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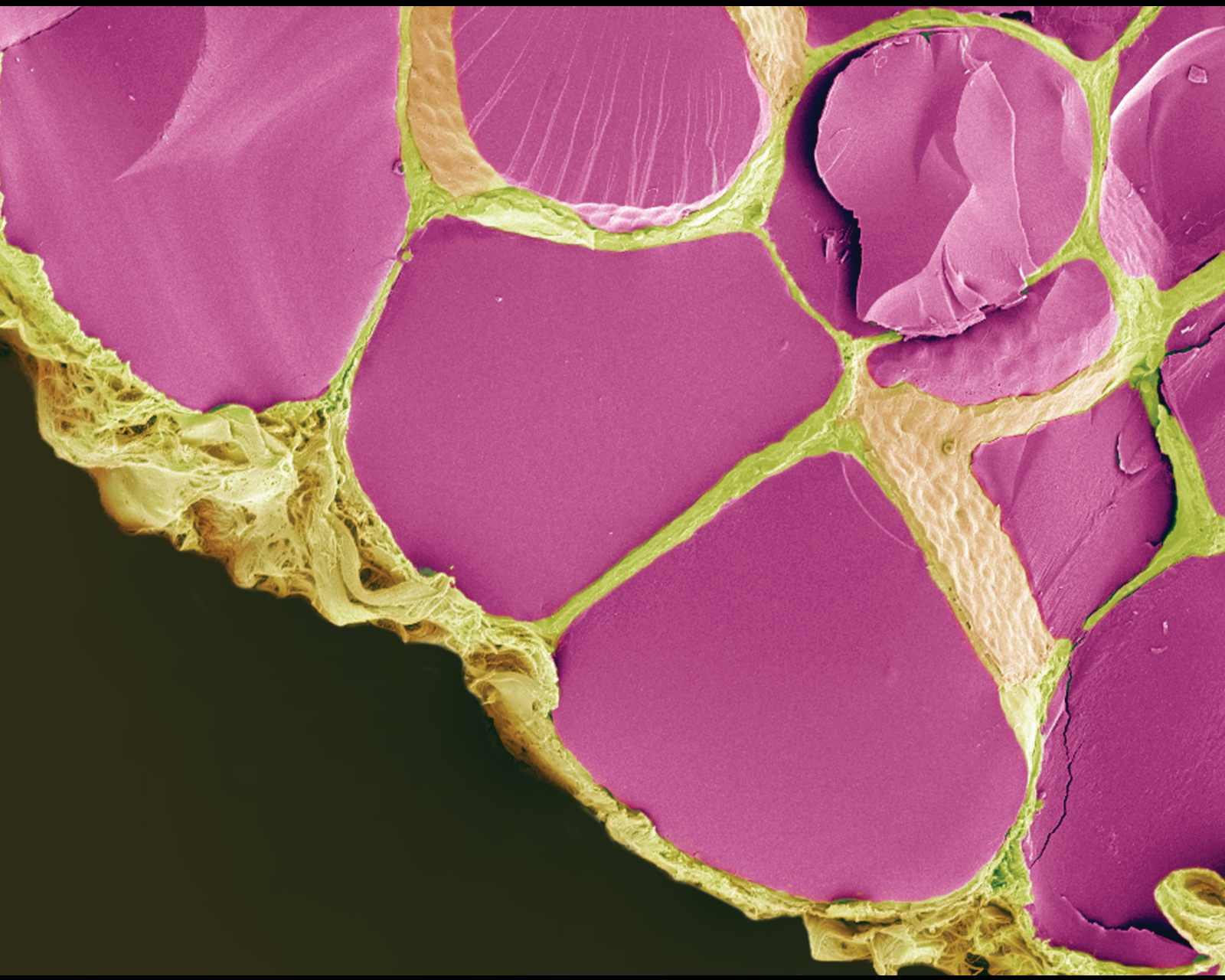

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Contents

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Review Article

Vitamin D Deficiency and the Presentation of Primary Hyperparathyroidism: A Mini Review

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The clinical presentation of primary hyperparathyroidism (PHPT) has evolved over the years from a symptomatic disorder to a predominantly asymptomatic condition. Altered vitamin D metabolism seems to play a role in the presentation of PHPT and may exacerbate the severity of disease. The epidemiology of PHPT differs in the developing versus the developed world, where more severe phenotypes occur in regions where vitamin D deficiency is common. Although it has been validated that patients with PHPT should be vitamin D sufficient, the threshold to supplement in relation to the severity of PHPT and the degree of vitamin D deficiency remains controversial. This review will highlight some of the controversy regarding vitamin D deficiency and the different phenotypes of PHPT.

1. Introduction

Primary hyperparathyroidism (PHPT) is the most common cause of hypercalcemia and is characterized by elevated or inappropriately normal parathyroid hormone (PTH) levels. There are three phenotypes of PHPT [1]. There is classic or symptomatic PHPT which presents with renal or skeletal complications such as nephrolithiasis or osteitis fibrosa cystica. There is asymptomatic PHPT which presents with no obvious signs or symptoms but may still have some renal or skeletal involvement. Also, the newest classification is normocalcemic PHPT (NPHPT) which is defined by normal albumin-corrected or ionized calcium levels with elevated PTH levels. Patients who fit this category may or may not have renal or skeletal complications. Vitamin D is a fat-soluble secosteroid that plays a central role in calcium homeostasis and bone metabolism through the feedback of calcium, phosphate, and PTH. Vitamin D deficiency can cause secondary hyperparathyroidism which must be differentiated from PHPT. The presentation of vitamin D deficiency in PHPT varies geographically. In the Western world, where biochemical screening is common, asymptomatic or NPHPT is more frequently noted. In contrast, symptomatic disease with renal and/or skeletal manifestations is the more predominant phenotype in developing countries [2]. As compared to the general population, vitamin D deficiency is more common in PHPT patients and has been associated with more severe disease [3, 4]. The mechanism behind the association of vitamin D deficiency and PHPT is not clear and remains controversial [5]. In this article, we aim to review the recent research looking into the relationship between the phenotypes of PHPT and 25(OH)D levels as well as vitamin D-binding protein.

1.1. Epidemiology and Prevalence of Vitamin D Deficiency in PHPT. Primary hyperparathyroidism and vitamin D deficiency are common conditions, and their effects are interrelated. The operational definition of vitamin D deficiency as given by the Institute of Medicine is a 25(OH)D level ≤20 ng/ml (50 nM/l) [6]. It has also been suggested to define vitamin D insufficiency as 25(OH)D levels between 20 and 30 ng/mL

and deficiency as <20 ng/mL [2, 4]. These cutoffs are established for optimal bone health and not in the context of PHPT [4]. The likelihood of 25(OH)D insufficiency (81%) and 25(OH)D deficiency (33%) in PHPT is reported to be much higher than in sex- and age-matched control populations (60% for insufficiency and 20% for deficiency) and remains so with seasonal variations [7, 8]. While 25(OH)D levels are highest in the late summer months (July-August) for people with or without PHPT, the average 25(OH)D level is still overall reduced in PHPT patients [7]. Recent trends show a decrease in the prevalence of vitamin D deficiency in PHPT, possibly from increased supplementation [6, 9]. It is estimated that the prevalence of vitamin D deficiency and insufficiency in PHPT has decreased by 50% and 30%, respectively, with a corresponding decline in PTH levels [10].

1.2. Geographic Variation of 25(OH)D Levels in PHPT. With the advent of increased access to population lab screening, developed nations have seen a shift in the clinical presentation of PHPT from a symptomatic disorder to that of a largely asymptomatic condition. In a study comparing PHPT patients in New York to those in Shanghai, the average 25(OH)D levels were found to be significantly lower (13 ng/mL) in the Shanghai cohort versus the New York cohort (36.7 ng/mL, $p < 0.001$) [11]. The patients in the Shanghai cohort also showed biochemical evidence of more severe PHPT with significantly higher PTH (402.1 pg/mL vs. 67.5 pg/mL, $p < 0.001$), alkaline phosphatase (112 U/L vs. 75 U/L, $p < 0.001$), and serum calcium (11.72 mg/dL vs. 10.6 mg/dL, $p < 0.001$) levels compared to the New York cohort. There were no cases of NPHPT in the Shanghai cohort which was partly attributed to the relatively higher prevalence of vitamin D deficiency [11]. Similar trends have been noted in other studies done in China, India, and Brazil, where higher rates of symptomatic disease were noted with lower 25(OH)D levels [12–14]. However, cases of milder or asymptomatic PHPT are rising in developing countries in more recent years [11]. One study in 2021 noted that while the majority (>90%) of PHPT patients in India still present with symptomatic disease, there is a rising prevalence of asymptomatic disease with lower PTH levels and an improved vitamin D status [15].

1.3. Vitamin D Deficiency and Classic PHPT. Prior to the ubiquitous screening of calcium in the last several decades, classic PHPT was described as a symptomatic condition with renal and/or skeletal manifestations at the time of diagnosis [9]. Most notably, osteitis fibrosa cystica, which clinically presents as bone pain and is radiographically characterized by a "salt and pepper" appearance of the skull, brown tumors, subperiosteal bone resorption, and osteolytic lesions, is now considered a very rare complication in the developed world [9, 16]. Low bone mineral density (BMD) and fragility fractures are now the more common skeletal signs of symptomatic disease. Classic renal manifestations include hypercalciuria, nephrolithiasis, and nephrocalcinosis.

There have been several studies that suggest vitamin D deficiency (defined as a 25(OH)D level at least <20 ng/mL) is associated with a more severe presentation of PHPT. This is thought to be due to higher PTH levels with higher calcium levels, increased bone turnover markers with lower bone mineral density, and increased parathyroid mass [11, 12, 17–19]. Patients who present with osteitis fibrosa cystica have been shown to have significantly lower 25(OH)D levels than those with asymptomatic disease (16.7 ± 1.1 ng/mL versus 29.9 ± 2.9 ng/mL, $p < 0.02$). They also have significantly higher PTH levels compared to asymptomatic PHPT patients (1352.8 ± 297.2 pg/mL versus 145.0 ± 43.7 pg/mL, $p < 0.02$) [12]. This has also been demonstrated in a cohort of patients with PHPT in India where a majority of patients (90%) present with osteitis fibrosa cystica and have severe vitamin D deficiency with an average vitamin D level of 8.4 ± 5.1 ng/mL [17]. In a study comparing features of PHPT in New York and Shanghai, the patients in China who often presented with symptomatic disease had significantly higher calcium and PTH levels as well as lower vitamin D levels [9]. In an Italian cohort of PHPT patients, those who had 25(OH)D levels below 20 ng/mL were noted to have significantly higher PTH, alkaline phosphatase, and bone turnover markers and significantly lower BMD than patients with 25(OH)D levels above 20 ng/mL [18]. In another Italian cohort of women with sporadic PHPT, it was found that the lowest quartile of 25(OH)D levels correlated with higher PTH levels, higher bone-specific alkaline phosphatase levels, and lower estimated glomerular filtration rates (GFRs). No association was found with serum and urine calcium levels, fracture risk, or nephrolithiasis [20]. Of note, another study similarly showed that a 25(OH)D level less than 20 ng/mL was associated with higher PTH levels but showed no clinical difference in bone mineral density, osteoporosis, fracture, nephrolithiasis, or urinary calcium [10].

1.4. Vitamin D Deficiency and Asymptomatic PHPT. In the 1970s, the clinical presentation of PHPT in the Western world shifted from the symptomatic disorder previously described to an asymptomatic condition likely due to widespread biochemical screening. Asymptomatic PHPT is a unique phenotype characterized by mild hypercalcemia without the classic symptoms. Calcium levels are usually less than 1 mg/dL above the upper limit of normal, and the PTH level is less than 2-fold above the upper range of normal [21]. Vitamin D insufficiency or deficiency is commonly noted in asymptomatic PHPT and is associated with higher PTH levels and higher markers of bone turnover such as bone-specific alkaline phosphatase [10, 22]. Low 25(OH)D levels have also been associated with more cortical bone loss and preservation of trabecular bone in these patients [22, 23]. It is, therefore, recommended that 25(OH)D levels be checked in all cases of asymptomatic PHPT and cautious repletion with vitamin D supplementation be initiated if 25(OH)D levels fall below 20 ng/mL [24, 25]. In a study that followed patients with asymptomatic PHPT over the course of 15 years, about a third of patients had progressive disease that eventually met criteria for surgery [26]. Despite the lack

of symptoms, patients with seemingly asymptomatic PHPT can still have renal or skeletal involvement which has led to recommendations to continue monitoring asymptomatic disease with radiographic imaging for renal stones, osteoporosis, or fractures [2, 27]. Vitamin D deficiency or insufficiency is less prevalent in asymptomatic PHPT patients due to increased routine vitamin D supplementation [24]. There may also be a role for vitamin D-binding protein (DBP). One study reported significantly lower DBP levels in a cohort of postmenopausal women with PHPT compared to age- and BMI-matched controls. Calculated free 25(OH)D levels and $1,25(OH)_2D$ levels were not significantly different between the two groups [28].

1.5. Vitamin D Deficiency and Normocalcemic PHPT. In the early 2000s, NPHPT was recognized as a new entity of PHPT with elevated PTH levels in the absence of hypercalcemia [29]. These patients have both normal ionized calcium and total albumin-corrected calcium levels. An increase in PTH measurements as part of the workup for low bone mass and diagnosis of osteoporosis helped discover this biochemical phenotype where calcium levels consistently remain normal [9]. To evaluate this diagnosis, secondary causes of hyperparathyroidism, such as vitamin D deficiency, chronic kidney disease, malabsorption, and medication use such as lithium, diuretics, bisphosphonates, and denosumab, must also be ruled out.

It is possible that slightly elevated PTH levels may be a part of the normal distribution curve in the spectrum of PTH ranges. It is estimated that about 2.5% of the normal population can be a part of this spectrum [9]. Another perspective is that while the increase in PTH may actually cause an increase in the serum calcium level, the concentration may stay within the normal cutoff limits and thus not be detected [30].

There is evidence to suggest that NPHPT patients develop complications of PHPT despite having normal calcium levels and that it does not represent a mild, asymptomatic form. One study concluded that patients seen with NPHPT have more substantial skeletal involvement compared to PHPT and develop more complications in the course of the disease [30, 31]. Another study found higher incidence of kidney stones in NPHPT and similar fracture history in comparison to PHPT. It was hypothesized that NPHPT patients may exhibit resistance to calcium at the bone and kidney levels leading to a higher PTH concentration [32].

The levels of 25(OH)D that would define deficiency in NPHPT are not fully delineated and both >20 ng/mL or >30 ng/mL have been used to rule out deficiency (Table 1). The Institute of Medicine's current cutoff for the diagnosis of vitamin D deficiency is 25(OH)D < 20 ng/mL, which applies to the general population as previously discussed. Some experts suggest repletion to 25(OH)D levels above 30 ng/mL (75 nmol/L), as there is evidence that even vitamin D insufficiency increases PTH levels [9, 33]. Once the 25(OH)D level is in the 30–40 ng/ml range, its previous exponential effect on PTH flattens [37]. Some have even recommended repletion to >40 ng/mL to see the effects on PTH levels [9, 33, 37].

A report that studied free versus total 25(OH)D levels in NPHPT showed that measured free 25(OH)D levels were 20% lower in NPHPT patients than in healthy age-, sex-, and BMI-matched controls (5 vs. 6.2 pg/mL, $p = 0.013$). The total 25(OH)D levels were >30 ng/dL in both the NPHPT and control groups and were not significantly different [35]. This again raised the question of whether total 25(OH)D levels are truly representative of the actual vitamin D status in NPHPT patients and warrants further investigation [37].

1.6. Vitamin D Levels in Multiple Endocrine Neoplasia (MEN) Disorders and Familial Hypocalciuric Hypercalcemia (FHH). It is estimated that about 1–18% of the patients with PHPT have underlying multiple endocrine neoplasia type 1 (MEN1) disease [38]. It presents as a multiglandular entity compared to sporadic PHPT that presents as a single gland adenoma 80–85% of the time. It is associated with recurrent hyperparathyroidism even after presumed successful surgery and the recurrence rates can be up to 50% by 12 years [38–40]. The MEN1 gene product "menin" directly interacts with the vitamin D receptor (VDR) and enhances gene transcription, leading to lower VDR expression in adenoma cells [41]. One study found that 21 out of 31 patients with MEN1 had 25(OH)D levels <10 ng/mL, 9 had levels between 10 and 30 ng/mL, and 1 patient had normal levels, showing the degree of vitamin D deficiency in MEN1 [42]. In addition, PHPT can occur in 20–30% of typical multiple endocrine neoplasia type 2A (MEN2A) syndrome [43].

Familial hypocalciuric hypercalcemia (FHH) is a rare inherited disorder of the calcium-sensing receptor gene, *CASR*. FHH can be biochemically similar to PHPT with an elevated calcium level associated with a normal or high PTH level, a normal 25(OH)D level, and a high 1,25-dihydroxyvitamin D level. The 24-hour urinary calcium excretion, however, is low in FHH. The calculated calcium/creatinine clearance ratio should be <0.01 [9]. It is important for patients to be vitamin D replete for diagnosis as the calcium/creatinine ratio can be <0.01 for patients with PHPT and vitamin D deficiency. Less conclusive studies suggest that vitamin D supplementation in familial PHPT syndromes may show beneficial effects on serum PTH and bone mineral density (BMD); however, more studies are needed [44].

