OH)D detection.

KEYWORDS

25 hydroxyvitamin D, venous blood, capillary blood, detection methods, consistency evaluation

1 Introduction

Vitamin D exists in many forms in the body's circulation and participates in the body's metabolism. The two important forms of vitamin D are 25(OH)D2(Ergocalciferol) and 25(OH)D3(Hydroxycholecalciferol). The body cannot synthesize 25(OH)D2 and can only obtain it through food or supplements. Therefore, most of the 25(OH)D detected in serum is 25(OH)D3, and only a small number of people whose serum contains 25(OH)D2 can reach detectable levels (1, 2).

1, 25-dihydroxyvitamin D [1,25 (OH) 2D] is the active form of vitamin D in the body, but its content in the human body is very small and difficult to detect. Circulating 25-hydroxyvitamin D [25 (OH)D, including 25(OH)D2 and 25(OH)D3] is one of the main metabolic forms of vitamin D in the human body (3). The stable form and long half-life are the best indicators to evaluate the nutritional status of vitamin D in the human body (4). At present, the detection methods of serum 25(OH)D include liquid chromatography-tandem mass spectrometry, LC-MS/MS), electrochemiluminescence immunoassay (ECLISA), etc. LC-MS/MS can simultaneously detect multiple vitamin D variants such as 25(OH)D2 and 25(OH)D3, with strong specificity and high sensitivity. It is currently recognized as the gold standard method for 25(OH)D detection, and the measurement results are accurate and reliable. However, the requirements for laboratory equipment, personnel and measurement support conditions are high, and the detection technology is more complex and relatively expensive. Benefits of ECLIA include high throughput, labor savings, and real-time reporting of results. At present, the ECLIA method is widely used in medical institutions and is the only method for the detection of 25(OH)D in capillary serum analyzers. The specificity and detection principle of the two methods for detecting venous serum 25(OH)D mentioned in this paper are different.

To determine 25(OH)D levels in body fluids, serum samples must be collected for analysis. For sample collection, different blood collection methods can be used clinically to obtain two blood samples: venous blood and capillary blood. Venous blood is widely used in clinical practice and is a long-acting blood collection method. However, for patients who are not suitable or unable to perform intravenous blood collection, such as children, extremely obese people, patients with severe burns, and patients with advanced cancer, it is more convenient to use the capillary tips of the heels and fingertips for blood collection. Different collection methods may affect the interpretation of the final data (Figure 1).

Relevant studies have shown that there is a good correlation between the levels of metabolites in venous blood and fingertip blood. However, the specific values that need to be converted may differ (5). It is important to explore effective and convenient methods to determine vitamin D status in order to timely detect vitamin D deficiency and supplement vitamin D. Therefore, our study aimed to analyze the effectiveness of two blood collection methods for 25(OH) D detection in healthy people during vitamin D supplementation, and to evaluate the consistency of the two detection methods, so as to provide references for clinicians.

This study was based on the practicability of vitamin D supplementation strategies in healthy people. Therefore, daily, 10-day, and monthly vitamin D supplementation groups were set, and the selection of monitoring time points was based on the comprehensive consideration of pharmacokinetics and practicability of real-world studies.

2 Materials and methods

2.1 Research participants

The study participants were 42 healthy adults recruited from the Second Hospital of Hebei Medical University in October 2022. The study subjects were 42 healthy adults recruited by our advertisement in the Second Hospital of Hebei Medical University in October 2022.The inclusion criteria were as follows: (1) healthy adults, (2) age >18 years old, and (3) no special vitamin D supplementation has been given in the past 6 months from the first visit. The exclusion criteria are as follows: (1) presence of congenital or hereditary diseases (phenylketonuria, nodular sclerosis, neurofibromatosis, etc.); (2) significant history of traumatic brain injury and other neurological functional diseases; (3) severe visual and hearing impairment; (4) heart, lung, liver, kidney, endocrine, or autoimmune diseases; (5) gastrointestinal diseases, difficulty eating, and malnutrition; (6) acute or chronic infectious diseases in recent 3 months; and (7) intake of nutritional supplements such as vitamins and minerals in the past 6 months or taking medication recently. For each participant, detailed clinical examinations were conducted, and personal medical history records, including height, weight, body mass index, etc., were collected.

This study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University (Ethical batch no. 2022-R766), the participants signed the informed consent form, and the original study was registered with the China Clinical Trial Registration Center (Registration no. ChiCTR230069387).

2.2 Research methods

2.2.1 25(OH)D serum sample collection

Serum samples were collected and sent to the subjects on day 1, day 14, day 28, month 6, and month 12 after the formal start of the study. On the day of sampling, all subjects were in a fasting state, and there was no inflammation or edema in the blood collection area. Venous blood collection and capillary blood collection were carried out successively by the same group of medical personnel. After routine disinfection, disposable venous blood collection needle (sphenwing type, Shandong Zhu Pharmaceutical) was used to puncture the left elbow vein of the subject, and vacuum negative pressure collection vessel was connected (special coagulant and special separation glue were added, Shanghai Aoxiang Medical), 2 ml venous blood was collected and injected into the collection vessel, and centrifuged for 1 h (4,000 r/min, 10 min) and stored away from light. Select the inner tip of the middle or ring finger for capillary blood collection, massage the blood collection site, after routine disinfection, use a disposable blood collection needle (Shandong Zhushe Pharmaceutical) to Pierce the skin 2 mm, wipe off the first drop of blood, use a disposable micro-blood collection straw (Changsha Global Medical Equipment Co., LTD.) to collect 100 μl capillary blood. The blood is squeezed from a microsampling

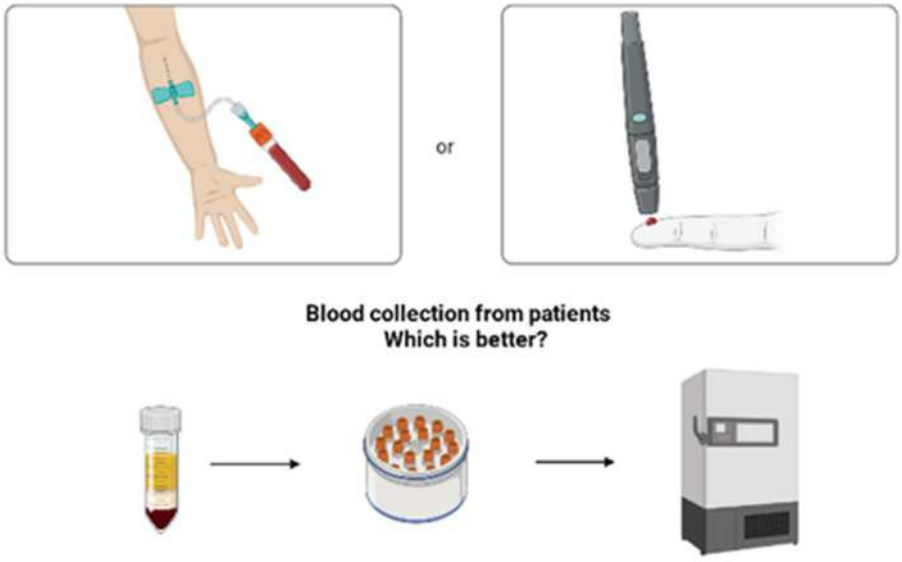

FIGURE 1
Deciding the better alternative for blood collection.

pipette into an EP tube (Eppendorf) containing the anticoagulant EDTA, immediately mixed at least 10 times, and stored at room temperature away from light. All samples will be labeled and stored for future reference, and tested at low temperature on the same day.

From 1 day, 14 days, 28 days, and 6 months after recruitment, two samples of venous serum and two samples of capillary blood were collected on an empty stomach for testing. Venous blood is collected from the cubital vein or jugular vein, with a volume of 2 ml, and injected into a vacuum test tube. Select the inner side of the middle or ring finger tip for capillary blood collection, and collect a blood volume of 100 μl using a disposable micro blood collection pipette. The blood sample was stored at room temperature and away from light. After 1 h, it was centrifuged (4,000 r/min, 10 min) and kept for collection. It was stored at 4°C before being sent for testing. Low-temperature transportation testing was contacted on the same day. Then, 100 μL of capillary blood was collected using a disposable micro blood collection pipette. The blood was squeezed from the micro blood collection vessel into the EP (Eppendorf) tube containing anticoagulant ethylenediaminetetraacetic acid and immediately mixed at least 10 times. The sample was marked and kept for future reference, and low-temperature transportation testing was contacted on the same day.

2.2.2 25(OH)D detection

To reduce the effect of testing instruments and methods on data accuracy, venous serum samples were sent to the laboratory of Beijing Jinyu Testing Center for unified testing. Liquid chromatography-tandem mass spectrometry (LC-MS/MS, 4500MD, AB SCIEX, USA) was used for the detection of serum 25(OH)D levels. Serum 25(OH)D levels in venous and capillary blood samples were measured by chemiluminescence immunoassay using automatic chemiluminescence analyzer (MCL60, Nanjing Renpulse Biotechnology Co., Ltd.). LC-MS/MS is defined as the most recognized "gold standard" for the measurement of 25(OH)D. Internal quality control (IQC) is carried out on all test samples by the tester to ensure that the laboratory test results can be subsequent statistical analysis.

2.2.3 25(OH)D standard deviation score calculation

Using the reference range of serum 25(OH)D levels in the *Chinese Journal of Pediatrics* "Practice Guidelines for Clinical Issues Related to Vitamin D Nutrition in Children" to grade vitamin D nutritional status: Based on serum 25(OH)D levels, vitamin D nutritional status is classified as vitamin D deficiency [25(OH)D < 30 nmol/L] and vitamin D insufficiency [25(OH)D 30–50 nmol/L]. There are four levels of vitamin D adequacy [25(OH)D > 50–250 nmol/L] and vitamin D toxicity [25(OH)D > 250 nmol/L] (3). By establishing a mathematical model, generate polynomial equations for the mean and standard deviation, with SDS = (measured value average)/s.

2.3 Statistical methods

Statistical analysis was conducted using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test (KS test) was used to test the normality of the three groups of data. Pearson correlation analysis was used to analyze the correlation between the results of the two detection methods and the results of the two samples. Pearson correlation analysis and linear regression analysis were used to detect the correlation and determine the correction algorithm. Bland-Altman plot and Kappa statistic were used to analyze the consistency. The concepts of approximate entropy and least squares were added to the curve

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TABLE 1 Basal metabolic data and body composition analysis data of the subjects.