1.7. Total or Free 25(OH)D and Vitamin D-Binding Protein in PHPT. Total 25(OH)D can be both free or bound to protein. The vast majority of circulating 25(OH)D is bound to vitamin D-binding protein (DBP) and not biologically available. At present, total 25(OH)D levels are checked to assess the 25(OH)D status and designate deficiency or insufficiency. Most 25(OH)D is bound to DBP, 10–15% is albumin bound, and <1% is unbound in the serum. Different clinical conditions can affect DBP levels, 25(OH)D binding, and total 25(OH)D levels [45]. In a study of 88 patients with PHPT matched with related and unrelated family members without PHPT, it was found that 25(OH)D levels as well as

Table 1: NPHPT with 25(OH)D levels: age (yo) is expressed as the range or mean ± SD years old; n = PHPT patients; N = survey subjects; prevalence is expressed as % = n/N; 25(OH)D cutoff: diagnostic criteria of vitamin D deficiency in the study = expressed as range or mean ± SD ng/dl.

Authors	Age (yo)	n/N Prevalence	% Female	25(OH)D cutoff (ng/mL)	Study population
Lowe et al. [30]	32–78	37/?	95	25D > 20 20–54	Referral center USA
Cusano et al. [33]	70 ± 6	9/2364 0.38%	0	25D > 20 25.2 ± 5	Community based USA
Chen et al. [34]	60 ± 18.5	11/940 1.12%	46	25D > 20 26.5 ± 6	Referral center China
Wang et al. [35]	59.9 ± 5.4	10/940 1.11%	90	25D > 30 31.9 ± 1.7	Community based USA
Schini et al. [36]	57–88	11/6280 0.18%	91	25D > 20	Metabolic bone center UK

DBP and free and bioavailable (albumin-bound vitamin D plus free vitamin D) 25(OH)D levels were low in PHPT patients. The authors suggested that although low DBP is found in PHPT, it alone cannot be responsible for low vitamin D levels [46]. However, since most of 25(OH)D is DBP bound, lower DBP may cause low total 25(OH)D levels in asymptomatic PHPT patients [47].

Several studies have shown that although total 25(OH)D levels are lower in PHPT compared to controls, the DBP levels are also lower, so the calculated free 25(OH)D levels remain unchanged [28, 47, 48]. Another report showed that calculated free $1,25(OH)_2D$ levels are higher in postmenopausal PHPT patients and correlate better with PTH levels in comparison to total $1,25(OH)_2D$ [49]. These studies raise the question of whether free 25(OH)D levels may be a better indicator of the vitamin D status in PHPT but this needs further exploration.

1.8. Potential Mechanisms of Low 25(OH)D in PHPT. There is evidence that vitamin D deficiency acts as an inciting factor that worsens the clinical disease status of PHPT [9]. The exact mechanism behind the higher prevalence of low 25(OH)D levels in PHPT is multifactorial, involving low vitamin D intake or production, disturbed 25(OH)D metabolism, and interactions with vitamin D-binding proteins (Figure 1).

PTH enhances the conversion of 25(OH)D to 1,25-dihydroxyvitamin D through increased 1-alpha hydroxylase activity in the kidneys via the CYP27B1 activity. Active vitamin D increases serum calcium via its action on vitamin D receptors (VDRs), which is sensed by the calcium-sensing receptor (CaSR) and feeds back to PTH. Active vitamin D regulates VDR levels in the parathyroid and has an ability to prolong VDR half-life. It also regulates the response of the parathyroid gland to calcium by inducing CaSR gene transcription [50]. Active vitamin D inhibits 1-alpha hydroxylase and activates 24-alpha hydroxylase via the CYP24A1 activity (in the kidneys) to regulate serum calcium concentrations [51]. It can thus be conflicting to have low 25(OH)D levels with normal or even high 1,25-dihydroxyvitamin D levels [9].

The elimination half-life of 25(OH)D is significantly shortened in PHPT through increased inactivation of 25(OH)D by 24-hydroxylation to $24,25(OH)_2D$, its inactive form [51]. This can be accounted for by an increased excretion of inactivated vitamin D derivatives in the feces [4, 51].

In countries such as India, traditional clothing also limits sun exposure and the prevalence of a vegetarian diet adds to nutritional 25(OH)D deficiency [52].

Elevated PTH also decreases DBP production in the liver which in turn leads to decreased total 25(OH)D levels. In a study of 50 PHPT patients, DBP and DBP-bound 25(OH)D levels were found to be lower in PHPT patients compared to healthy controls, while free and bioavailable levels were similar between the groups [47]. DBP and 25(OH)D levels have been noted to revert back to normal after parathyroidectomy [47, 48]. Different gene types of DBP can affect total 25(OH)D levels in PHPT patients [46].

The abovementioned studies provide some insight into the pathophysiology of vitamin D metabolism and lower total 25(OH)D levels in PHPT (Figure 1). They confirm that PHPT is associated with lower total vitamin D levels and that these low levels can affect the biochemical presentation. However, there needs to be more studies to assess clinical endpoints such as fracture risk, incidence of nephrolithiasis, and optimal levels of supplementation [4, 9, 20, 46].

2. Supplementation of Vitamin D in PHPT

As previously discussed, low 25(OH)D levels can be associated with a more severe presentation of PHPT with higher PTH and bone turnover marker levels and a larger parathyroid adenoma size than expected [19, 53, 54]. Several studies have tried to analyze the benefits of 25(OH)D supplementation in PHPT with outcomes ranging from hypercalcemia to no significant influence on serum calcium levels or clinical benefits. In a study investigating the effects of vitamin D repletion in patients with mild PHPT for one year, it was noted that mean serum calcium and phosphate levels did not change with the normalization of serum 25(OH)D levels [55]. PTH levels fell by 26% at one year and the serum alkaline phosphatase and urine N-telopeptidelevels were

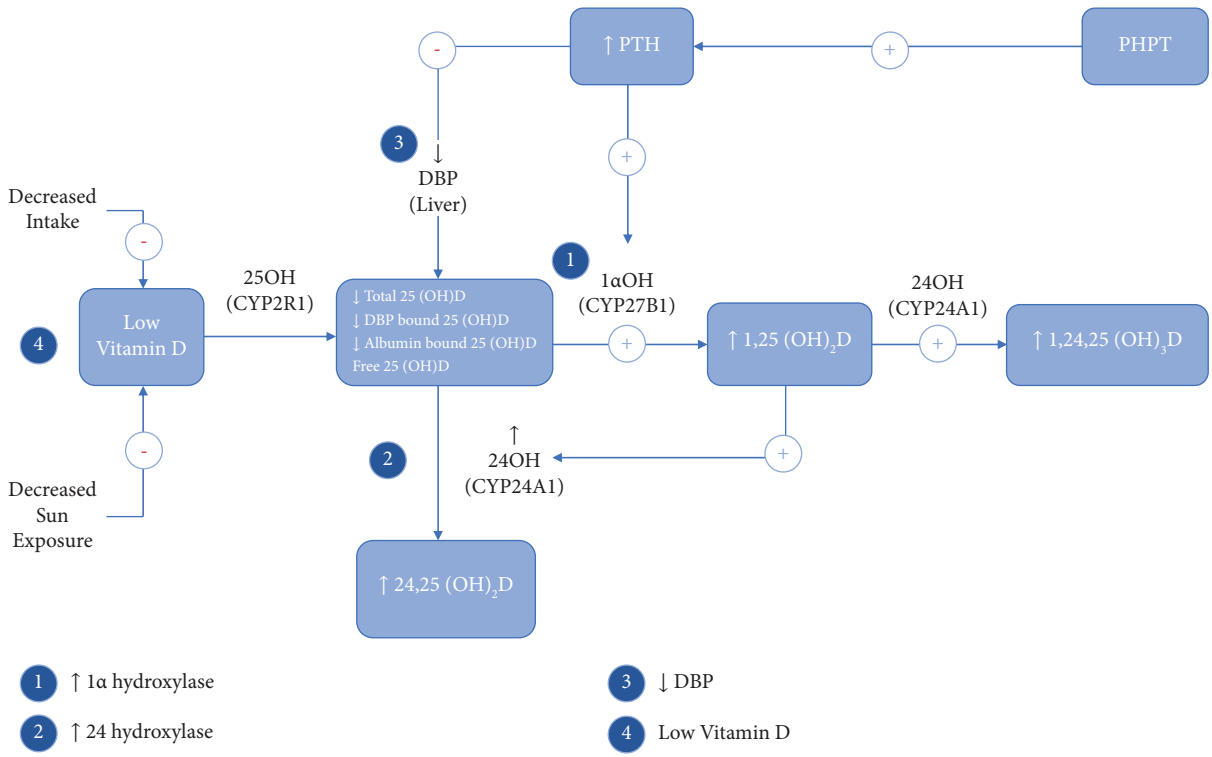

Figure 1: Potential mechanisms of low 25(OH)D levels in PHPT. PHPT: primary hyperparathyroidism; PTH: parathyroid hormone; DBP: vitamin D-binding protein; 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; $24,25(OH)_2D$: 24,25-dihydroxyvitamin D; 1αOH: 1-alpha hydroxylase; 24 OH: 24 hydroxylase.

lower than the baseline. Group mean 24-hour urinary calcium excretion showed no changes. This study concluded that 25(OH)D repletion in PHPT does not exacerbate hypercalcemia and could be beneficial by decreasing PTH levels and bone turnover [55]. A meta-analysis of observational studies evaluating the safety of vitamin D replacement in patients with PHPT also showed that vitamin D supplementation can significantly reduce PTH levels without a significant rise in serum or urinary calcium levels [56]. A more recent meta-analysis of over 300 patients with PHPT and vitamin D levels below 30 ng/mL further supported that vitamin D supplementation can significantly decrease PTH and alkaline phosphatase levels without exacerbating hypercalcemia or hypercalciuria [57].

In a randomized clinical trial of PHPT patients who were supplemented with 2800 IU cholecalciferol daily for 52 weeks, an increase in 25(OH)D levels from 23 ng/mL to 38 ng/mL was associated with lower PTH and c-telopeptide levels as well as improvement in the lumbar spine bone mineral density (BMD) without significant changes in serum or urinary calcium [58]. However, in an unblinded study of PHPT patients who were supplemented with calcifediol, 12 out of 27 patients had to stop supplementation due to either hypercalcemia or hypercalciuria [59]. The Fourth International Workshop recommended a threshold 25(OH)D level greater than 20 ng/ml in PHPT [25]. However, the same publication acknowledged the controversy regarding what the optimal cutoff for 25(OH)D should be in the management of PHPT. More recent consensus guidelines, including the Fifth International Workshop, now suggest the repletion of 25(OH)D levels above 30 ng/mL based on the abovementioned studies [1, 60].

We believe that in PHPT with severe hypercalcemia, vitamin D supplementation has to be done with caution to avoid a hypercalcemic crisis. In asymptomatic PHPT patients, vitamin D should be supplemented, while monitoring serum calcium and urinary calcium excretion and with an aim to keep 25(OH)D levels at least between 20 and 30 ng/ml [4, 9, 53]. Vitamin D2 or D3 can be given starting at 1000 IU per day [24]. The repletion of vitamin D to a target level of 40 ng/mL or more may help diagnose NPHPT with more accuracy in select patients. Current guidelines only utilize total 25(OH)D levels, but there have been studies that suggest free 25(OH)D levels may be a better marker for the vitamin D status in NPHPT [35, 37].

2.1. Supplementation with Vitamin D before and after Parathyroid Surgery. A recent study compared serum calcium concentrations, PTH levels, calculated free and bioavailable 25(OH)D, and DBP levels before and after parathyroidectomy [48]. The investigators found that serum calcium and PTH levels revert to normal after surgery. DBP as well as DBP-bound 25(OH)D levels increased after surgery likely due to the normalization of PTH. It is important to note that patients in the study received calcitriol after surgery as part of standard postoperative care [48]. In

a retrospective cohort study, it was found that vitamin D repletion in PHPT patients undergoing parathyroidectomy decreases hypocalcemia and reduces the length of hospital stay [61].

Vitamin D levels should be measured and supplemented to adequate levels prior to parathyroid surgery [9]. In order to avoid hypocalcemia, we suggest supplementing to a target of 25(OH)D >20 ng/ml for symptomatic patients and to >30 ng/ml for asymptomatic patients. We suggest that the careful repletion of vitamin D to a 25(OH)D level of 40 ng/mL before surgery for NPHPT can be attempted to rule out possible secondary hyperparathyroidism.

3. Conclusions

In recent years, the presentation of PHPT has shifted in the Western world to a generally asymptomatic condition with less vitamin D deficiency. Increased rates of screening and supplementation of vitamin D has changed the prevalence of vitamin D deficiency in PHPT and has introduced NPHPT into the clinical spectrum. Vitamin D repletion is recommended in all forms of PHPT, but the threshold to replace to is controversial and should be based on the severity of hyperparathyroidism. Recent studies and meta-analysis suggest that a 25(OH)D threshold level of >30 ng/mL is reasonable to prevent secondary stimulation of PTH secretion and to maintain stable serum and urinary calcium levels. Standard measurements and guidelines only include total 25(OH)D levels, but this may not always be a reliable indicator of the vitamin D status. It may be prudent to also check free 25(OH)D levels along with vitamin D-binding protein levels in the selected patients. Further studies are needed to understand the optimal total 25(OH)D level cutoffs in the different phenotypes of PHPT.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Mechanisms Linking Vitamin D Deficiency to Impaired Metabolism: An Overview

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Vitamin D deficiency is a common health problem worldwide. Despite its known skeletal effects, studies have begun to explore its extra-skeletal effects, that is, in preventing metabolic diseases such as obesity, hyperlipidemia, and diabetes mellitus. The mechanisms by which vitamin D deficiency led to these unfavorable metabolic consequences have been explored. Current evidence indicates that the deficiency of vitamin D could impair the pancreatic β-cell functions, thus compromising its insulin secretion. Besides, vitamin D deficiency could also exacerbate inflammation, oxidative stress, and apoptosis in the pancreas and many organs, which leads to insulin resistance. Together, these will contribute to impairment in glucose homeostasis. This review summarizes the reported metabolic effects of vitamin D, in order to identify its potential use to prevent and overcome metabolic diseases.

1. Introduction

Vitamin D (calciferol) refers to a group of fat-soluble secosteroids that exists in two forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 is derived from plant's ergosterol upon exposure to ultraviolet B (UVB) light [1], whereas vitamin D_3 is derived from 7-dehydrocholesterol (7DHC) found in the human and animal skin following exposure also to UVB light [2]. The main exogenous sources of vitamin D_2 are plants, plankton, and fungi [3], whereas the main sources of vitamin D_3 are dairy products, fish, meat, and poultry [4, 5]. Additionally, both types of vitamin D are currently available as dietary supplements and in the form of fortified foods [6]. In the body, vitamin D_2 and vitamin D_3 will undergo hydroxylation in the liver involving the 25-hydroxylase (CYP2R1) enzyme to form ercalcidiol ($25(OH)D_2$) and calcidiol (25(OH)D) [6, 7] where these will then bind to vitamin D binding protein (DBP) in the circulation [8]. Calcidiol is the major circulating form of vitamin D [9], and therefore, its serum concentration can be used to measure the vitamin D status [10]. In the kidney, calcidiol undergoes another hydroxylation process mediated by the 1α-hydroxylase (CYP27B1) enzyme to form calcitriol ($1,25(OH)_2$), which is a fully active hormone that is responsible for most (if not all) of the vitamin D biological actions [6, 7]. At tissue levels, vitamin D in its active form (calcitriol) binds to the vitamin D receptor (VDR), which is an intracellular protein, and the vitamin D-VDR complex translocates into the nucleus to bind to target genes and modify the gene expression [11, 12]. Additionally, vitamin D could also induce VDR expression and activation by auto-regulating the expression of the *Vdr* gene and inducing the transcription of *Vdr* gene itself [11, 13]. Classically, vitamin D is known for its role in maintaining bone health by regulating calcium homeostasis and by exerting direct action on the cartilage and bones to promote normal skeletal development [7]. However, in the past few decades, researchers have begun to explore the extra-skeletal effects of vitamin D [14–16] including metabolism, in view that VDR and the enzymes that are linked to vitamin D have been found in many tissues of the body [17].

1.1. Vitamin D and Metabolisms. There is growing evidence that vitamin D has a significant role in metabolism, in particular in regulating the glucose homeostasis [18–21]. Vitamin D deficiency has been identified as a global health issue by epidemiological studies [22, 23] and is defined by serum calcidiol levels of lower than 50 nmol/L or 20 ng/ml [24]. In addition to higher risk of development of rickets and osteomalacia in the growing skeleton [7], osteoporosis in the aging skeleton [25], and stress fractures in the physically active individuals [26], recent studies found that vitamin D deficiency could cause impairment of insulin secretion and trigger development of insulin resistance [27, 28]. In fact, insufficient blood levels of vitamin D have been associated with impaired glucose tolerance and hyperinsulinemia [29, 30], often caused by obesity, and these pose major risk for diabetes mellitus (DM) development [31]. *Vice versa*, studies have also found that high incidence of DM was observed in individuals who are deficient in vitamin D [32–35].

Apart from impaired glucose metabolism, vitamin D deficiency has also been associated with obesity [36] and the deficiency of vitamin D was found to be more prevalent in obese individuals [37, 38]. Furthermore, lower serum vitamin D levels were observed in a person with higher body mass index (BMI) [39] and weight loss was found to lead to higher serum vitamin D levels [40]. Alternatively, vitamin D supplementation would significantly decrease the body weight, BMI, as well as waist and hip circumferences [40, 41]. It is unclear what contributes towards the inverse relationship between serum vitamin D levels and BMI; however, it is likely that the larger volume of body fluid in overweight and obese subjects would result in lower serum concentrations of vitamin D [42]. Other mechanisms, however, have not been extensively explored. In addition to causing impaired glucose metabolism, deficiency in vitamin D could also cause dyslipidemia, a condition frequently linked to impaired glucose homeostasis [43]. Vitamin D supplementation was found to lower the blood total cholesterol, low-density lipoproteins (LDL), and triglycerides (TG) levels [44]. Besides, a study has found that LDL and TG levels were inversely correlated with serum vitamin D levels [43] and low high-density lipoproteins (HDL) levels were associated with low serum vitamin D levels [43, 45]. In view of these, vitamin D has been proposed to be clinically useful as an adjuvant therapy for statin in the treatment of hypercholesterolemia [46, 47]. Although the exact mechanisms are unclear, it was suggested that vitamin D could affect cholesterol metabolism by either decreasing the cholesterol absorption or reducing the endogenous cholesterol production [47]; however, these have yet to be proven.