	Group A			Group B			Group C			
	Baseline	12 m	*P*-value*	Baseline	12 m	*P*-value*	Baseline	12 m	*P*-value*	*P*-value**	Cohen's d
Height, cm	168.00 ± 7.63	168.00 ± 7.62	0.854	167.56 ± 3.25	167.55 ± 3.12	0.873	168.21 ± 5.42	168.20 ± 5.41	0.862	0.234	0.00
BMI, kg/m^2	25.35 ± 0.18	25.30 ± 0.22	0.01	22.46 ± 0.35	22.44 ± 0.46	0.04	21.35 ± 0.26	21.30 ± 0.11	0.001	0.001	0.10
Body fat, kg	15.14 ± 3.38	16.22 ± 4.24	<0.001	18.46 ± 2.54	18.57 ± 2.44	0.03	19.33 ± 3.24	17.24 ± 3.22	<0.01	0.01	0.14
Body fat%	33.74 ± 10.25	36.58 ± 0.54	<0.001	34.33 ± 4.24	34.45 ± 5.66	0.01	31.25 ± 11.22	29.51 ± 9.0.35	<0.01	0.001	0.01
Total body water, kg	34.46 ± 5.62	34.50 ± 4.82	0.24	34.25 ± 3.65	34.17 ± 2.51	0.38	34.68 ± 2.65	34.70 ± 2.51	0.28	0.09	0.07
Waist-to-hip ratio	0.87 ± 0.18	0.88 ± 0.17	0.45	0.85 ± 0.25	0.85 ± 0.65	0.77	0.83 ± 0.31	0.82 ± 0.27	0.64	0.15	0.06
Bone mass, kg	3.07 ± 0.49	3.44 ± 0.58	<0.01	3.24 ± 0.22	3.36 ± 0.68	<0.01	3.80 ± 0.25	3.85 ± 0.22	<0.01	<0.01	0.16

*$p < 0.05$, **$p < 0.01$.

fitting. The weighted Kappa coefficient of 25(OH)D classification was calculated to analyze the consistency of the three groups of data. Using receiver operating characteristic (ROC) curve method to evaluate the sensitivity and specificity. The results were analyzed according to the p value and kappa value, and the difference was statistically significant (two-tailed $P < 0.05$). We use Cronbach α The coefficient (a commonly used reliability analysis method) is used to compare the consistency or stability of the results obtained from the 25(OH)D test of venous blood serum samples and capillary blood serum samples. If the reliability coefficient is above 0.8, the reliability of the experiment or scale is very good; A reliability coefficient above 0.6 is acceptable; If it is below 0.6, the scale needs to be redesigned.

3 Results

3.1 Basic information

The serum samples of 42 participants [including 14 men (33%) and 28 women (65%)], including 168 venous blood serum samples and 168 capillary blood serum samples, were statistically compared using 168 pairs. In the 42 samples from day 1 of the study, according to the "Practice Guidelines for Clinical Issues Related to Vitamin D Nutrition in Chinese Children," 27 participants had vitamin D deficiency, 10 had vitamin D insufficiency, and 5 had vitamin D adequacy (3). Data were analyzed for normality using the Jarque Bra test, and the results showed a normal distribution of 25(OH)D values in the venous blood (Table 1, Figure 2). The mean values of the three groups were: 30.00, 29.93, 29.33 nmol/L, there was no statistical significance ($P > 0.05$). The three groups all reached a stable peak at 6 months, and the average levels of the three groups were 49.21, 42.50, and 43.025 nmol/L, respectively. The average levels of group A were higher than those of group B and group C, and the difference was statistically significant ($P < 0.001$, Table 2).

The levels of vitamin D in venous blood with two different detection methods and capillary blood with chemiluminescence method were analyzed by paired t-test. The paired t-test showed that the vitamin D levels in venous blood detected by mass spectrometry and capillary blood detected by chemiluminescence method were significantly different ($t = 8.326$, $p < 0.01$) (Table 3). Paired t-test showed that the difference of 25(OH)D levels in venous blood and capillary blood detected by chemiluminescence method was also statistically significant ($t = 5.636$, $P < 0.05$), and the mean value of capillary 25(OH)D level was significantly higher than that of venous 25(OH)D level detected by the two methods ($P < 0.05$).

3.2 Consistency analysis of the two detection samples

The linear fitting formula of scatter data from venous blood detected by chemiluminescence and mass spectrometry was as follows: venous blood 25(OH)D(CLIA) nmol/l =1.128*venous blood 25(OH)D(LC-MS) + 3.628 nmol/l $R^2 = 0.702$. Normality test was carried out on the three groups of data, and all of them were normal distribution. Pearson correlation analysis showed that

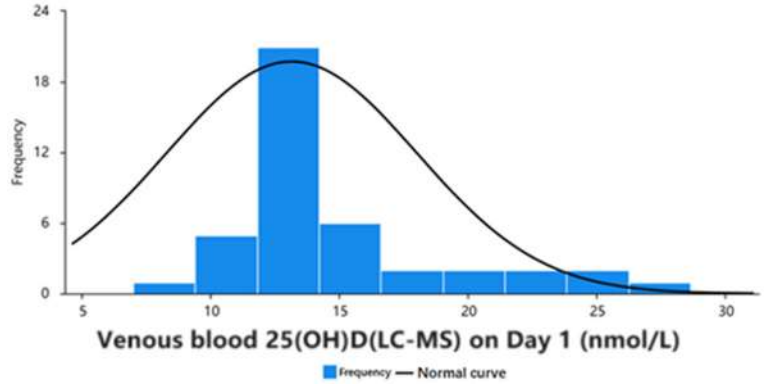

FIGURE 2
Frequency distribution and normality curve of initial venous blood 25(OH)D concentration in 42 participants.

TABLE 2 Summaries of statistical results from each time period (nmol/L).

	Group A	Group B	Group C	Total
Day 1	30.00 ± 3.472	29.93 ± 3.64	29.33 ± 2.88	29.912 ± 3.468
Day 14	38.52 ± 3.63	39.54 ± 3.26	39.66 ± 2.90	39.712 ± 3.208
Day 28	41.32 ± 2.87	40.74 ± 2.866	40.98 ± 2.45	40.886 ± 3.104
Month 6	49.21 ± 2.74	42.50 ± 3.29	43.03 ± 2.03	44.943 ± 2.930
Month 12	48.75 ± 3.58	42.88 ± 4.11	43.22 ± 1.58	45.136 ± 3.393

TABLE 3 Paired *T*-test analysis results for venous blood and capillary blood 25(OH)D concentration.

Item	Mean value	Standard deviation	t	P
Venous blood 25(OH)D LC-MS nmol/L	45.32	8.56		
			8.326	<0.01
Capillary blood 25(OH)D LC-MS CLIA nmol/L	52.03	7.44		
			5.636	<0.05
Venous blood 25(OH)D LC-MS nmol/L	49.88	12.66		

25(OH)D concentration.

there was a good correlation between 25(OH)D levels measured by the two methods ($r = 0.732$, $P < 0.001$). When the concentration of 25(OH)D in venous and capillary samples was measured by chemiluminescence, the concentration of 25(OH)D in capillary blood was higher than that in venous (Figures 3, 4). The linear fitting formula of scatter data measured by chemiluminescence was as follows: concentration of 25(OH)D in venous blood (nmol/L) = 1.105 * concentration of 25(OH)D in capillary blood −7.532 nmol/L, $R^2 = 0.625$.

Taking into account that the variability of differences increases with amplitude, we plotted the percentage difference on the Bland-Altman. Analysis revealed a significant positive bias in venous blood 25(OH)D measured by CLIA compared to LC-MS. The results of CLIA were significantly higher than those of LC-MS. The standard deviation of the difference was 4.259 nmol/ml (95% confidence interval [CI] −2.013 to 8.465), which translates to a percentage difference of 6.259%. Bland-Altman analysis showed that the serum 25(OH)D of capillary blood and venous blood measured by CLIA method had a significant positive bias, and the detection result of capillary blood was significantly higher than that of venous blood. The standard deviation of the difference was 2.234 nmol/mL (95% confidence interval [CI] −10.662–15.130), which translates to a percentage difference of 1.266% (Figures 5, 6).

In Cronbach's reliability analysis, the reliability coefficient value was 0.765 which was >0.6, indicating that the reliability quality of the research data was acceptable. For the "CITC value," the values of the analysis items were all >0.4, indicating a good correlation between the analysis items and a good level of reliability. In summary, the reliability coefficient value of the research data was >0.6, indicating that the quality of data reliability was acceptable.

We used approximate entropy, a non-linear dynamic complexity measure, to evaluate the efficacy of vitamin D treatment. The approximate entropy of other variables to vitamin D ratio was measured, with a *T*-value of 0.4614 for bone density, 0.5864 for vitamin A, and 0.7462 for capillary blood vitamin D. Among other possibly related analysis results, the correlation between vitamin D in the capillary blood and venous blood was good.

Kappa statistic analysis was used to evaluate the consistency of the results of vitamin D detection among the above three groups of data. When the data were classified as vitamin D sufficient, insufficient, and deficient, paired analysis was performed for the comparison between the three groups of data (Table 4). The *p* values of the three groups of data were all <0.01, and the Kappa value was >0.7, indicating that the results of 25(OH)D analysis in venous blood by chemiluminescence method were consistent with the results of 25(OH)D analysis in capillary blood by mass spectrometry. Combined with the Kappa coefficient analysis, the Kappa coefficient of the results of venous blood 25(OH)D analysis by chemiluminescence method and capillary blood 25(OH)D analysis by mass spectrometry was 0.723, which was between 0.6