In view of the evidence pointing towards the metabolic effect of vitamin D, this review aims at summarizing the current understanding on the role of vitamin D in body metabolism and how its deficiency could impair the glucose homeostasis that would lead to insulin resistance. Several evidences have shown the association between serum vitamin D levels and insulin resistance, which pointed towards the possible protective effects of vitamin D on pancreatic β-cell function [48]. Additionally, vitamin D could also help to enhance insulin sensitivity [49] and ameliorate chronic inflammation [50], oxidative stress [51], and apoptosis [52] in the pancreas and other insulin-responsive tissues [20, 30].

In order to gain understanding on the mechanisms linking vitamin D deficiency to impaired metabolism, the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed) was used to search for articles published between 1995 and 2021 based on the following keywords or their combinations: vitamin D; vitamin D deficiency; insulin resistance; obesity; diabetes; glucose; metabolism; insulin secretion; insulin sensitivity; inflammation; oxidative stress; or apoptosis. In this narrative review, the relevant articles and other related reference lists were evaluated. The findings from animal experiments, human clinical trials, and *in vitro* studies are conveyed, whereas the molecular mechanisms proposed by the researchers were evaluated and discussed accordingly.

2. Studies Linking Vitamin D Deficiency to Impaired Metabolism

2.1. Animal Studies. Lower serum glucose levels, improved activities of enzymes related to glucose metabolic pathways, restoration of glucose homeostasis, and reduced pancreatic and liver damage were observed following intraperitoneal injections of vitamin D (7 ng/gm) daily to alloxan-induced diabetic female albino mice for fifteen (15) consecutive days [53]. In addition, a study has also found enhanced glucose uptake, improved glucose metabolism, and reduced body oxidative stress level following oral supplementation of 67 IU/kg/day cholecalciferol for 8 weeks to diet-induced vitamin D-deficient obese C57BL/6J male mice [54]. A study also indicated reduced body weight as well as reduced blood glucose level, and complete restoration of insulin sensitivity following the treatment of diet-induced obese, insulin-resistant C57BL/6J male mice with 3000 IU/kg/day with vitamin D for seven (7) consecutive days [55]. In streptozotocin (STZ)-induced diabetic male Sprague-Dawley (SD) rats, intraperitoneal injections of 20,000 IU/kg cholecalciferol resulted in a significant decrease in the fasting plasma glucose (FPG), HbA1c, and an improvement in insulin and IGF-1 levels [56]. In the meantime, intraperitoneal injection of 7 μg/kg vitamin D at a frequency of three (3) times a week for eight (8) weeks to high-fat, high-sugar (HFHS) diet-induced obese C57BL/6J male mice resulted in reduced body weight, improvement in overall systemic glucose tolerance, restoration of insulin signaling, and reverted hepatic myosteatosis [57].

In another study, enhanced insulin resistance, which was evidenced by elevated homeostatic model assessment for insulin resistance (HOMA-IR) index, was observed when STZ-induced male SD rats were fed with vitamin D-deficient diet, and this was later reversed by the consumption of normal diet containing vitamin D [58]. In the meantime, 9–12-week treatment of 20 μg/kg calcipotriol to diet-induced obese C57BL/6J wild-type (WT) mice was associated with suppressed liver inflammation and hepatic steatosis as well as improvement in the overall insulin sensitivity [59]. In contrast to the above studies, a study by Liu et al. showed that no direct association was observed between vitamin D

deficiency and obesity, insulin resistance, and hepatic lipid accumulation in HFD-induced vitamin D-deficient young ICR male mice [60]. Furthermore, they proposed that vitamin D deficiency could lead to enhance fatty acid β-oxidation and increases the adipose tissue energy expenditure, which might result in the overall reduction in body weight, increased plasma insulin levels, and increased hepatic lipid accumulation [60].

2.2. Human Studies. Vitamin D supplementation was reported to help to improve the metabolic parameters associated with insulin resistance and DM in human subjects [61]. In a double-blinded, randomized, placebo-controlled trial in obese subjects, weekly supplementation with 25,000 IU cholecalciferol orally for 3 months together with hypocaloric diet resulted in improved insulin sensitivity [62]. In the meantime, another double-blind, randomized clinical study on prediabetic, vitamin D-deficient human subjects revealed significant improvement in insulin sensitivity and reduced progression toward overt DM following six (6)-month treatment with oral vitamin D_3 (50,000 IU), weekly for three (3) months, followed by once-a-month treatment for the next three (3) months [63]. In a randomized controlled trial, daily supplementation with 2000 IU cholecalciferol orally for 3 months to human subjects with type 2 DM (T2DM) with vitamin D deficiency has resulted in decreased HOMA-IR index as well as HbA1c levels, a marked increase in HDL level, a decreased in TG/HDL ratio, and a reduced level of endogenous and oxidative DNA damages in the lymphocytes [64]. Additionally, a double-blind randomized clinical trial revealed that vitamin D-deficient obese individuals with T2DM who received weekly treatment with 50,000 IU oral vitamin D for eight (8) weeks had a significant decrease in HbA1c levels; however, no significant changes in FPG, insulin, HOMA-IR index, and quantitative insulin sensitivity check index (QUICKI) were observed [65].

A double-blind, placebo-controlled, randomized clinical trial involving human subjects with serum 25(OH)D level $\leq$50 nmol/L and a BMI of 30–40 kg/m^2 showed that weekly supplementation with 50,000 IU vitamin D_3 for twelve (12) weeks resulted in a significant decrease in the BMI and fasting blood glucose (FBG) levels [66]. The benefits of vitamin D in improving the metabolic parameters were also seen in post-menopausal women, where a significant reduction in the metabolic syndrome risk profile including hyperglycemia, hypertriglyceridemia, and HOMA-IR was observed following daily supplementation with oral vitamin D_3 (1000 IU) for nine (9) months [67]. Furthermore, premenopausal women with vitamin D insufficiency receiving 20,000 IU oral cholecalciferol weekly for twenty-four (24) weeks had a significant improvement in insulin resistance indices including HOMA-IR and QUICKI, despite no significant changes in the area under the curve (AUCgluc) for glucose tolerance [68].

In contrast to the benefits of vitamin D in improving the metabolic profiles in obese and post-menopausal subjects, a single-blind randomized control trial in diabetic pregnant women with vitamin D deficiency, however, reported no improvement in insulin resistance or glycemic control following 60,000 IU of oral vitamin D_3 supplementation once a month until delivery [69]. Similarly, a randomized, placebo-controlled, double-blind trial on women suffering from polycystic ovarian syndrome (PCOS) with vitamin D deficiency demonstrated no significant changes in the fasting serum insulin and FBG levels and the HOMA-IR index after supplementation with 50,000 IU oral vitamin D_3 once every 20 days for two (2) months [70]. Table 1 summarizes the reported effects of vitamin D on metabolism in animals, while Table 2 presents a list of interventional clinical trials of the benefits of vitamin D supplementation in improving metabolic profiles in humans.

3. Mechanisms Underlying the Action of Vitamin D in Improving Impaired Metabolism

3.1. Vitamin D Improves Pancreatic β-cell Functions. Functional, pancreatic β-cells play important role in maintaining the blood glucose homeostasis [71, 72]. These cells adapt to an excessive blood glucose level by increasing the insulin secretion, and the latter is further exaggerated in the state of insulin resistance [73, 74]. Compensatory hyperinsulinemia will result in β-cell hyperplasia and hypertrophy [75], which helps to maintain the blood glucose levels up to a point where β-cells could no longer produce sufficient insulin to keep pace with the demand [76, 77]. Chronic exposure to high glucose and free fatty acids (FFA) levels could cause progressive β-cell dysfunction, which may eventually lead to β-cell apoptosis in DM [77, 78]. Research has suggested that insulin secretion from pancreatic β-cells is influenced by plasma vitamin D levels [79, 80]. It is proposed that there is a strong correlation between serum vitamin D levels and insulin secretion as well as insulin sensitivity [81, 82]. The role of vitamin D in pancreatic β-cell function is supported by the discovery of 1α-hydroxylase enzyme, which is classically found in the kidney [83]. Specific staining for 1α-hydroxylase was detected in the pancreas and other extra-renal tissues including the colon and brain [83]. Bland et al. reported that 1α-hydroxylase is able to convert vitamin D to its active form within the pancreatic islet cells, and this suggests that local production of calcitriol could occur in the pancreatic islet cells [79].

Although the mechanisms underlying the role of vitamin D in pancreatic β-cells insulin secretion are not well understood, a few proposals have been made [84]. Kjalarsdottir et al. have shown that *Vdr* mRNA expression in the pancreatic islet cells is glucose responsive [85]. Mice lacking functional VDR are unable to synthesize adequate insulin in response to hyperglycemia [86, 87], and their pancreatic β-cells showed a lower amount of stored insulin [87], suggesting vitamin D-dependent insulin synthesis and secretion. In addition to this, a study has demonstrated that VDR expression was reduced when a mouse insulinoma cell line (MIN6) was cultured in a high-glucose environment,

Table 1: Animal experimental studies on the effects of vitamin D on insulin resistance.

Model	Treatment	Findings	References
HFD-induced C57BL/6J male mice	Oral 150 IU/kg per day calcitriol orally (in 1 mL coconut oil) by oral gavage for 16 weeks	Gradual decrease in weight in HFD-fed mice treated with calcitriol, reduced concentrations of various inflammatory markers including TNF-α, CRP, and IL-6, lower the levels of C-peptide and insulin, improved liver structure	Alkharfy et al. [115]
Diet-induced obese C57BL/6J WT mice	12 weeks of 60% HFD with 9–12 weeks of calcipotriol (TOCRIS) treatment (20 μg/kg body weight)	VDR activation by calcipotriol suppressed liver inflammation and hepatic steatosis, therefore significantly improving insulin sensitivity	Dong et al. [59]
Vitamin D-deficient C57BL/6 male mice	Intraperitoneal injections of cholecalciferol 50 ng·3x/week for 6 weeks	Improved insulin sensitivity in vitamin D-deficient lean mice but no significant improvement in insulin resistance in obese mice	Mutt et al. [19]
Diet-induced obese C57BL/6J male mice	Intraperitoneal injections of 7 μg/kg calcitriol 3x/week for 8 weeks	Reduced body weight, improved overall systemic glucose tolerance, restored insulin signaling, and reverted hepatic myosteatosis	Benetti et al. [57]
Diet-induced obese and insulin-resistant C57BL/6J adult male mice	Oral calcitriol 3000 IU/kg/day (75 mg/kg/day) for 7 consecutive days	Complete restoration of insulin sensitivity, reduced body weight, and glycemia, but with severe kidney damage	Gaspar et al. [55]
Diet-induced vitamin D-deficient obese C57BL/6J male mice	Oral cholecalciferol 67 IU/kg/day for 8 weeks	Upregulated glucose uptake, improved glucose metabolism, prevented oxidative stress via novel molecular mechanisms	Manna et al. [54]
Diet- and STZ-induced diabetic male SD rats	Oral vitamin D 0.03 μg/kg/day for 8 weeks	Protective effects against diabetes-induced liver complications by attenuating the crosstalk between inflammation and insulin resistance, and ameliorating hyperglycemic state	Liu et al. [137]
STZ-induced diabetic male SD rats	Intraperitoneal injections of 20,000 IU/kg of cholecalciferol on days 1 and 14	Significant decrease in fasting plasma glucose, decline in HbA1c, improved insulin, and IGF-1 levels	Derakhshanian et al. [56]
Alloxan-induced diabetic female albino mice	7 ng/gm/day of 1,25(OH)2D3 dissolved in propylene glycol given intraperitoneally for 15 days	Lowered serum glucose, improved activities of enzymes of glucose metabolic pathways, restored glucose homeostasis, and reduced pancreatic and liver damage.	Meerza et al. [53]

and subsequent treatment with vitamin D_3 improved insulin to the levels seen in normal functional islets in addition to increase in VDR activity as well as prevented the pathological dedifferentiation of β-cells [88]. Hence, VDR could be a crucial transcription factor that protects the β-cells against dysfunction and maintains its insulin secretion by preventing cell dedifferentiation that precedes β-cell failure at the onset of DM. Thus, vitamin D could have a role in maintaining pancreatic β-cell function and help to enhance insulin secretion through VDR, and therefore, supplementation with vitamin D could prevent β-cell loss and delays the onset of DM. Since insulin secretion is calcium-dependent [89], vitamin D, which is known to be involved in calcium homeostasis, could play an indirect role in pancreatic β-cells' insulin secretion. Insulin release requires calcium influx and the opening of voltage-gated calcium channels (VGCCs) upon glucose stimulation [90]. The active form of vitamin D, calcitriol, regulates extracellular calcium levels and calcium influx into β-cells via VGCC [91]. It has also been proposed that vitamin D upregulates the *Cacna1e* gene, which encodes the Cav2.3 subunit of R-type VGCC [85]. In a study by Kjalarsdottir et al. on cultured human and mouse pancreatic islets, pre-incubation with vitamin D enhances glucose-stimulated insulin secretion and increases glucose-stimulated calcium influx [85]. Hence, these results suggest that vitamin D could enhance calcium influx through VGCC, which in turn modulates the ability of the pancreatic β-cells to secrete insulin [85].

Although VGCC represents the most common pathway for insulin secretion [92], some studies suggested that calcium-binding proteins or calbindin might have a role in the regulation of intracellular calcium responses in β-cells as it is one of the cytosolic vitamin D-dependent calcium-binding proteins found in these cells [93]. Li et al. proposed that calbindin modulates intracellular calcium levels by suppressing calcium-dependent depolarization after-potentials and hence regulates hormonal secretion by acting as an endogenous calcium buffer and controlling depolarization-induced release of a hormone, including insulin [94]. Therefore, vitamin D could indirectly modulate insulin secretion by regulating the levels of calbindin, which controls depolarization-induced insulin release via the regulation of cytosolic calcium concentrations [93]. Although the exact mechanisms of vitamin D regulation of calbindin are not clearly understood, calbindin has been identified as one of the target genes of the *Vdr* [95] and the transcription of vitamin D-dependent calbindin proteins is mediated by the binding of VDR to the functional vitamin D response

Table 2: Interventional clinical trials on the effects of vitamin D supplementation on insulin sensitivity.

Study design	Population of study	Intervention	Findings	References
Double-blind, placebo-controlled, randomized clinical trial	44 participants with serum 25(OH)D level ≤50 nmol/L and BMI 30–40 kg/m^2	Weight reduction diet with 50,000 IU vitamin D_3 pearl once a week for 12 weeks	Improved fasting serum glucose and matrix metalloproteinase 9 (MMP-9) levels; no significant differences for glycemic markers (serum insulin, HOMA-IR)	Aliashrafi et al. [66]
Single-blinded randomized control trial	100 diabetic pregnant women	60,000 IU of oral vitamin D_3 once a month till delivery	No improvement in insulin resistance or glycemic control in diabetic pregnant women with vitamin D deficiency	Bhavya Swetha et al. [69]
Double-blind, placebo-controlled clinical trial	160 post-menopausal women aged 50–65 years old	Daily 1000 IU of oral vitamin D_3 for 9 months	Reduction in metabolic syndrome risk profile in younger post-menopausal women with vitamin D deficiency	Ferreira et al. [81]
Single-center, double-blind, randomized placebo-controlled trial	150 healthy premenopausal women with vitamin D insufficiency	20,000 IU of oral cholecalciferol weekly for 24 weeks	Significant improvement in HOMA-IR and QUICKI, no significant effect on AUCgluc.	Trummer et al. [68]
Double-blind randomized clinical trial	90 obese type 2 diabetes patients	50,000 IU of oral vitamin D pearls weekly for 8 weeks	Significant decrease in HbA1c and improved T2D but no significant changes in glucose indices (FPG, insulin, HOMA-IR, QUICKI)	Safarpour et al. [65]
Double-blind, randomized, placebo-controlled trial	18 obese, nondiabetic, vitamin D-deficient volunteers	25,000 IU oral cholecalciferol weekly For 3 months and lifestyle modification	Improved insulin sensitivity and body composition but no improvements in pancreatic β-cell function	Cefalo et al. [62]
Double-blind randomized clinical trial	162 prediabetic, vitamin D-deficient subjects	50,000 IU of oral vitamin D_3 pearls weekly for 3 months, followed by 1 pearl per month	Improved insulin sensitivity and decreased rate of progression toward overt diabetes	Niroomand et al. [63]
Randomized controlled trial	92 vitamin-D-deficient subjects	Daily 2000 IU oral cholecalciferol for 3 months	Decreased level of DNA damage, reduced insulin resistance parameters, and improved glucose and lipid metabolisms	Wenclewska et al. [64]
Randomized, placebo-controlled, double-blinded trial	50 female subjects (20 to 40 years old) with PCOS and vitamin D deficiency	50,000 IU of oral vitamin D3 or placebo, once every 20 days for 2 months	There were no significant changes in fasting serum insulin and glucose levels, and HOMA-IR	Ardabili et al. [70]

element (VDRE) within the gene promoter regions [96]. Figure 1 shows the proposed mechanisms underlying vitamin D action in stimulating insulin secretion by the pancreatic β-cells.

3.2. Vitamin D Improves Insulin Sensitivity. There are several mechanisms by which vitamin D affects insulin sensitivity in insulin target tissues. When vitamin D is deficient, insulin sensitivity will begin to decline, thus setting the stage for the onset of DM and other DM-related illnesses [97]. Firstly, vitamin D modulates the secretion of insulin-sensitizing hormones such as adiponectin and leptin [61] and increases the expression of disulfide-bond A oxidoreductase-like (DsbA-L) protein, a key regulator for adiponectin production [98]. Lower levels of adiponectin have been reported in vitamin D-deficient, obese children [98], whereas higher adiponectin levels were observed in patients with type 2 DM (T2DM) receiving vitamin D-fortified food containing 500 IU vitamin D_3 daily for twelve (12) weeks [99]. Besides, vitamin D maintains the insulin signaling pathway by increasing the expression of insulin receptors (IRs) [100]. A deficiency in vitamin D could see a decline in IR expression, leading to the onset of insulin resistance [101]. Previous studies found that vitamin D-treated U-937 human promonocytic cells have higher VDR protein expression and *Ir* mRNAs, suggesting that vitamin D is capable of inducing the transcriptional activation of the human *Ir* gene in insulin-responsive cells [101]. Another study has identified the presence of VDR in the human *Ir* gene promoter region in vitamin D-treated U-937 human promonocytic cells, which suggested that vitamin D in the form of calcitriol might enter the insulin-responsive cells and bind to cytosolic VDR prior to the nuclear translocation to further bind to nuclear retinoic acid X-receptor (RXR) in order to form calcitriol-VDR-RXR complex [102, 103]. This complex then binds to the VDRE in the human *Ir* gene promoter region to enhance mRNA expression and transcriptional activation of the *Ir* genes [61]. The increased expression of *Ir* genes could lead to the upregulation of IR, which increases the total number of IR to enhance IR capacity and maintain the insulin sensitivity [101].

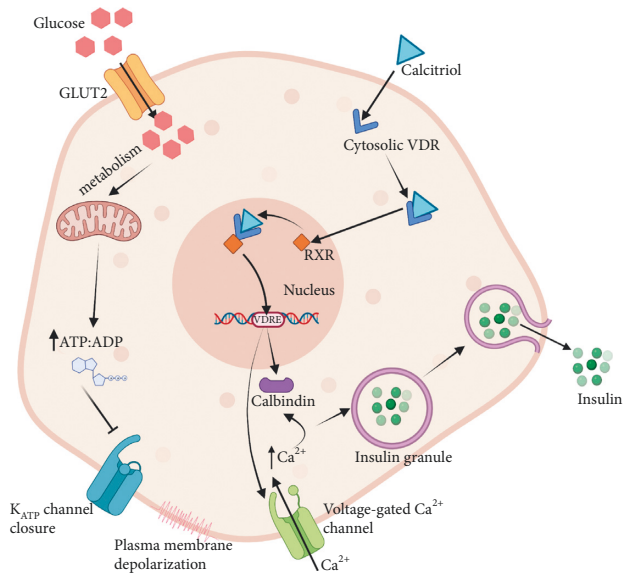

FIGURE 1: The mechanism underlying glucose-stimulated insulin secretion in pancreatic β-cell. Glucose is transported into the cell via GLUT2. Glucose metabolism leads to a high ATP : ADP ratio, which triggers the closure of ATP-sensitive potassium channel (K_{ATP} channel). The resulting plasma membrane depolarization stimulates the opening of the voltage-gated calcium channels (VGCCs) and calcium influx. High intracellular calcium level induces the exocytosis of the insulin secretory granule and insulin secretion. Vitamin D in its active form calcitriol binds to cytosolic VDR. Calcitriol-VDR complexes are translocated into the nucleus and bind to RXR to form calcitriol-VDR-RXR complexes. These complexes then bind to VDRE within the calbindin gene promoter regions to stimulate the transcription of cytosolic calcium-biding proteins, calbindins. calbindins regulate cytosolic calcium concentration and indirectly modulate calcium-dependent insulin secretion. Here, vitamin D also indirectly upregulates the Vgcc genes and thus could help to enhance calcium influx through VGCC.

Vitamin D has also been found to enhance the insulin action in cells through the PI3K-dependent insulin signaling [103]. The activation of IR will stimulate the phosphorylation of tyrosine residues in the insulin receptor substrate (IRS) protein where its key functions is to regulate the PI3K [104]. Therefore, by modulating the *Ir* gene and upregulating the IR, vitamin D could indirectly boost the IRS-associated PI3K activity, which is involved in the glucose uptake in insulin-responsive tissues [105]. In addition, vitamin D might help to improve the insulin sensitivity by activating peroxisome proliferator-activated receptor delta (PPAR-δ), a transcription factor that is involved in the metabolism and mobilization of FFA in the target tissues [61]. A study reported that PPAR-δ (NR1C2) knockout mice was found to be glucose intolerant and metabolically less active, while treatment with PPAR-δ agonist to diabetic *db*/*db* mice increases insulin sensitivity in all major insulin-responsive tissues [106]. PPAR-δ has been reported to ameliorate hyperglycemia by increasing the glucose flux through the pentose phosphate pathway (PPP), thereby enhancing carbohydrate catabolism and suppressing the glucose production in the liver [107]. Additionally, it also helps to increase the β-oxidation of FFA in the skeletal muscles, inhibiting FFA release from the adipocytes, and therefore improving the metabolic homeostasis and enhancing the systemic and peripheral insulin sensitivity [107]. Insulin resistance could then trigger lipoprotein lipase to hydrolyze stored TG in inflamed adipose tissue and release the resulting FFA into the circulation, which would then be taken up by other organs such as skeletal muscles and liver, causing excessive fat accumulation and lipotoxicity, which are responsible for the development of insulin resistance [108]. Although the role of vitamin D in activating PPAR-δ is unclear, evidences suggested that PPAR-δ is the primary vitamin D-responding gene, while PPAR-δ and VDR signaling pathways are interconnected by cross-regulation at the level of their transcription factor mRNA [109].

The role of vitamin D in maintaining insulin sensitivity could also be related to its association with VDR and forkhead box protein O1 (FoxO1) protein, where the latter is an important downstream negative regulator in the insulin signaling pathway [110]. Previous study has suggested that FoxO1 regulates IRS-2 protein tyrosine phosphorylation, and thus enhances insulin signal transduction and improves insulin sensitivity [111]. In a study on skeletal muscle-specific VDR-null mice, the mice were found to develop insulin resistance and glucose intolerance with elevated FoxO1 expression and activity [86], which might be responsible for insulin resistance and impaired glucose metabolism in the skeletal muscle [86]. The treatment of C2C12 myoblasts with calcitriol reduced FoxO1 nuclear translocation, expression, and activity, which possibly be VDR-dependent [86]. Thus, vitamin D might play an important role in maintaining peripheral insulin sensitivity through the presence of VDR and indirectly modulating the expression of FoxO1 in insulin-responsive tissues.

Vitamin D could help to upregulate the expression of glucose transporters and its translocation onto the cell membrane, which are essential for the glucose uptake into cells [112]. Evidences have shown that the treatment of L6 myotubes with calcitriol leads to a significant increase in the expression of glucose transporter-1 (GLUT1) and glucose transporter-4 (GLUT4) [113]. Moreover, a study has revealed that vitamin D could help to increase the glucose consumption via inducing SIRT1 activation, which regulates the activation of IRS-1 and GLUT4 translocation in the murine C2C12 myotubes [114]. A study has demonstrated that vitamin D could directly upregulate GLUT4 expression in 3T3L1 adipocyte cell lines and improves the insulin sensitivity [112]. Meanwhile, in the liver, an increase in hepatic insulin signals including phosphorylated Akt (pAkt), phosphorylated FoxO1 (pFoxO1), and phosphorylated glycogen synthase kinase 3 beta (pGSK3β), which are involved in glucose transport into the hepatocytes, are observed in C57BL/6 male mice receiving intraperitoneal injections of 50 ng cholecalciferol three (3) times/week for six (6) weeks [19]. Nevertheless, a study performed on C57BL/6J mice showed an increased expression of IRS-1 in the skeletal muscle and increased expression of VDR in the liver, but there was no significant correlation between vitamin D supplementation and GLUT4 expression in other

target tissues [115]. In insulin-responsive tissues, a narrow range of intracellular calcium is required for insulin-mediated functions and insulin-associated intracellular processes [116]. High intracellular calcium would enhance GLUT4 translocation onto the cell membrane of the skeletal muscle cells and increases the glucose uptake [117]. Thus, low intracellular calcium in insulin target tissues may impair insulin signal transduction, and this could lead to peripheral insulin resistance [118]. In view of this, vitamin D might affect insulin sensitivity through its role in the regulation of extracellular calcium as well as calcium flux through the cell membranes [93]. Figure 2 shows the proposed mechanisms by which vitamin D acts in order to enhance the glucose uptake in insulin-responsive target tissues.

Apart from these, vitamin D deficiency could also lead to elevated levels of parathyroid hormone (PTH), which has been documented to reduce insulin-stimulated glucose uptake [119]. Although the exact mechanisms are unclear, it is proposed that low serum calcidiol levels could reduce calcium absorption, hence causing secondary hyperparathyroidism [120, 121], which will exacerbate insulin resistance by decreasing the number of GLUT1 and GLUT4 in the adipose tissue, liver, and skeletal muscles [49, 122]. Moreover, PTH treatment in 3T3-L1 adipocytes has been shown to suppress insulin-stimulated glucose uptake and insulin signaling through IRS-1 phosphorylation at serine 307 via cyclic adenosine 3,5-monophosphate (cAMP) pathway [123]. Reduced IRS-1 and GLUT4 expressions will contribute towards lower insulin-induced glucose transport, and these could explain the link between vitamin D deficiency, high serum PTH levels, and insulin resistance [123].

In the meantime, vitamin D deficiency could also indirectly affect insulin resistance through the renin-angiotensin-aldosterone system (RAAS). VDR-knockout mice showed higher expressions of renin and angiotensin II, but their levels were reduced following vitamin D administration [124]. Besides, RAAS inhibition could help to improve insulin resistance and glucose intolerance in nondiabetic patients with cardiovascular diseases as well as improve the cardiovascular and renal outcomes in DM [125]. Zhou et al. reported that the inhibition of insulin action in peripheral tissues by RAAS might be mediated via the regulation of intracellular calcium levels where a reduced calcium level will inhibit insulin action [125]. Vitamin D has a genomic effect against RAAS through the suppression of *Renin* gene expression via transcription factor cAMP response element-binding protein (CREB) [126]. Figure 3 summarizes the mechanisms of vitamin D action in various tissues to enhance insulin action and reduces insulin resistance.

3.3. Vitamin D Ameliorates Chronic Inflammation. During the development of insulin resistance, chronic low-grade inflammation occurs [127], which can cause impairment in adipose tissue function by causing mitochondrial dysfunction and triggering endoplasmic reticulum (ER) stress—all of which would contribute towards insulin resistance [128, 129]. Although it is unclear whether insulin resistance or inflammatory response occurs first, it was

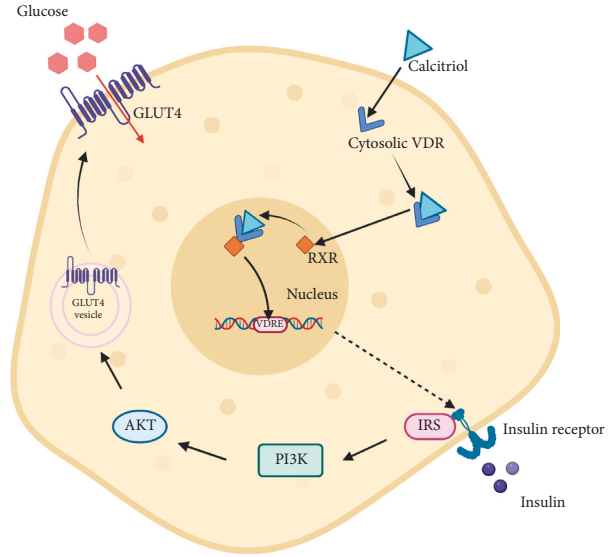

Figure 2: The mechanism underlying insulin-induced glucose uptake in a target cell. vitamin D in its active form, calcitriol binds to cytosolic VDR, which are then translocated into the nucleus and bind to RXR to form calcitriol-VDR-RXR complexes. The complexes then bind to VDRE within the Ir gene promoter regions to stimulate the transcription and upregulation of insulin receptor gene. The binding of insulin to IR stimulates a cascade of process involving multiple downstream mediators, including insulin receptor substrate-1 (IRS-1) and PI3K. The resulting activation of protein kinase B (Akt) stimulates the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane, which facilitates the uptake of circulating glucose into the cell.

suggested that inflammation in T2DM is the causative factor for insulin resistance [130]. The onset of insulin resistance is believed to occur with the dysregulation of metabolic pathways in the adipose tissue [131]. In obesity-related insulin resistance, poor blood flow in hypertrophied adipose tissue leads to macrophages infiltration due to tissue hypoxia and subsequently inflammation [132]. Adipocyte hypertrophy could result in increased secretion of pro-inflammatory adipokines including tumor necrosis factor α (TNF-α), interleukins (IL-6, IL-8), monocyte chemoattractant protein 1 (MCP-1), and resistin [133]; and the decrease in the release of anti-inflammatory adipokines such as adiponectin [134]. Studies have shown that vitamin D may act as an anti-inflammatory agent and modulates inflammatory responses by promoting the secretion of anti-inflammatory cytokines and suppressing the secretion of pro-inflammatory cytokines [135, 136].

In a study using diabetic male SD rats, oral supplementation of 0.03 μg/kg/day vitamin D for eight (8) weeks resulted in lower expression of pro-inflammatory cytokines as well as reduced hyperglycemia as reflected by a decrease in FPG levels and HOMA-IR [137]. Moreover, oral supplementation of 150 IU/kg calcitriol per day for sixteen (16) consecutive weeks in high-fat diet (HFD)-induced diabetic C57BL/6J male mice lowered the concentrations of various inflammatory markers including TNF-α, C-reactive protein (CRP) and IL-6, and the levels of C-peptide and insulin [138]. These findings were attributed to vitamin D role in

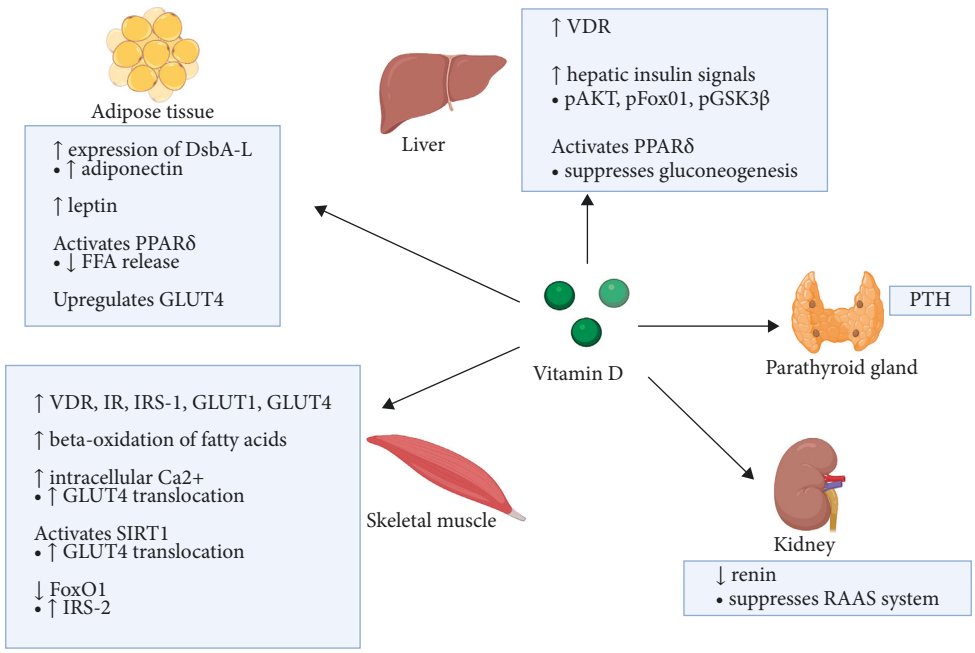

Figure 3: Schematic diagram summarizing the roles of vitamin D in maintaining insulin sensitivity. In the liver, vitamin D increases VDR expression, enhances hepatic insulin signals, and activates PPAR-δ to suppress hepatic glucose production. In the adipose tissue, vitamin D increases the production of insulin-sensitizing hormones adiponectin and leptin, activates PPAR-δ to reduce the release of FFA into the circulation, and upregulates GLUT4. In the skeletal muscles, vitamin D increases the levels of VDR, IR, IRS-1, GLUT1, and GLUT4, enhances β-oxidation of fatty acids, increases intracellular calcium levels, and activates SIRT1 to enhance the translocation of GLUT4 to the plasma membrane for glucose uptake, and increases the level of IRS-1 by suppressing FOXO1. Vitamin D also suppresses the gene expression of renin, thereby preventing inhibitory effects of RAAS against insulin action in peripheral tissues. On the other hand, secondary hyperparathyroidism as a result of vitamin D deficiency could exacerbate insulin resistance by reducing the glucose uptake. Vitamin D could maintain insulin sensitivity by increasing calcium absorption and preventing the secondary elevation of PTH.

modulating inflammatory responses, which attenuated the crosstalk between inflammation and insulin resistance [137]. Apart from this, vitamin D has been reported to suppress the release of several other pro-inflammatory cytokines including IL-6, IL-1, IL-8, COX-2, intercellular adhesion molecule 1 (ICAM-1), and B7-1 protein [139]. Streptozotocin-induced diabetic rats with vitamin D deficiency had enhanced insulin resistance with high proportion of phospho-p65 (p-p65)/RelB in which RelB is an anti-inflammatory molecule, while p-p65 is a pro-inflammatory molecule. An overproduction of pro-inflammatory cytokines not only causes inflammation but also leads to the dysregulation of the glucose and lipid metabolisms [61]. For instance, pro-inflammatory cytokines involved in the activation of IκB kinase beta (IKK-β)/nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase 1 (JNK1) pathways cause serine kinase phosphorylation of IRS-1 or IRS-2, which attenuate insulin signaling and consequently leads to the development of insulin resistance [140].