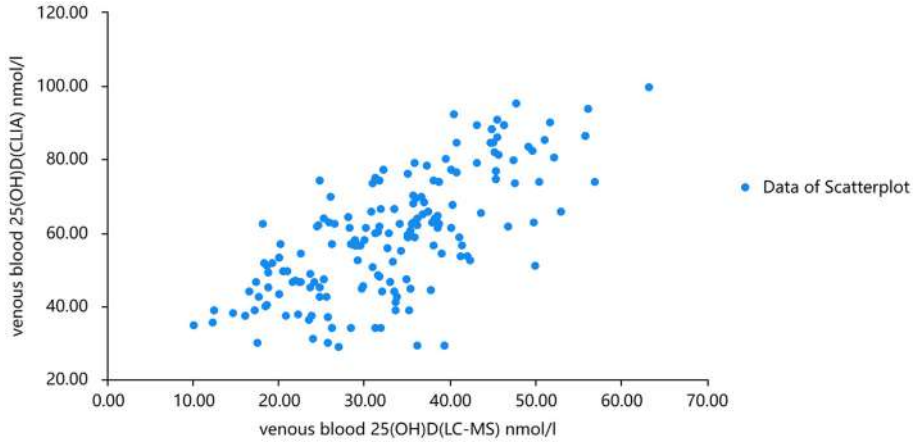

FIGURE 3
Scatter plots of serum 25(OH)D levels analyzed by mass spectrometry and chemiluminescence, respectively.

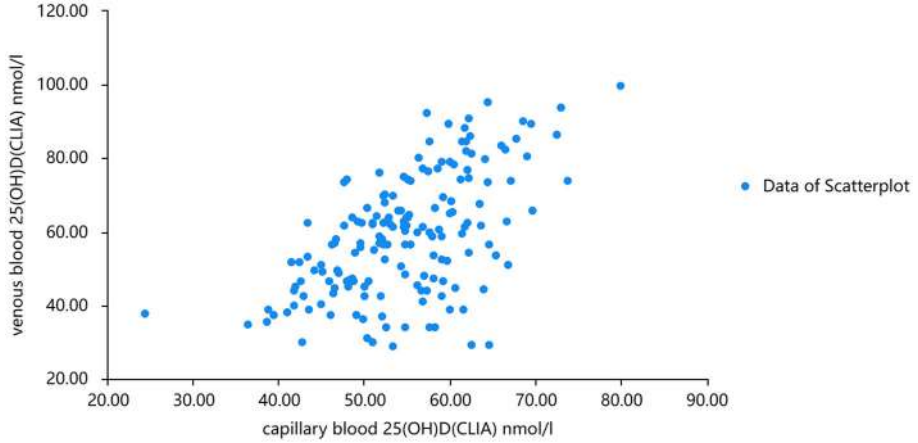

FIGURE 4
Chemiluminescence was used to analyze the scatter plots of venous 2 and capillary 25(OH)D concentrations.

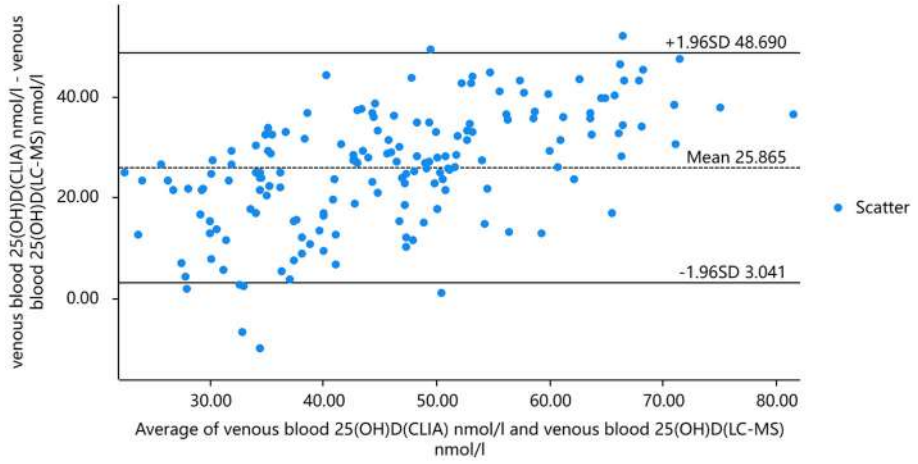

FIGURE 5
The results of 25(OH)D in venous blood were analyzed by mass spectrometry and chemiluminescence and Bland-Altman analysis.

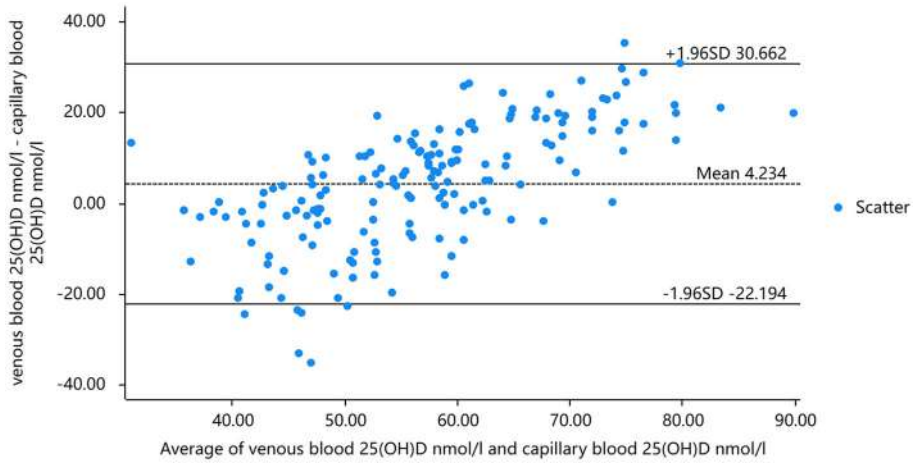

FIGURE 6
Bland-Altman analysis of 25(OH)D concentration in venous and capillary blood by chemiluminescence method.

and 0.8, indicating that the results of the two groups had a strong consistency in judging whether vitamin D deficiency and the results were reliable.