Studies have demonstrated that vitamin D has an anti-inflammatory role through both NF-κB and p38 MAPK inflammatory pathways [141, 142] with NF-κB being an essential component of the inflammatory pathways in the adipose tissue [143]. The translocation of both NF-κB and p38 MAPK is related to the degradation of inhibitor kappa B-alpha (IκB-α) and the transcription of pro-inflammatory genes including IL-6, TNF-α, and interleukin 1β (IL-1β) [144, 145]. Vitamin D upregulates IκB-α by reducing IκB-α phosphorylation and thereby reduces the nuclear translocation of NF-κB and p38 MAPK, downgrading their pro-inflammatory activities [146].

Evidences also showed that VDR is expressed in immune cells such as the macrophages and dendritic cells [147]. VDR deficiency is linked to inflammation in several diseases, including DM [148]. In fact, vitamin D and VDR have anti-inflammatory and immunosuppressive effects in autoimmunity by increasing the phagocytic ability of monocytes to modulate the innate immune system as well as by promoting the ability of dendritic cells to modulate regulatory T-cell differentiation [149]. In addition, both macrophages and dendritic cells express 25-hydroxylase and 1α-hydroxylase enzymes, indicating their role in calcitriol production [150]. Macrophages are also known for cytokine production, and one of the most important inflammatory cytokines secreted by macrophages is TNF-α [151]. In dendritic cells, vitamin D elevates the production of anti-inflammatory IL-10 and reduces the release of pro-inflammatory cytokines including TNF-α, IL-12, and IFN-γ [61]. In addition to this, vitamin D may reduce tissue inflammation by inhibiting the secretion of TNF-α-induced chemokine MCP-1 that is responsible for macrophage and monocyte infiltration [58]. In human monocytes, vitamin D suppresses the mRNA expression of Toll-like receptor 2 (*Tlr-2*) and Toll-like receptor 4 (*Tlr-4*) proteins [152], which are important regulators of metabolic

inflammation during the development of metabolic disorders [153]. Hence, vitamin D deficiency may exacerbate insulin resistance through an increase in tissue inflammation.

3.4. Vitamin D Attenuates Oxidative Stress. Oxidative stress is recognized as a key mechanism in insulin resistance [154]. Among the endogenously produced oxidative stress agents are the reactive oxygen species (ROS), which include superoxide, hydrogen peroxide, and hydroxyl radicals [155]. ROS possesses physiological significance even at low levels, especially to the signaling pathways [156]. The main source for ROS is NADPH oxygenase (NOX) [157] and malondialdehyde (MDA) [158]. The oxidative processes are regulated by antioxidants such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and catalase [159]. Higher production of ROS and declining antioxidative capacity may lead to excessive lipids, proteins, and DNA oxidation products [160]. In oxidative stress, oxidative degradation of lipids causes damage to cell membranes [161], which will eventually lead to cell damage and disruption to the signaling pathways. A study on C2C12 muscle cells showed that vitamin D deficiency could cause mild oxidative stress and an increased muscle proteolysis, while pretreatment with vitamin D could help to reverse oxidative stress and total protein degradation, and reduce muscle atrophy [162]. Besides, vitamin D could help to diminish the ROS formation by downregulating NOX through the suppression of *Nox* gene expression [163, 164]. Studies supported the antioxidant properties of vitamin D where in vitamin D-deficient mice, the inhibition of oxidative stress could improve insulin resistance [165]. In addition to this, a study on SD male weanling rats reported that vitamin D deficiency is linked to a decreased SOD and catalase enzymes in the rat skeletal muscles, and there were higher nitrate levels indicating nitrosative stress in the tissue [162].

Meanwhile, in a randomized double-blind placebo-controlled clinical trial on overweight and vitamin D-deficient women with polycystic ovarian syndrome (PCOS), vitamin D treatment for eight (8) weeks resulted in lower MDA levels and increased production of GSH, which enhances ROS removal [166]. Vitamin D also enhances GSH production by upregulating genes for the key enzymes that are involved in GSH synthesis, such as glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), and glutamate-cysteine ligase (GCL) [167, 168]. A randomized placebo-controlled trial on women with gestational diabetes mellitus (GDM) reported a significant increase in plasma GSH, lower plasma MDA, and improved metabolic profile with calcium-vitamin D co-supplementation [169]. Another randomized, double-blind, placebo-controlled clinical trial in atopic dermatitis patients confirmed that vitamin D supplementation increases the SOD and catalase activities in erythrocytes [170]. Indeed, a cell culture study using human placental umbilical cord vein endothelial cells reported that oxidative stress downregulates VDR expression in endothelial cells, whereas vitamin D treatment enhances antioxidant enzyme Cu, Zn-superoxide dismutase (SOD1) expression in these cells [171]. A clinical trial on T2DM patients reported a significantly lower vitamin D and GPx levels when compared to healthy individuals; however, there is no statistical correlation between serum vitamin D levels and SOD activity was observed [172].

The mechanisms by which vitamin D alleviates oxidative stress are still a matter of debate. Vitamin D could act by utilizing the genomic mechanisms in ameliorating oxidative stress where an increase in ROS formation could induce the hypermethylation of the gene promoter regions, which could adversely affect the genes that are responsible in the protection against oxidative stress [173] such as the peroxiredoxin 2 (*Prdx2*) gene that encodes a family of antioxidative enzymes and the scavenger receptor class a member 3 (*Scara3*) gene that encodes a scavenger protein that depletes the ROS radicals [174]. By maintaining the expression of DNA demethylases, which reduces the hypermethylation of gene promoter regions, vitamin D could indirectly play a role in reducing ROS levels and provide protection against oxidative stress in the tissue in the insulin resistance state [175].

3.5. Vitamin D Abrogates Apoptosis. In the pancreas, unresolved inflammation in the insulin resistance state could enhance the immune cell infiltration, which leads to the dysfunction of insulin-secreting β-cells and ultimately cell death [176]. Markedly increased caspase activation and adipocyte apoptosis have been observed in insulin-resistant adipose tissue [177]. In the skeletal muscle, an increase in circulating saturated fatty acids along with poor fatty acid handling results in increased levels of ceramide [178], which acts as a second messenger in triggering an apoptotic response via the mitochondrial system [179]. In the liver, insufficient unfolded protein response (UPR) to elevated ER stress leads to adverse effects such as hepatic fat buildup, inflammation, and cell death [180]. A study on streptozotocin-induced type 1 diabetic FVB mice demonstrated enhanced C/EBP homologous protein (CHOP) and caspase-12 cleavage in response to ER stress in the liver, which resulted in hepatocyte apoptosis [181]. Vitamin D has been reported to be involved in regulating cell proliferation, differentiation, and apoptosis in numerous tissues, including in the insulin-responsive tissues [182]. Firstly, the actions of vitamin D against apoptosis might be attributed to its calcium regulatory role as the molecular targets of vitamin D-mediated apoptosis are calcium-dependent protease calpain and calcium/calpain-dependent caspase-12, which are the primary calcium-activated apoptotic factors [183]. In insulin resistance state, sustained increase in intracellular calcium activates calcium-dependent calpain, which subsequently activates calcium/calpain-dependent caspase-12, resulting in apoptosis [184]. Vitamin D might reduce the intracellular calcium levels, which subsequently prevents calcium-dependent calpain activation and subsequently ameliorating apoptosis.

Besides, vitamin D could protect the cells against cytokine-induced apoptosis by directly modulating the expression and activity of inflammatory cytokines [139] as well

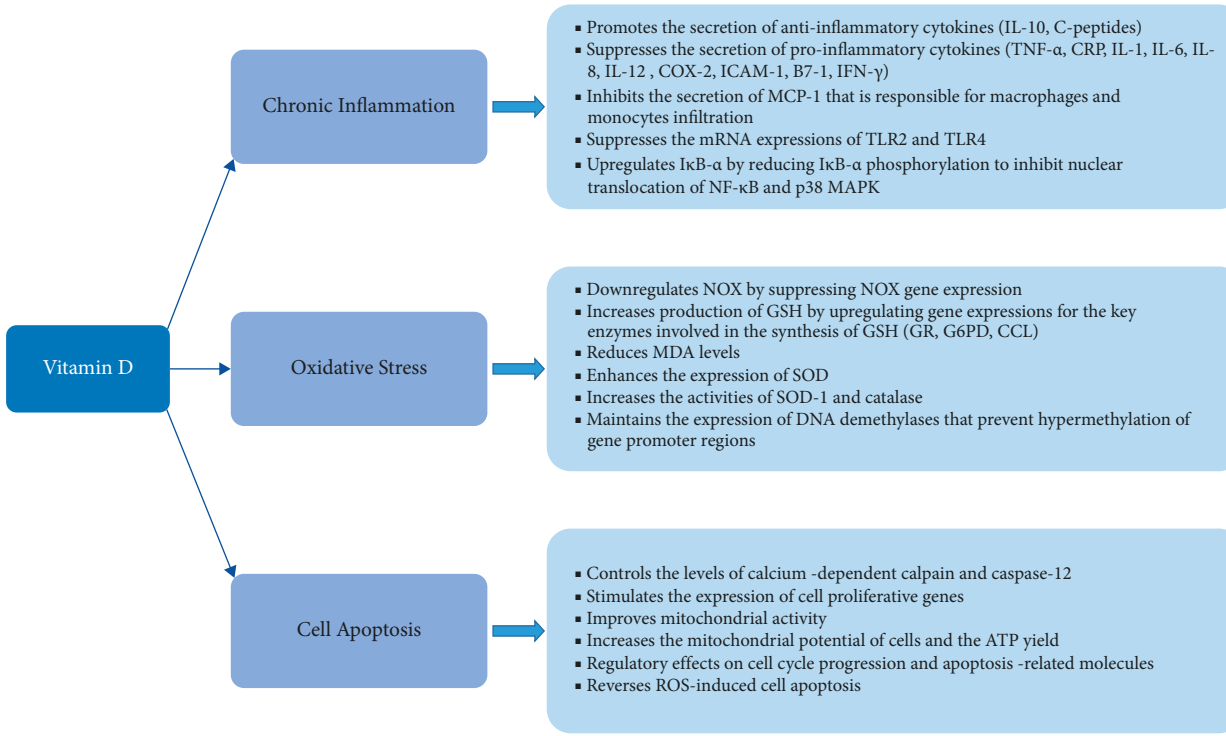

Figure 4: The roles of vitamin D in chronic inflammation, oxidative stress, and cell apoptosis.

as stimulates the expression of genes that favored cell proliferation in adipocytes [185]. Furthermore, vitamin D may inhibit apoptosis [186] by improving the mitochondrial activity [51] and increasing the mitochondrial potential of cells and adenosine triphosphate (ATP) yield [187], and through the regulatory effects on cell cycle progression and apoptosis-related molecules [52]. These mechanisms may involve the presence of VDR and the regulation of FoxO1. A recent study has demonstrated that VDR gene silencing is associated with reduced cell survival and overexpression of *FoxO1* mRNA and protein, which imply that VDR plays important role in reducing cell death [13]. Vitamin D treatment and high VDR expression have been shown to induce cell survival and mitigate FoxO1-induced cell apoptosis, whereas vitamin D treatment and FoxO1 gene silencing reverse ROS-induced cell apoptosis [13]. Figure 4 shows the roles of vitamin D in ameliorating inflammation, oxidative stress, and apoptosis in tissues.

4. Conclusion

Vitamin D seems to have a significant role in metabolism, and its deficiency could be linked to the pathogenesis of insulin resistance. Insufficient levels of vitamin D are associated with hyperglycemia, low insulin sensitivity, chronic inflammation, oxidative stress, and apoptosis. Normal vitamin D levels are associated with normal glucose homeostasis, insulin sensitivity, improved pancreatic β-cell function and insulin secretion, and other improvement in metabolic parameters. Therefore, prompt detection and effective management of vitamin D deficiency in individuals with insulin resistance could be an easier, cost-effective approach that may improve the health outcomes and help to reduce the risk of developing DM and other related metabolic disorders.

Data Availability

The datasets supporting the conclusions of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Research Article

Serum 25(OH)D Levels Modify the Association between Triglyceride and IR: A Cross-Sectional Study

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Background. Triglycerides and 25(OH)D had been reported as correlates of IR, but the results suggest substantial heterogeneity across races. In addition, little research reported on whether different 25(OH)D levels affect triglycerides and IR. Therefore, a similar study on the US population would be a great addition to the current one. This study investigated the association between triglycerides and IR at different 25(OH)D levels. *Methods*. A total of 19,926 participants were included, each containing specific indicators for the study project. IR was estimated as a HOMA-IR index ≥2.73. Four multivariate logistic regression models were developed to analyze the association between TG and IR and whether different 25(OH)D levels influenced this association. Smoothed fitting curves were plotted. *Results*. Triglyceride was significantly associated with IR (OR: 1.3, 95 CI %), while this association received different 25(OH)D levels (P for interaction <0.001). The effect value OR was 1.33 with the high levels, and its effect value OR was 1.28 with the low levels. *Conclusion*. This study demonstrates that triglyceride levels are significantly associated with insulin in the US adult population and can be used as a predictor of IR. This correlation was compromised at different 25 (OH)D levels, so future studies need to be explored in more ethnically diverse contexts.

1. Introduction

Insulin resistance (IR) is defined as reduced insulin sensitivity (IS) and refers to an increased amount of insulin needed to perform its metabolic actions [1]. According to previous research, IR is a hallmark of human obesity and is associated with the risk of developing diabetes, breast cancer, cardiovascular disease [2, 3], pancreatic cancer [2, 4], and liver cancer [5]. Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and is closely associated with insulin resistance [6, 7]. Moreover, a recent study suggests that risk factors for insulin resistance include 25(OH)D deficiency, secondary hyperthyroidism, exercise intolerance, obesity, high triglyceride (TG) levels, and so on [8]. It can be deduced that 25(OH)D3 is a protective factor for IR, and TG is a risk factor for IR.

Vitamin D (25(OH)D), also known as cholecalciferol, is not really a vitamin but the precursor to the potent steroid hormone calcitriol (also named as 1,25-dihydroxy25(OH) D3 (1,25(OH)2 D3)), which has extensive roles through many tissues of the body. 25(OH)D was synthesized in human skin under the exposure to ultraviolet or through dietary uptake is well-adapted to the human body [9]. 25(OH)D3 deficiency may also be linked to autoimmune and infectious diseases such as osteoporosis [10], inflammatory bowel disease [11], glomerular disease, and chronic renal insufficiency [12]. In addition, 25(OH)D deficiency increases mortality from certain diseases such as systemic lupus erythematosus [13], liver cirrhosis [14], and diabetes [15]. Currently, there have been some studies showing that 25(OH)D promotes the synthesis and secretion of insulin by pancreatic β-cells [10, 16] and has a role in combating type I

and II diabetes, and 25(OH)D3 deficiency is thought to be a significant factor in the development of type II diabetes.

Triacylglycerols (TG), fatty acyl ester derivatives of glycerol, are a class of neutral lipids that represent the most important storage form of energy for eukaryotic cells [17]. The primary function of TG is to serve as a vital energy substance for the body [18]. However, recent studies have shown that elevated TG levels can increase the severity of diseases, such as accelerating cardiovascular diseases and atherosclerosis [19], exacerbating gout symptoms [20], causing recurrent acute pancreatitis and persistent organ failure [21], and complicating dialysis patients with increased TG levels that also cause diabetes mellitus [22], coronary artery disease [23], male sexual dysfunction [24], nontraumatic fractures in middle-aged women [25], Alzheimer's disease [26], hypothyroidism [27], and one of the factors in the occurrence of diseases, especially diabetes mellitus. In addition, it has been demonstrated that increased muscle TG levels contribute to IR by attenuating insulin signaling [28].

It has been found that IR increases the risk of developing type II diabetes [29]. In recent years, type II diabetes (T2D) has become a global challenge with a tremendous economic burden for society and public health systems. More than 90% of patients with diabetes have type II diabetes and it is of great necessity to investigate the association between TG and IR at different 25(OH)D3 levels. It has been shown that TG is associated with IR [28]. However, there are not enough evidence to show whether 25(OH)D levels influence this association. With the present study, we sought to determine the effects of 25(OH)D levels and TG levels on IR using data from the 2009–2018 NHANES.

2. Methods

2.1. Data Sources. This study was conducted using a cross-sectional study of nine cycles (2001–2018) of data from the NHANES database (https://www.cdc.gov/nchs/nhanes/) of the National Center for Health. It is a study focusing on health screening and healthy eating in the United States. After applying its recommended population weights weighting, it applied a multistage stratified probability design to collect data. Its sample is representative of the overall sample of noninstitutionalized US citizens. These data included demographic data, dietary data, physical measurements, laboratory data, and questionnaire data. All NHANES-based studies were approved by the US National Health Statistics Research Ethics Review Board. Ethical approval and more detailed information can be found on the NHANES Ethics Review Committee's website (https://www.cdc.gov/nchs/nhanes/irba98.htm) [30].

2.2. Study Design and Participant Population. This study was a cross-sectional study and used data from the NHANES official website: https://www.cdc.gov/nchs/nhanes/NHANES. The target-independent variable was the level of serum triglyceride levels when the participants were tested. The dependent variable was IR, as defined by the participant's HOMA-IR index [31]. Less than 50 mmol/L was defined as the 25(OH)D deficient group, and ≥50 mmol/L was defined as the 25(OH)D level adequate group according to the internationally recommended definition of 25(OH)D levels.

Participants aged 20 years or older who completed an interview and examination at the Mobile Examination Center (MEC) from 2001 to 2018 were recruited for this study. Participants who do not meet the following criteria were excluded: (1) have data on fasting insulin, fasting glucose, and fasting triglycerides, (2) age ≥20 years, and (3) not taking medications that affect insulin, glucose metabolism, or lipid metabolism.