There was a significant positive correlation between capillary blood serum 25(OH)D and venous blood serum 25(OH)D. When the corrected capillary serum 25(OH)D was used to diagnose vitamin D deficiency, the capillary serum 25(OH)D result was able to identify subjects with vitamin D deficiency (clinical thresholds 30.00 and 50.00 nmol/L) with sensitivity of 0.97–0.98,. The specificity was 0.89–0.94, and the ROC AUC was 0.96–0.97 ($P < 0.001$, Figures 7, 8).

4 Discussion

Vitamin D is an important nutrient that maintains bone metabolism in the human body. Vitamin D deficiency is closely related to chronic diseases such as multiple sclerosis, type I and II diabetes (6). Conversely, vitamin D deficiency increases the risk of preeclampsia, and lower serum 25(OH)D concentrations are associated with cardiovascular diseases in pregnant women and their offspring (7). 25(OH)D is an active form of vitamin D produced by liver hydroxylation in the body. It is relatively stable in the blood and has no significant pulse secretion or circadian rhythm changes. It is the best indicator for evaluating the vitamin D nutritional status in the body (8). Many studies on this topic have been conducted in different directions both domestically and internationally (9–11). The accurate detection of 25(OH)D and the correct interpretation by clinical doctors are of great significance to monitor the efficacy and safety of vitamin D deficiency-related diseases in the diagnosis and treatment process.

There are consistent assessments of serum 25(OH)D levels in venous and capillary blood, and consistent assessments of 25(OH)D levels in the same blood sample by different assays. A study assessing vitamin D nutritional status in newborns indicated that two common immunoassays lead to very different classifications of vitamin D status. May be related to interference with other vitamin D metabolites (12). In other studies, although there was good agreement between capillary blood and venous blood measurements, the subjects were mostly children, and capillary blood 25(OH)D measurements were lower than venous blood measurements (13).

The detection methods for serum 25(OH)D range from initial radioimmunoassay and enzyme-linked immunosorbent assays to high-performance liquid chromatography, electrochemiluminescence immunoassay (ECLISA), and LC-MS/MS, which have become increasingly accurate methods for detecting serum 25(OH)D levels. At present, the mainstream detection methods are ECLISA and LC-MS/MS. LC-MS has higher specificity in detecting 25(OH)D, which can more accurately distinguish 25(OH)D from other similar compounds, improving the sensitivity and accuracy of detection. This reduces the possibility of cross-reactivity and non-specific binding, and the measurement results are generally considered to be closer to the actual level. CLIA uses antibodies, and other vitamin D metabolites with structures similar to 25(OH)D are also likely to cross-react, leading to higher CLIA measurements. Therefore, there may be significant differences in the results of 25(OH)D determination between the two methods.

Some clinicians use immunoassay to detect 25(OH)D. If the serum 25(OH)D standard used in the determination of vitamin D nutritional level is consistent with CLIA method, the vitamin D status of patients may be misjudged. Therefore, caution must be exercised when interpreting the serum 25(OH)D detection report and the detection method must be clearly defined. In this trial, we tried to establish a linear relationship between the different results in order to obtain a more accurate vitamin D level.

According to the test results, the result of serum 25(OH)D in venous blood measured by CLIA method was greater than that of capillary blood serum 25(OH)D measured by CLIA method. The clinician may not be able to determine the vitamin D level of the patient due to different methods or different samples using the same judgment criteria, so we suggest using the truncation value derived from the linear equation for vitamin D status assessment:

TABLE 4 Kappa coefficient table for the consistency evaluation of the three 25(OH)D results.

Item	Kappa value	Standard error	*Z* value	*p* value*	95%CI
Venous blood 25(OH)D(LC-MS) & capillary blood 25(OH)D(CLIA)	0.723	0.066	4.890	0.01	0.203–0.443
Venous blood 25(OH)D(LC-MS) & venous blood 25(OH)D(CLIA)	0.895	0.071	8.431	0.01	0485–0.704
Venous blood 25(OH)D(LC-MS) & capillary blood 25(OH)D(LC-MS)	0.793	0.063	6.730	0.01	0.403–0.653

The 95% confidence interval calculation formula in Table 4 has been added to the note of the table. 95%CI = [Kappa value ± 1.96* (progressive standardized error)]. *$p < 0.05$.