2.3. Data Collection. All data were collected by trained professionals. The data utilized in this study included demographics (age, gender, race, education, etc.), anthropometric measurements (height, waist circumference, weight, body mass index (BMI), etc.), health-related behaviors (smoking and alcohol consumption), and biochemical tests (HDL, ALT, AST, TG, etc.). All information is collected, and blood samples are collected in a mobile testing center (MEC) where basic information is collated immediately and serum samples are sent to the Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, and designated authorized institutions for analysis after scientific storage management.

2.3.1. Measurement of 25(OH)D. Immediately after serum samples are taken at the MEC, they are stored frozen at −30°C. Samples for 25(OH)D measurement are then transported uniformly to the CDC Environmental Health Laboratory in Atlanta, Georgia. 25(OH)D levels (ng/ml) were defined as the sum of 25(OH)D3 and D2. Laboratory analysis was performed by ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) [32].

2.3.2. Measurement of Triglycerides. The method for triglycerides was done based on Wahlefeld's method using lipases derived from microorganisms to facilitate the rapid and complete hydrolysis of triglycerides to glycerol and subsequent oxidation to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, the peroxide reacts with 4-aminophenazone and 4-chlorophenol in a Trinder reaction to a colorimetric endpoint.

2.3.3. HOMA-IR Index and Reduced Insulin Sensitivity. The HOMA-IR index is considered to be a good index for evaluating insulin sensitivity (PMID: 31958717). The formula for calculating HOMA-IR is: fasting blood glucose level (FPG, mmol/L) × fasting insulin level (FINS, μU/mL)/22.5. In this study, based on previous studies, the HOMA-IR index in the US adult population was calculated as an IR index greater than or equal to 2.73 was defined as positive for IR in the US adult population, based on previous studies. Participants with a HOMA-IR index lower than 2.73 were defined as IR negative [33].

2.3.4. Definition of Some Other Variables. Diabetes mellitus: fasting blood glucose was multiplied by 0.056 (rounded to retain three decimal places) to convert the unit from mg/dl to mmol/l [34]. Diabetes was diagnosed by fasting glucose ≥7.0 mmol/l, OGTT ≥11.1 mmol/l, physician diagnosis, self-reported, or taking diabetes medication.

Race: Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black, and other races.

Education: High school graduate, high school graduate, college graduate, or higher.

Smoking: smoked, quit smoking, and never smoked. Participants who smoked ≥100 cigarettes or more in total in the past and reported smoking on a few days or every day at the time of the interview were considered current smokers. Participants who smoked <100 cigarettes in the past but were not currently smoking were considered ex-smokers. Participants who smoked <100 cigarettes in the past were considered nonsmokers.

Alcohol consumption: drinking versus nondrinking. Heavy drinkers were defined as >1 drink/day for women and >2 drinks/day for men.

BMI: based on height and weight. Height was measured by the researchers using an electronic sports measuring device (Seca Ltd, Medical Scales and Measurement Systems, Birmingham, UK) with an accuracy of millimeters. Body weight was measured by researchers using a digital scale (Toledo Scale; Mettler-Toledo, LLC, Columbus, OH, USA), and after measurement, pounds were converted to kilograms. The formula for BMI is: BMI = weight (kg)/height (M^2) [35].

2.4. Statistical Methods. All data were analyzed using R version 4.1.2, with continuous variables represented by detailed sample descriptions with a mean confidence interval of 95%. Categorical variables were represented by counts and weighted percentages. Skewed distributions were based on median and Q1–Q3. Normal distributions were described by median and standard deviation. Continuous variables were compared between groups using the Student *t*-test or Mann-Whitney *U* test based on the normality of the distribution, and within-group comparisons were made using Fisher's exact probability method. Covariates were selected based on potential confounders that may be associated with triglycerides and IR. Gender, age, race, smoking, BMI, obesity, and education were selected as covariates based on a combination of previous literature, international standards, and relevant clinical experience. Multiple interpolation was used to fill in the missing covariates with the aim of maximizing statistical power and minimizing bias. In addition, sensitivity analyses were conducted to see if the resulting complete data differed significantly from the original data. These studies showed that the data after multiple interpolation did not differ significantly from the original data and were not statistically significant ($p > 0.05$). Therefore, all results of our multivariate analysis were based on the data set after multiple interpolation according to Rubin's criterion. Four multivariate logistic regression models were developed to analyze the relationship with IR at different 25(OH)D levels, and smooth fitted curves were constructed. $p < 0.05$ (two-sided) was considered statistically significant.

3. Results

3.1. Description of the Basic Information of the Population. There were 19,926 participants in the 9 NHANES cycles (2001–2018; Figure 1). The basic information of the included participants is detailed in Table 1. The participants were grouped according to the occurrence of IR and divided into IR positive group and IR negative group. The mean age of all participants was 47.4 ± 19.2 years. The distribution of the two groups differed in gender, race, education, hypertension, diabetes, and alcohol consumption. BMI, waist circumference, TG, ALT, and AST were higher in the IR positive group than in the IR negative group. In contrast, HDL, TBIL, and 25(OH)D were higher in the IR negative group than in the IR positive group.

3.2. Univariate Regression Analysis. Univariate logistic regression showed the factors associated with IR in this study as shown in Table 2. Race, education, alcohol consumption, HDL, TBIL, ALB, and 25(OH)D were negatively associated with IR. In contrast, BMI, waist circumference, hypertension, diabetes, TG, ALT, AST, and BUN were positively associated with IR.

3.3. Multifactorial Regression Analysis. This study constructed four logistic regression models to analyze the independent association between triglyceride levels and IR. As shown in Table 3, the model-based effect values OR indicated a corresponding increase in the risk of IR for each unit increase in triglycerides. For example, in the unadjusted model (Model 1), the total effect value was 2.02 (1.94 to 2.09). Each 1 unit increase in triglycerides implies a 102% increase in the risk of developing IR. In the adjusted basic informatics model (Model 2), the effect value was OR: 2.07 (1.99 to 2.15). In the model adjusted for characteristic informatics (Model 3), the effect value was OR: 1.53 (1.46 to 1.59). In the fully adjusted model (Model 4), the OR and 95% CI were 1.3 and 1.25 to 1.36, respectively. The results suggest that triglycerides and IR were independently and negatively associated after controlling for potential confounders.

In addition, we also grouped according to 25(OH)D levels and observed whether triglycerides and IR received an effect of this association at different 25(OH)D levels (Table 3). The association between triglycerides and IR was strengthened within the high 25(OH)D subgroup (Model 1: 2.13 vs. 1.97, Model 2: 2.12 vs. 2.01, Model 3: 1.54 vs. 1.52, and Model 4: 1.33 vs. 1.28).

3.4. Curve-Fitting Analysis. The current study better explains the association between triglycerides and IR at different 25(OH)D levels. We plotted the curve fit. As shown in Figures 2 and 3, there is a difference in the association

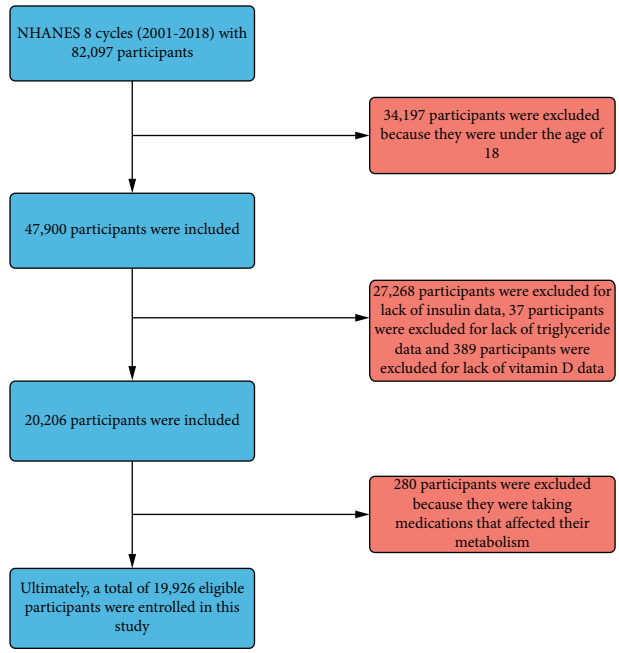

Figure 1: Flowchart of patient selection.

Table 1: Baseline characteristics of the study participants.

Variables	Total ($n = 19,926$)	IR negative ($n = 11,251$)	IR positive ($n = 8,675$)	P value
Age, mean ± SD	47.4 ± 19.2	46.1 ± 19.5	49.1 ± 18.7	<0.001
Gender, n (%)				
Male	9,563 (48.0)	5,285 (47)	4,278 (49.3)	0.001
Female	10,363 (52.0)	5,966 (53)	4,397 (50.7)	
Race, n (%)				
Mexican American	3,578 (18.0)	1,745 (15.5)	1,833 (21.1)	
Other Hispanic	4,007 (20.1)	2,162 (19.2)	1,845 (21.3)	
Non-Hispanic white	8,981 (45.1)	5,449 (48.4)	3,532 (40.7)	<0.001
Non-Hispanic black	1,656 (8.3)	831 (7.4)	825 (9.5)	
Other races	1,704 (8.6)	1,064 (9.5)	640 (7.4)	
Education, n (%)				
Poorly educated	2,330 (11.7)	1,151 (10.2)	1,179 (13.6)	
Moderately educated	7,544 (37.9)	4,099 (36.4)	3,445 (39.7)	<0.001
Highly educated	10,052 (50.4)	6,001 (53.3)	4,051 (46.7)	
BMI, mean ± SD	28.6 ± 6.7	25.8 ± 4.9	32.2 ± 7.1	<0.001
Waist, mean ± SD	97.9 ± 16.2	90.9 ± 12.8	107.0 ± 15.6	<0.001
Hypertension, n (%)				
No	12,444 (62.5)	7,902 (70.2)	4,542 (52.4)	<0.001
Yes	7,482 (37.5)	3,349 (29.8)	4,133 (47.6)	
DM, n (%)				
No	16,537 (83.0)	10,394 (92.4)	6,143 (70.8)	<0.001
Yes	3,389 (17.0)	857 (7.6)	2,532 (29.2)	
Alcohol, n (%)				
No	15,810 (79.3)	8,547 (76)	7,263 (83.7)	<0.001
Yes	4,116 (20.7)	2,704 (24)	1,412 (16.3)	
Smoke, n (%)				
Never smoking	10,890 (54.7)	7,898 (70.2)	2,646 (30.5)	
Former smokers	4,942 (24.8)	2,813 (25)	2,134 (24.6)	<0.001
Current smokers	4,094 (20.5)	540 (4.7)	3,895 (44.9)	
HDL, median(IQR), mmol/L	1.3 (1.1, 1.6)	1.4 (1.2, 1.8)	1.2 (1.0, 1.4)	<0.001
TG, median (IQR), mmol/L	1.2 (0.8, 1.8)	1.0 (0.7, 1.4)	1.5 (1.0, 2.2)	<0.001
ALT, median (IQR), U/L	20.0 (16.0, 28.0)	19.0 (15.0, 25.0)	23.0 (17.0, 32.0)	<0.001
AST, median (IQR), U/L	23.0 (19.0, 27.0)	22.0 (19.0, 26.0)	23.0 (19.0, 28.0)	<0.001
TBIL, median(IQR), μmol/L	12.0 (8.6, 15.4)	12.0 (10.3, 15.4)	12.0 (8.6, 13.7)	<0.001
BUN, median(IQR), mmol/L	4.3 (3.6, 5.4)	4.3 (3.2, 5.4)	4.3 (3.6, 5.7)	<0.001
ALB, median (IQR), g/L	42.0 (40.0, 45.0)	43.0 (41.0, 45.0)	42.0 (40.0, 44.0)	<0.001
25(OH)D, median (IQR), ng/ml	59.1 (43.3, 76.2)	62.2 (45.9, 80.1)	54.8 (40.0, 70.8)	<0.001

DM, diabetes mellitus; HDL, high-density lipoprotein; TG, triglyceride; ALT, alanine transaminase; AST, aspartate transaminase; TBIL, total bilirubin; BUN, blood urea nitrogen; ALB, albumin; and vitd, 25(OH)D.

Table 2: Univariate analysis for IR.

Variable	OR (95% CI)	*P* value
Age	1.01 (1.01 ~ 1.01)	<0.001
Gender		
Male	1	
Female	0.91 (0.86 ~ 0.96)	0.001
Race		
Mexican American	1	
Other Hispanic	0.81 (0.74 ~ 0.89)	<0.001
Non-Hispanic white	0.62 (0.57 ~ 0.67)	<0.001
Non-Hispanic black	0.95 (0.84 ~ 1.06)	0.342
Other races	0.57 (0.51 ~ 0.64)	<0.001
Education		
Poorly educated	1	
Moderately educated	0.82 (0.75 ~ 0.9)	<0.001
Highly educated	0.66 (0.6 ~ 0.72)	<0.001
BMI	1.22 (1.21 ~ 1.23)	<0.001
Waist	1.09 (1.08 ~ 1.09)	<0.001
Hypertension		
No	1	
Yes	2.15 (2.03 ~ 2.28)	<0.001
DM		
No	1	
Yes	5 (4.6 ~ 5.44)	<0.001
Alcohol		
No	1	
Yes	0.61 (0.57 ~ 0.66)	<0.001
HDL	0.16 (0.15 ~ 0.17)	<0.001
TG	2.02 (1.94 ~ 2.09)	<0.001
ALT	1.02 (1.02 ~ 1.03)	<0.001
AST	1 (1 ~ 1.01)	<0.001
TBIL	0.96 (0.95 ~ 0.96)	<0.001
BUN	1.04 (1.03 ~ 1.05)	<0.001
ALB	0.94 (0.94 ~ 0.95)	<0.001
25(OH)D	0.99 (0.99 ~ 0.99)	<0.001

DM, diabetes mellitus; HDL, high-density lipoprotein; TG, triglyceride; ALT, alanine transaminase; AST, aspartate transaminase; TBIL, total bilirubin; BUN, blood urea nitrogen; and ALB, albumin.

between different 25(OH)D levels and IR. Also, at higher triglycerides, higher 25(OH)D levels are more likely to be insulin resistant, which represents that high levels of triglycerides may weaken the protective effect of 25(OH)D on IR.

4. Discussion

To our knowledge, this study examined the association between triglyceride and IR using univariate logistic regression. It found that HDL, total bilirubin, albumin, and 25(OH)D may be protective factors for IR. In contrast, smoking, BMI and waist circumference above normal, hypertension, diabetes mellitus, triglycerides, glutathione, glutathione, and blood urea nitrogen may be risk factors for IR. In addition, men were found to have a higher probability of IR than women, in which the probability of IR varied significantly by race and the population's education level was negatively associated with the probability of IR. In the present study, we found that the odds of IR were lower in the drinking population than in the nondrinking population. However, this does not mean that we support alcohol drinking. More correlational studies need to evaluate the combined effects of alcohol consumption on humans.

The effect of confounding was explored by adjustment for a wide range of potentially confounding factors in regression models. In a fully adjusted model excluding other confounders (Model 4; Table 3), we found a strong correlation between TG and IR (OR: 1.3, 95% CI 1.25 to 1.36). Our results are largely consistent with those of other studies. For example, a cross-sectional study conducted by Boursier [36] in 2017 on an obese population ($n = 498$) found that triglycerides are an independent correlate of IR (OR: 3.0, 95% CI 2.0 to 4.5). In addition, Lin et al. [37] also found higher triglycerides in the Chinese population ($n = 9764$) with IR. In these studies, we found there are still some differences between their results and ours. The reasons for the differences may be due to the different populations selected for the studies (different races) and differences in the way the data were processed. Also, this study further found that the association between TG and IR differed significantly at different 25(OH)D3 levels, and the reason for the difference was that high levels of TG could disrupt the protective effect of 25(OH)D3 on IR.

Current mainstream studies report that 25(OH)D3 may improve IR by increasing insulin sensitivity through inhibition of inflammation [38–40]. 25(OH)D may reduce the extent of IR-related pathology by enhancing insulin signaling. There are various pathways through which 25(OH)D enhances insulin signaling, such as maintaining normal resting levels of ROS and Ca2+ [41], exerting autocrine and paracrine effects [42], and reducing oxidative stress [43]. It is thus clear that 25(OH)D may inhibit IR through multiple pathways.

On the other hand, some studies have shown that excess triglycerides in the body promote ectopic lipid accumulation in the liver, skeletal muscle, and heart triggering impairment of insulin signaling pathways and hence inducing IR [44, 45]. This is due to white adipocyte tissue (WAT) dysfunction caused by lipid accumulation. WAT dysfunction increases proinflammatory adipokines, and activation of oxidative stress and the renin-angiotensin-aldosterone system (RAAS) promotes IR [44, 46].

Notably, several studies have shown that high levels of TG lead to a decrease in 25(OH)D3 levels [47–50], suggesting that TG is negatively correlated with 25(OH)D3, which may be explained by the fact that cholecalciferol load is distributed in a larger volume, resulting in its release into the adipose tissue stores of 25(OH)D in circulation more slowly [50]. From these studies, we hypothesized that elevated TG levels would disrupt the protective effect of 25(OH)D3 on IR, leading to a stronger relationship between triglycerides and IR.

In addition, there is still some discussion about the link between insulin resistance and 25(OH)D. In today's world, the most prevalent chronic liver disease is chronic hepatitis due to nonalcoholic fatty liver disease. Most studies suggest that metabolic pathways such as 25(OH)D levels and insulin resistance may underlie nonalcoholic steatohepatitis. However, the conclusions of these studies are not absolute; for example, the findings of Yuval et al. [51] in 2016 suggest

Table 3: Multiple logistic regression analysis of the association between TG and IR.