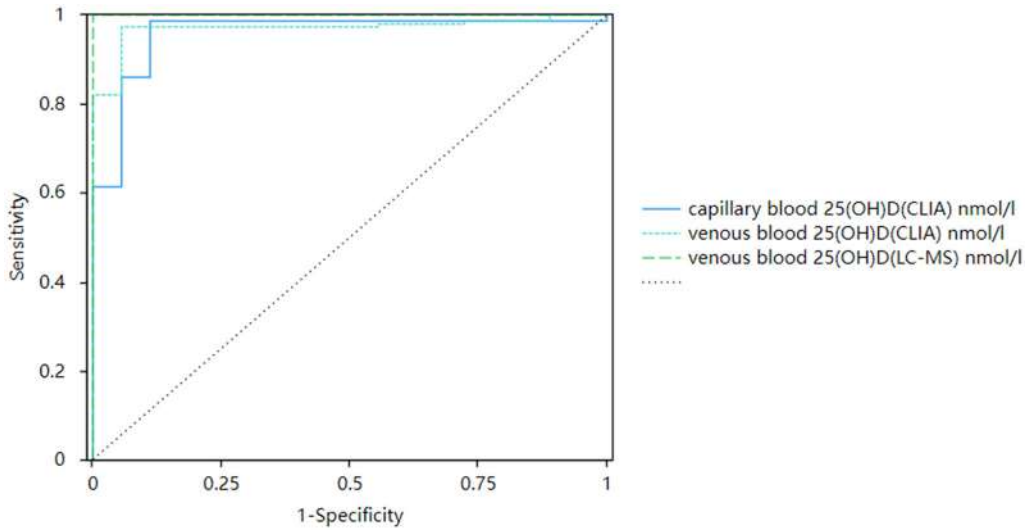

FIGURE 7
Corrected receiver operating curves of capillary and venous 25(OH)D with 30 nmol/L as the cutoff value.

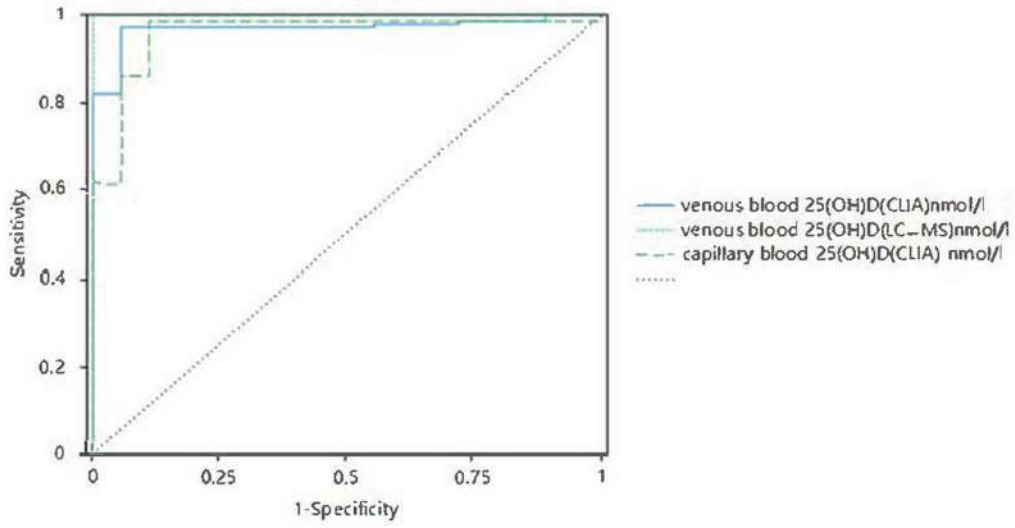

FIGURE 8
Corrected receiver operating curves of capillary and venous 25(OH)D with 50 nmol/L as the cutoff value.

The cut-off values of 25(OH)D in venous blood were 30.00, 50.00, and 250.00 nmol/L by LC-MS, and the corresponding cut-off values of 25(OH)D in venous blood serum by CLIA method were 37.468, 60.028, and 285.628 nmol/L, respectively. The cut-off values of 25(OH)D in capillary blood by CLIA were 40.723, 66.844, and 265.303 nmol/L, respectively.

When detecting vitamin D, venous blood volume of 3–5 mL must be collected, and the capillary blood volume must be 20 μL.

The amount of capillary blood collected is small (13), the tools used are simple, the detection process is time-consuming, and it is more convenient when the patient cannot, or is not suitable, or have conditions that do not support it. Clinicians are faced with different blood collection methods, and their concentration relationships must be compared. In this study, the two detection methods showed good consistency when the measured value of 25(OH)D was >37.5 nmol/L, and the two can be mutually referenced. However, for capillary blood, during the collection process, owing to the possibility of infiltration of the squeezed fabric into the collected samples, different vitamin D levels in different body parts, and the susceptibility of capillary blood examination results to environmental temperature, humidity, and other factors may lead to certain deviations in the test results, and the results cannot be distinguished between D2 and D3, which must be taken into consideration.

5 Conclusions

In summary, the detection results of 25(OH)D in the capillary and venous blood are comparable. When venous blood collection is inconvenient or there are special requirements for blood collection volume and frequency, the vitamin D level in capillary blood samples can be measured to replace the venous blood. The obtained results can be calculated using a common formula to determine the vitamin D content in the venous blood or converted into a reference range for the capillary blood. However, owing to the small sample size of this study, the normal distribution of 25(OH)D in the serum of the participants did not show extreme values. The detection method, data conversion, and result interoperability must be confirmed, and the feasibility still needs to be further expanded to provide more accurate references for clinical practice.