Variable	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
TG	2.02 (1.94~2.09)	<0.001	2.07 (1.99~2.15)	<0.001	1.53 (1.46~1.59)	<0.001	1.3 (1.25~1.36)	<0.001
25(OH)D group								
<50 nmol/L	1.97 (1.84~2.1)	<0.001	2.01 (1.87~2.15)	<0.001	1.52 (1.41~1.63)	<0.001	1.28 (1.19~1.37)	<0.001
≥50 nmol/L	2.13 (2.03~2.23)	<0.001	2.12 (2.02~2.23)	<0.001	1.54 (1.47~1.62)	<0.001	1.33 (1.26~1.4)	<0.001

Model 1: nonadjusted; Model 2: adjusted age, gender, and race; Model 3: adjusted age, gender, race, and education, BMI, waist, hypertension, DM, and alcohol; Model 4: adjusted age, gender, race, education, BMI, waist, hypertension, DM, alcohol, ALT, AST, TBIL, BUN, ALB, and HDL.

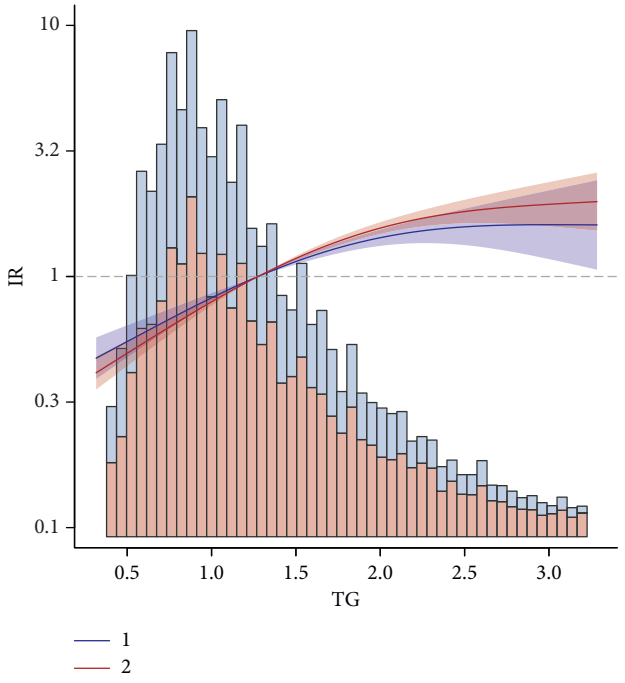

Figure 2: Association between TG and IR (total).

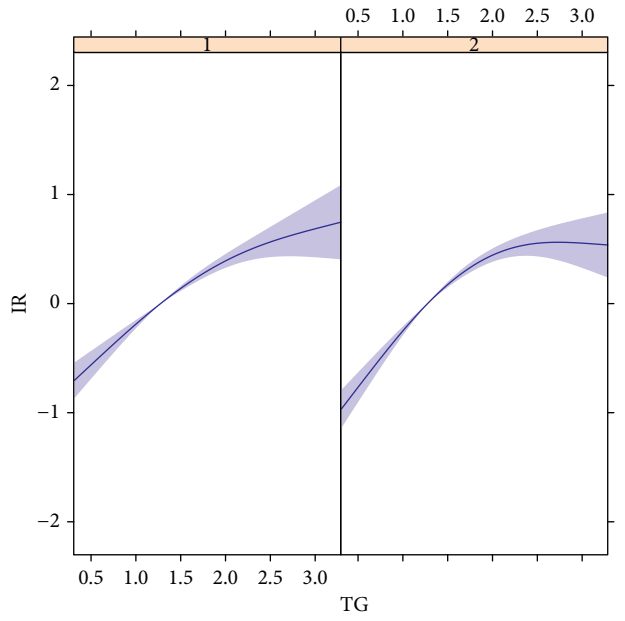

Figure 3: Association between TG and IR (individual).

that 25(OH)D levels do not appear to be associated with NAFLD. Thus, despite preclinical evidence linking 25(OH)D to the pathogenesis of NAFLD, in humans, 25(OH)D deficiency does not appear to be associated with the severity of NAFLD. As Tarantino et al. [52] showed in 2019, this may be a critical point, as there is clear evidence that we are far from having a clear understanding of the underlying mechanisms of NAFLD.

We found shortcomings in the present study. For example, this study did not address some special populations, such as pregnant women and children. Whether the results apply to these special populations is currently unknown. More studies should be conducted in the future to demonstrate the applicability of the results to these specific populations. A 2014 study of 25(OH)D by Youselzadeh et al. [53] concluded that factors such as race and obesity were affected by 25(OH)D and 25(OH)D-binding proteins. At the same time, a 2017 study by Jassil et al. [54] found that non-Hispanic blacks had lower 25(OH)D, which more favourably demonstrated the link between 25(OH)D and 25(OH)D-binding proteins. In future research, we will also conduct research on 25(OH)D-binding proteins. However, there are clear advantages to this study. First, the sample size was large (19,926) and spanned a period of 18 years. Furthermore, the stratified fit curves were developed to better illustrate the association between triglycerides and IR. Moreover, we analyzed the association between triglycerides and IR using multiple regression, which allowed us to exclude further factors that were not strongly associated with IR. Only then did we finally conclude that high levels of triglycerides undermine the protective effect of 25(OH)D on IR.

5. Conclusion

We found an independent negative correlation between triglycerides and IR in the US population using a cross-sectional study. In addition, the association between TG and IR was enhanced under high-level 25(OH)D conditions when classified according to 25(OH)D levels. The study results may provide a reference for the analysis of factors in the pathogenesis of IR and suggest 25(OH)D levels and TG as markers of IR.

Abbreviations

BMI: Body mass index
FBG: Fasting blood glucose
VitD3: Vitamin D3

Hb-A1c: Glycosylated hemoglobin
TC: Total cholesterol
ALT: Alanine aminotransferase
BUN: Blood urea nitrogen
WC: Waist
TG: Triglyceride
CI: Confidence interval
OR: Odds ratio
HUA: Hyperuricemia.

Data Availability

All data were downloaded from NHANES' official website: https://www.cdc.gov/nchs/nhanes/. NHANES was a large multipurpose cross-sectional survey that provided comprehensive data on various aspects of nutrition and health.

Ethical Approval

Not applicable.

Consent

Not applicable.

Disclosure

Rongpeng Gong and Xin Tang are co-first authors.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Rongpeng Gong and Tang Xin conceived the idea; Rongpeng Gong, Xing Tang, and Ziying Jiang wrote the manuscript; Chaofan Dong and Gang Luo collected and read the literature and revised the article; and Xiuxia Han read through and corrected the manuscript. All authors read and approved the final manuscript.

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Research Article

Association between Serum Magnesium and Hemoglobin in Patients with Primary Hyperparathyroidism

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Background. There is a positive association between serum magnesium and hemoglobin levels in the general population. However, no studies have evaluated the association between serum magnesium and hemoglobin levels in patients with primary hyperparathyroidism (PHPT). We aimed to investigate whether there is a relationship between serum magnesium and hemoglobin levels in the patient population with PHPT. *Methods*. This retrospective study included 307 hospitalized PHPT patients who were continuously admitted to the Second Xiangya Hospital of Central South University, from January 2010 to August 2020. Laboratory and demographic data of patients were collected. Hypomagnesemia was defined as serum magnesium <0.75 mmol/L. Patients with a hemoglobin level below 130 g/L in males and below 120 g/L in females were accepted as the anemic group. *Results*. Among the 307 patients with PHPT included in our study, 77 (25.1%) patients (33 (32.4%) males and 44 (21.5%) females) had hypomagnesemia. A total of 138 (45.0%) patients (49 males (48.0%) and 89 females (43.4%)) had anemia. Compared with the nonanemic group, the anemic group had lower average albumin, eGFR, and serum magnesium levels in both males and females. In contrast, average creatinine, PTH, and corrected calcium were significantly higher in the anemic group than in the nonanemic group in both males and females. Lower serum magnesium levels were associated with lower hemoglobin levels independent of serum calcium, albumin, eGFR, and PTH in PHPT patients. *Conclusions*. Hypomagnesemia is a common electrolyte disorder in PHPT patients. Hypomagnesemia is independently associated with lower hemoglobin levels in patients with PHPT.

1. Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disease characterized by hypercalcemia and high or inappropriately normal levels of parathyroid hormone (PTH) [1–3]. In PHPT, there is a range of symptoms caused by hypercalcemia that affect the skeletal, renal, and digestive systems [4]. Anemia has been recognized as a possible complication of PHPT [5–7]; however, the exact pathogenesis of anemia is still unknown. It is possible that patients develop anemia when the kidneys do not produce sufficient erythropoietin (EPO) levels to promote erythropoiesis [8]. The bone and bone marrow, although often regarded as separate systems, function as a single unit. The bone marrow contains hematopoietic cells, and bone marrow suppression causes anemia [9]. Anemia is also a common complication of gastrointestinal tract diseases [10]. In addition, high PTH levels may play a role in anemia in

PHPT patients. Researchers have reported that EPO synthesis, erythroid progenitor production, and red blood cell (RBC) survival are negatively impacted under high PTH [11–13]. Similarly, high serum levels of PTH negatively affect serum hemoglobin levels in patients with chronic kidney disease (CKD) [14, 15]. There is indirect evidence of restoration of hematocrit levels following control of secondary hyperparathyroidism or parathyroidectomy in uremic patients due to restoration of bone marrow space and increase in immunoreactive EPO levels [16–19].

Magnesium, which is the second most important intracellular cation, has received considerable interest within the scientific community. The relationship between magnesium deficiency and anemia was first reported in animal studies [20, 21]. In 1973, Piomelli et al. observed a significant decrease in hemoglobin levels in rats fed with a magnesium-deficient diet. This anemia was accompanied by severe reticulocytosis, reduction in hemoglobin and hematocrit levels, and decrease in RBC lifespan and half-life. The authors argued that magnesium deficiency contributed to hemolytic anemia by affecting energy metabolism in RBCs [21]. A study conducted with 8,511 participants showed that high magnesium levels were associated with a lower risk of anemia [22]. Similarly, an inverse relationship was reported between magnesium and anemia in the elderly patient population [23]. Positive effects of high serum magnesium on hemoglobin have been reported in CKD patients [24, 25]. It is worth mentioning that magnesium supplementation increases hemoglobin levels in athletes [26].

The characteristics of serum magnesium in PHPT patients are still controversial. A previous study reported that serum magnesium was usually within the normal range in PHPT [27]; however, hypomagnesemia has been noted in PHPT patients, especially in association with very high serum calcium levels or kidney damage [28–30]. Therefore, the characteristics of serum magnesium in patients with PHPT remain unclear. To the best of our knowledge, no study has investigated the association between magnesium and hemoglobin levels in patients with PHPT. In this study, we investigated whether there is an association between serum magnesium and hemoglobin levels in PHPT patients.

2. Materials and Methods

2.1. Study Design and Patients. We performed a retrospective study of 307 hospitalized patients with PHPT, who were admitted to Second Xiangya Hospital of Central South University, which is a tertiary hospital in Changsha, Hunan Province, Central South of China, from January 2010 through August 2020. Patients diagnosed with secondary hyperparathyroidism, tertiary hyperparathyroidism, familial hypocalciuric hypercalcemia (FHH), osteitis fibrosa cystica (OFC), or gastrointestinal bleeding were excluded from the study. Hypomagnesemia was defined as serum magnesium <0.75 mmol/L [31]. Anemia was defined as hemoglobin <130 g/L in males and <120 g/L in females [32].

This study protocol was approved by the Ethics Committee. All the patients provided informed consent for participating in this study.

2.2. Medical History Collection and Anthropometric Information. Medical records were reviewed for age, sex, and disease duration. The patient height was measured to the nearest 0.1 cm, and the weight was recorded to the nearest 0.1 kg with the participant wearing light clothing. BMI was calculated as weight in kilograms divided by height in meters squared.

2.3. Biochemical Measurements. We recorded the levels of serum calcium, phosphorus, magnesium, albumin, creatinine, PTH, and 25(OH)D. Blood samples were collected after an overnight fast. The measurement of hemoglobin (Hb) levels was performed by using the automated hematology analyzer ADVIA 2120 (Siemens Healthcare Diagnostics, Germany). Serum albumin, calcium, phosphorus, and magnesium were determined using an automatic biochemical analyzer (Abbott Laboratories, North Chicago, IL, USA). Albumin-corrected serum calcium was calculated using the following formula, corrected calcium (mmol/L) = serum calcium ((mmol/L) + 0.02 × (40 − serum albumin (g/L)). Serum creatinine levels were determined by an enzymatic method (Kanto Chemical, Tokyo, Japan), and the estimated glomerular filtration rate (eGFR) was quantified using CKD-EPI 2009 equations [33]. Serum PTH was measured by automated chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Erlangen, Germany). Serum 25(OH)D was measured using an enzyme-linked immunosorbent assay (Immunodiagnostic Systems Limited, Boldon, UK). All interassay and intra-assay coefficients of variation were less than 10% [34].

2.4. Statistical Analysis. Data that were normally distributed were expressed as mean ± SD, while data that did not follow a normal distribution were expressed as median (range). Normal and nonnormal distributions between groups were compared using Student's *t*-test and Wilcoxon rank sum test, respectively. We used correlation coefficients, linear regression, and logistic regression to assess relationships. Statistical significant was set at $P < 0.05$ (two-sided).

3. Results

Table 1 shows the demographic data and biochemical parameters. Among the 307 hospitalized patients, 102 were males and 205 were females. The mean age of the study population was 52.2 ± 14.6 years. Among the patients, 237 (77.2%) had symptomatic PHPT, 138 (45.0%) had anemia, and 77 (25.1%) had hypomagnesemia.

We compared the parameters between the nonanemic and anemic group by sex (Table 2). A total of 138 patients (49 males (48.0%) and 89 females (43.4%)) had anemia, accounting for 45.0% of the total population. Average serum magnesium was significantly lower in the anemic group than in the nonanemic group in both males and females (0.75 ± 0.17 vs. 0.88 ± 0.19 mmol/L, $P < 0.05$; 0.80 ± 0.19 vs. 0.89 ± 0.16 mmol/L, $P < 0.05$, respectively). Furthermore, average albumin and eGFR levels were significantly lower in the anemic group than in the nonanemic group in both males

Table 1: Baseline characteristics of the subjects.

Number of subjects (males/females)	307 (102/205)
Age (years)	52.2 ± 14.6
Duration (years)	4.48 ± 7.06
BMI (kg/m^2)	22.4 ± 3.4
Hemoglobin (g/L)	120 ± 21
eGFR (mL/min/1.73 m^2)	76.0 ± 39.1
Creatinine (umol/L)	91.4 ± 76.2
Albumin (g/L)	38.5 ± 7.7
Serum calcium (mmol/L)	2.85 ± 0.44
Corrected calcium (mmol/L)	3.11 ± 0.57
Serum phosphorus (mmol/L)	0.78 ± 0.28
Serum magnesium (mmol/L)	0.85 ± 0.18
PTH (pg/mL)	68.0 ± 80.9
25(OH)D (ng/mL)	33.6 ± 17.3
Symptomatic patients, *n* (%)	237 (77.2)
Anemia, *n* (%)	138 (45.0)
Hypomagnesemia, *n* (%)	77 (25.1)

BMI, body mass index; eGFR, estimate glomerular filtration rate; PTH, parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D.

Table 2: Comparison of the parameters between the nonanemia group and the anemia group by sex.

	Males			Females		
	Nonanemia group ($n = 53$)	Anemia group ($n = 49$)	*P*	Nonanemia group ($n = 116$)	Anemia group ($n = 89$)	*P*
Age (years)	46.9 ± 15.5	49.8 ± 16.1	0.368	50.1 ± 14.1	57.3 ± 12.5	<0.001
Duration (years)	4.74 ± 8.68	4.69 ± 6.88	0.472	4.02 ± 5.68	5.21 ± 8.03	0.321
BMI (kg/m^2)	22.8 ± 2.9	21.7 ± 3.1	0.111	23.5 ± 3.6	21.8 ± 3.4	0.002
Hemoglobin (g/L)	145 ± 11	107 ± 17	<0.001	132 ± 8	104 ± 13	<0.001
eGFR (mL/min/1.73 m^2)	81.1 ± 39.7	58.4 ± 31.4	0.006	90.4 ± 37.3	69.9 ± 38.9	<0.001
Creatinine (umol/L)	88.8 ± 35.7	140.9 ± 108.3	0.003	62.7 ± 25.5	92.5 ± 89.0	0.001
Albumin (g/L)	39.6 ± 2.8	36.9 ± 4.5	0.002	40.1 ± 4.5	37.5 ± 11.0	0.039
Serum calcium (mmol/L)	2.82 ± 0.33	3.03 ± 0.48	0.011	2.81 ± 0.43	2.79 ± 0.45	0.783
Corrected calcium (mmol/L)	3.03 ± 0.55	3.35 ± 0.50	0.003	2.94 ± 0.49	3.17 ± 0.58	0.004
Serum phosphorus (mmol/L)	0.70 ± 0.19	0.76 ± 0.17	0.240	0.79 ± 0.25	0.81 ± 0.32	0.561
Serum magnesium (mmol/L)	0.89 ± 0.19	0.76 ± 0.17	0.001	0.90 ± 0.17	0.82 ± 0.17	0.001
PTH (pg/mL)	54.2 ± 60.0	114.7 ± 112.8	0.002	45.2 ± 60.4	72.8 ± 80.6	0.006
25(OH)D (ng/mL)	36.7 ± 20.4	32.5 ± 13.3	0.389	33.9 ± 19.2	32.8 ± 15.9	0.705
Hypomagnesemia *n* (%)	10(18.9)	23(47.0)	0.002	10(8.6)	34(38)	0.002

BMI, body mass index; eGFR, estimate glomerular filtration rate; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D. Hypomagnesemia was defined as serum magnesium <0.75 mmol/L.

and females. In contrast, average creatinine, PTH, and corrected calcium levels were significantly higher in the anemic group. Older patients and patients with comparatively lower BMI values were associated with anemia in females. Similarly, there was a trend in males, but the result was not statistically significant. Our data showed no statistically significant differences in serum phosphorus and 25(OH)D levels between the groups with and without anemia.