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 humans were approved by the Ethics Committee of the Second Hospital of Hebei Medical University. 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

YX: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. KW: Writing – original draft, Investigation, Software. XM: Writing – review & editing, Validation. HZ: Conceptualization, Visualization, Writing – review & editing. XT: Data curation, Project administration, Writing – review & editing.

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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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References

1. Grant WB, Schwalfenberg GK, Genuis SJ, Whiting SJ. An estimate of the economic burden and premature deaths due to vitamin D deficiency in Canada. *Mol Nutr Food Res.* (2010) 54:1172–81. doi: 10.1002/mnfr.200900420

2. Högler W. Complications of vitamin D deficiency from the foetus to the infant: One cause, one prevention, but who's responsibility. *Best Pract Res Clin Endocrinol Metab.* (2015) 29:385–98. doi: 10.1016/j.beem.2015.03.003

3. Subspecialty Group of Child Health Care, Society of Pediatrics, Chinese Medical Association, Editorial Committee of Chinese Journal of Pediatrics. Chinese practice guideline for clinical issues related to vitamin D nutrition in children. *Chin J Pediatrics.* (2022) 60:387–94. doi: 10.3760/cma.j.cn112140-20211230-01092

4. Zhang H, Yu X, Mao M, Li H. Interpretation of clinical practice guidelines for vitamin D nutrition related issues in Chinese children. *Chin J Pediatr.* (2022) 60:408–12.

5. Nishiumi S, Kohata T, Kobayashi T, Kodama Y, Ohtsuki S, Yoshida M. Comparison of venous and fingertip plasma using non-targeted proteomics and metabolomics. *Talanta.* (2019) 192:182–8. doi: 10.1016/j.talanta.2018.09.047

6. Holick MF. Vitamin D: a d-lightful solution for health. *J Investig Med.* (2011) 59:872–80. doi: 10.2310/JIM.0b013e318214ea2d

7. Carreras-Badosa G, Armero-Bujaldón C, Solé-Amat L, Prats-Puig A, Diaz-Roldan F, Soriano-Rodriguez P, et al. Serum 25-hydroxyvitamin D and cardiovascular disease risk factors in women with excessive weight gain during pregnancy and in their offspring at age 5-6 years. *Int J Obes (Lond).* (2018) 42:1019–28. doi: 10.1038/s41366-018-0101-6

8. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol.* (2010) 10:482–96. doi: 10.1016/j.coph.2010.04.001

9. Stoffman N, Gordon CM. Vitamin D and adolescents: what do we know. *Curr Opin Pediatr.* (2009) 21:465–71. doi: 10.1097/MOP.0b013e32832da096

10. Bacchetta J, Pelletier S, Jean G, Fouque D. Immune, metabolic and epidemiological aspects of vitamin D in chronic kidney disease and transplant patients. *Clin Biochem.* (2014) 47:509–15. doi: 10.1016/j.clinbiochem.2013.12.026

11. Bak M, Serdaroglu E, Guclu R. Prophylactic calcium and vitamin D treatments in steroid-treated children with nephrotic syndrome. *Pediatr Nephrol.* (2006) 21:350–4. doi: 10.1007/s00467-005-2118-z

12. Gallo S, Comeau K, Agellon S, Vanstone C, Sharma A, Jones G, et al. Methodological issues in assessing plasma 25-hydroxyvitamin D concentration in newborn infants. *Bone.* (2014) 61:186–90. doi: 10.1016/j.bone.2014.01.012

13. Jiao X, Yuan Y, Wang X, Li J, Liu B, Yuan T, et al. Development of a sensitive HPLC-MS/MS method for 25-hydroxyvitamin D(2) and D(3) measurement in capillary blood. *J Clin Lab Anal.* (2020) 34:e23451. doi: 10.1002/jcla.23451

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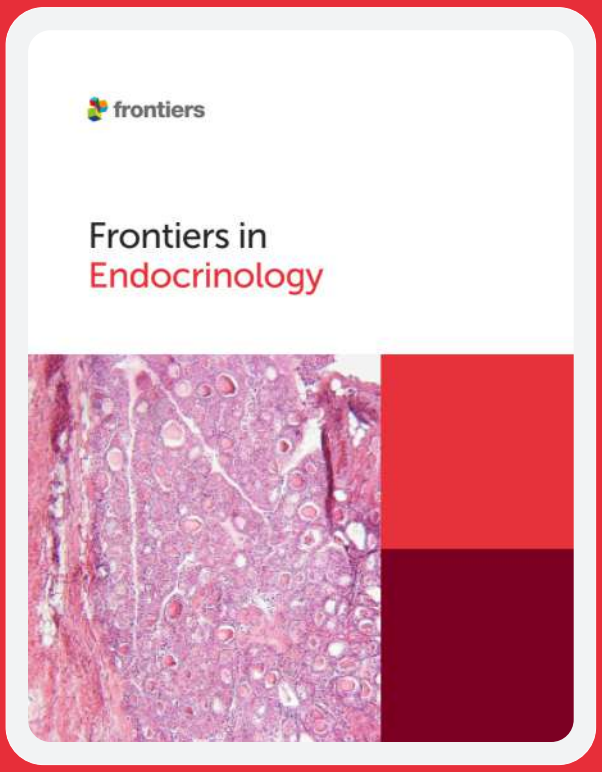

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