General regression analysis showed that serum magnesium was positively correlated with eGFR ($P < 0.05$; Figure 1(a)). eGFR and serum magnesium were positively correlated with hemoglobin ($P < 0.05$; Figures 1(b) and 1(d)). On the contrary, serum PTH was negatively correlated with hemoglobin ($P < 0.05$; Figure 1(c)). Consistently, this correlation remained significant after the adjustment of demographic data including age, sex, disease duration, and BMI (Table 3, Model 2). Serum albumin and calcium were correlated in both males and females (Table 2). After adjusting these indexes, the association between hemoglobin and PTH/magnesium remained significant (Table 3, Model 3). eGFR and PTH were correlated with hemoglobin; therefore, they were further selected as confounding factors for the multivariate regression analysis. High serum PTH and low serum magnesium were associated with low hemoglobin, independent of eGFR, PTH, or magnesium levels (Table 3, Models 4-5).

Our findings revealed that hypomagnesemia was associated with lower hemoglobin levels, independent of age, sex, disease duration, BMI, albumin, calcium, eGFR, and PTH in PHPT patients.

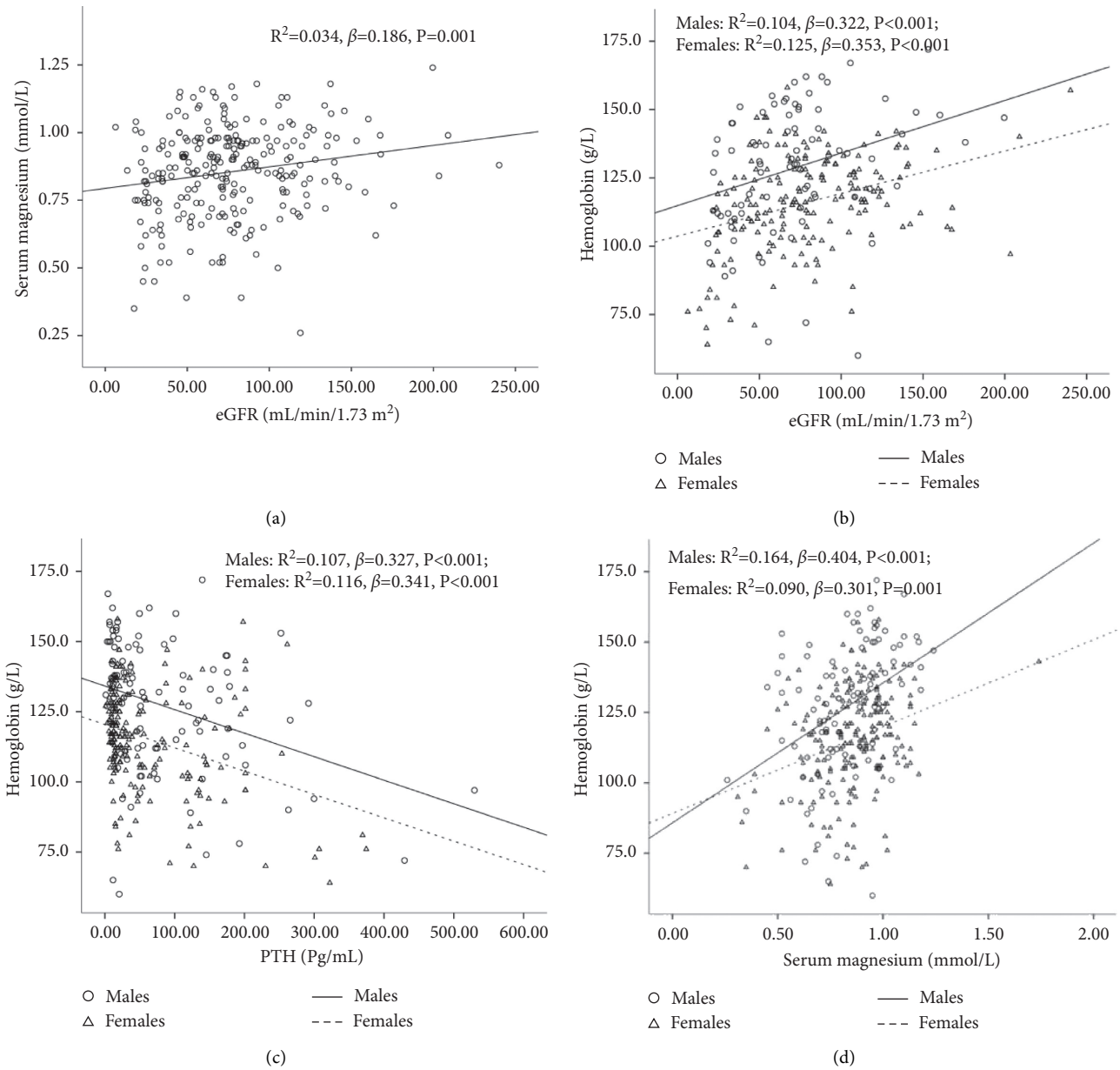

Figure 1: Relationship between serum hemoglobin/magnesium and various indicators in patients with PHPT.

Table 3: Association between magnesium/PTH and hemoglobin.

	Magnesium and hemoglobin ($n = 307$)			PTH and hemoglobin ($n = 307$)		
	β	R^2	P	β	R^2	P
Model 1	0.331	0.094	<0.001	−0.288	0.080	<0.001
Model 2	0.275	0.213	<0.001	−0.257	0.208	<0.001
Model 3	0.265	0.241	<0.001	−0.262	0.243	<0.001
Model 4	0.230	0.272	<0.001	−0.225	0.283	<0.001
Model 5	0.174	0.301	0.004	−0.176	0.301	0.003

Model 1: general regression analysis; Model 2: adjusted for age, sex, duration of disease, and BMI; Model 3: adjusted for age, sex, duration of disease, BMI, albumin, and calcium; Model 4: adjusted for age, sex, duration of disease, BMI, albumin, calcium, and eGFR; Model 5: adjusted for age, sex, duration of disease, BMI, albumin, calcium, eGFR, and PTH.

4. Discussion

The incidence of hypomagnesemia is approximately 2% in the general population [35]. In this study, hypomagnesemia had a high prevalence rate in PHPT patients (77/307; 25.1%). Magnesium is maintained within a normal range by a dynamic interplay among the intestines (absorption), bone (deposition), and kidneys (excretion), and disturbances in these organs may contribute to hypomagnesemia [36, 37]. Hypomagnesemia has been observed in PHPT patients, especially in association with high serum calcium levels or kidney damage [28–30]. High prevalence of hypomagnesemia in patients with CKD reveals that impairment in renal function may affect the absorption of magnesium and lead to hypomagnesemia [25, 38]. As seen in our study, serum magnesium was positively correlated with eGFR. Furthermore, hypercalcemia can result in hypomagnesemia by increased filtered calcium load to the loop of Henle, resulting in decreased reabsorption of magnesium [39]. The high prevalence of hypomagnesemia might be the result of our patient population having higher serum calcium levels, severe bone disease, and kidney stones. Our previous study showed that compared to PHPT patients from the USA, PHPT patients from Changsha have higher serum calcium, PTH, and alkaline phosphatase (ALP) levels; lower 25(OH) D levels and bone mineral density; and increased renal stone incidence, suggesting that they have more severe PHPT. It is unclear why there are differences in these parameters between PHPT patients from the USA and China. One possibility is that PHPT patients in the USA are diagnosed at the asymptomatic stage, because most individuals in the USA receive annual physical examination [40, 41]. In China, most PHPT patients are diagnosed when they were hospitalized due to the presence of kidney stones, skeletal lesions, or other symptoms related to hypercalcemia [42]. Other possible explanations include differences in ethnicity and nutritional status [43].

In this study, we assessed the prevalence of anemia and associations between biochemical indices of disease severity and anemia in PHPT patients. A total of 138 patients (49 males (48.0%) and 89 females (43.4%)) had anemia, accounting for 45.0% of the total population. Older patients and patients with lower BMI values were associated with anemia. This phenomenon was also observed in the elderly patient population [44]. The association between serum albumin and anemia may be due to a reduction in hepatic protein synthesis as a result of decreased food intake, malnutrition, advanced age, or sarcopenia [45].

There was a significant reduction in hemoglobin levels with decreasing eGFR values as previously reported [46]. The primary cause of anemia is a reduction in EPO synthesis due to loss of renal functional mass [47]. Impaired renal function leads to the accumulation of toxins. Imbalances in calcium/phosphate, acid/base, and electrolytes resulting from impaired renal function affect RBC shape and survival [48]. All these factors are responsible for low hemoglobin levels with poor kidney function.

Conversely, we obtained a significant negative association between PTH and hemoglobin, independent of age, sex, disease duration, BMI, albumin, eGFR, calcium, and magnesium. This finding is consistent with the fact that high PTH in secondary hyperparathyroidism results in anemia probably as a result of erythropoiesis inhibition, marrow fibrosis, and blood loss by reducing platelet aggregation [49].

In addition, we found a significant association between serum magnesium and hemoglobin levels, independent of age, sex, disease duration, BMI, albumin, calcium, eGFR, and PTH. A strong association between serum magnesium and hemoglobin levels has been reported in non-PHPT patients. The results of this study confirm such association in PHPT patients. The mechanism of the relationship between hemoglobin and magnesium is not clear. It is possible that magnesium deficiency causes hemolysis. After four to five weeks on a low-magnesium diet, magnesium levels in rats rapidly decreased and hemoglobin levels significantly decreased compared with the control group. Anemia was accompanied by severe reticulocytosis, reduction in hemoglobin and hematocrit levels, and reduced lifespan and half-life of RBCs. Hemolytic anemia in a state of magnesium deficiency may be caused by energy metabolism disorders in RBCs [21]. In a beta-thalassemia model, magnesium deficiency caused hemolytic anemia, and anemia improved upon magnesium supplementation [50]. In patients with sickle cell anemia, oral magnesium supplementation reduced dense erythrocyte, absolute reticulocyte, and immature reticulocyte counts and improved erythrocyte membrane transport abnormalities [51]. Furthermore, magnesium is the cofactor of several enzymes involved in protein and nucleic acid synthesis. Erythrocyte energy metabolism and hemoglobin synthesis may decrease in magnesium deficiency, thereby resulting in anemia [22]. Researchers have shown that high serum magnesium levels are correlated with an adequate EPO responsiveness in patients undergoing maintenance hemodialysis [24]. In addition, low magnesium levels cause inflammation and endothelial dysfunction [52], which are known risk factors for anemia.

There were some limitations in our study. First, ionized calcium was not assessed. Second, we were unable to obtain data on hemolysis and inflammation markers. Third, we did not include other clinical important risk factors for anemia. Fourth, these findings are related to populations with a severe PHPT. Thus, these relationships cannot be immediately translated to asymptomatic PHPT outpatients. Fifth, the lack of a control group is a weakness of the study. Indeed, we do not have information regarding whether or not this PHPT population had different Mg levels and prevalence of anemia as compared with non-PHPT-matched inpatients. Finally, the conclusions of this study are weakened by its retrospective design.

Despite these limitations, this study had several strengths. First, this study evaluated data from a large cohort of PHPT patients. Second, blood measurements were performed at the same hospital laboratory. Third, we assessed the prevalence of hypomagnesemia in patients with PHPT. Fourth, this is the first study that assessed whether there is any association between serum magnesium and hemoglobin levels in PHPT patients. Finally, we found that

hypomagnesemia, which is a frequent electrolyte disorder in PHPT patients, is associated with hemoglobin levels.

In conclusion, hypomagnesemia is a common electrolyte disorder in PHPT patients. Hypomagnesemia is associated with lower hemoglobin, independent of albumin, calcium, eGFR, and PTH in PHPT patients.

Data Availability

The data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Disclosure

The manuscript was presented in a conference, and a preprint has previously been published [53].

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Na Ding and Guo Tao contributed equally to this work. Ding Na and Guo Tao are directly responsible for patients' evaluations, data collection, and manuscript drafting; Liu Shu-Ying, Wang Qin-Yi, Qu Xiao-Li, and Li Yong-Fang helped in collecting patients' data; Ou Yang-Na is responsible for statistical analysis; Sheng Zhi-Feng and Yang Yan-Yi are responsible for the original idea. All authors approved the final manuscript and are responsible for taking final responsibility for the paper.

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Research Article

Sex-Specific Effects of Vitamin D Status on the Metabolic Profile in Prediabetic Subjects

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Introduction. We aim to investigate the effect of vitamin D on metabolic parameters in a population with prediabetes and to detect possible sex differences. *Methods*. In 621 patients with diagnosed prediabetes, glucose, lipid, and anthropometric parameters were measured. Furthermore, the interaction of 25-OH-vitamin D (25-hydroxyvitamin D) with metabolic and glucose metabolism parameters was analysed in the total prediabetic population, as well as after stratification by sex (female vs. male prediabetic subgroup), by logistic regression. *Results*. 25-OH-vitamin D was negatively related to cholesterol, BMI, fatty liver index, insulin, and HOMA-IR. Especially in the male prediabetic cohort, 25-OH-vitamin D levels negatively correlated with total cholesterol levels ($r = -0.17$, $p = 0.001$), with triglycerides ($r = -0.17$, $p = 0.001$), and with HbA1c levels ($r = -0.14$, $p = 0.010$). Only in the female cohort with prediabetes, we found a negative correlation of 25-OH-vitamin D levels with systolic ($r = -0.18$, $p = 0.005$) and diastolic blood pressures ($r = -0.23$, $p < 0.001$). *Conclusion*. In this study, in females with prediabetes, 25-OH-vitamin D was notably related to a more favourable metabolic profile, including lower total cholesterol and higher HDL cholesterol levels. On the contrary, in men with prediabetes, there was a stronger association between 25-OH-vitamin D and cholesterol-HDL quotient, as well as fatty liver index was observed in the male prediabetic subgroup. Therefore, sex differences should be considered in future studies on vitamin D and glucose tolerance status.

1. Introduction

Vitamin D is an important micronutrient for human health. Still, around 13% of the European population suffer from vitamin D deficiency [1]. Nowadays, various studies investigated the necessity of vitamin D for numerous non-skeletal effects, including the insulin secretion in the pancreas [2]. The vitamin D receptor (VDR) is present in the pancreatic β-cells and in tissue influenced by insulin as the skeletal muscle, myocardium, and adipose tissue [3, 4]. Altered insulin secretion and sensitivity may be associated with polymorphisms of VDR [5]. Vitamin D might also have a protective role against pancreatic β-cell inflammatory damage and death by immunomodulatory properties [6]. There is also evidence that low levels of serum 25-hydroxyvitamin D (25-OH-vitamin D), an accepted indicator of vitamin D status, are associated with impaired glucose tolerance and diabetes mellitus [7]. Furthermore, studies claim that vitamin D status is associated with diabetic complications including nephropathy, retinopathy, and neuropathy [8].

In 2017, the National Diabetes Statistics Report from the US Centers for Disease Control and Prevention estimates that approximately 34% of the US adults suffer from prediabetes [9]. Prediabetes is the prestage state for developing diabetes mellitus [10]. A Chinese study described that

participants with low levels of 25-OH-vitamin D have a higher risk of developing diabetes or prediabetes [11]. Furthermore, Gao et al. evidenced low levels of 25-OH-vitamin D already 4 years prior to the diagnosis of prediabetes or diabetes mellitus [11]. Also, a Swedish study observed that low levels of 25-OH-vitamin D are associated with higher incident diabetes mellitus in men and women with prediabetes [12]. However, after adjusting for all possible confounders, this effect was only significant in men, possibly based on differences in BMI [12]. Furthermore, a Chinese study reported that vitamin D has a negative impact on insulin resistance, but this could only be shown in male patients with newly diagnosed type 2 diabetes mellitus [13]. In the female subgroup and in the general population, no significant effect could be shown [13]. A prior study analysed that daily vitamin D supplementation in participants with prediabetes could reduce the risk for developing overt diabetes [14].

Nevertheless, the current knowledge on the impact of vitamin D on prediabetes is scarce, especially concerning the association with metabolic profile and possible sex-specific differences. Hence, the aim of the present study was to investigate the effect of vitamin D levels on the outcome of prediabetes and to explore sex-specific effects.

2. Subject, Materials, and Methods

2.1. Study Design. The present cross-sectional study is a retrospective data analysis of first visits to the prediabetes clinic at "Sanatorium Hera" in Vienna from October 2015 to September 2017. The patients underwent a standard medical checkup with physical examination, electrocardiogram, extensive blood analysis, and assessment of vital parameters after giving informed consent. Only patients with prediabetes were included ($n = 621$). In order to classify prediabetes, a glucose tolerance test (oGTT) was carried out, or fasting blood glucose levels and HbA1c values were used for the diagnosis. Prediabetes was defined according to the guidelines of the American Diabetes Association [15] if HbA1c was ≥5.7% and <6.5% and/or if they had fasting blood glucose levels ≥100 mg/dl and <126 mg/dl and/or ≥140 mg/dl and <200 mg/dl in a 2-hour oral glucose tolerance test.

2.2. Calculations. The homeostasis model assessment insulin resistance score (HOMA-IR) was calculated by using basal insulin and glucose levels (HOMA-IR = Ins x Gluc/405). The fatty liver index (FLI) was calculated by body mass index (BMI), waist circumference, γ-GT, and triglycerides ($\text{FLI} = (e^{0.953*\log e\,(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\,(\text{ggt}) + 0.053*\text{waist circumference} - 15.745})/(1 + e^{0.953*\log e\,(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\,(\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) * 100$) [16].

2.3. Statistical Analysis. At first, the data dictionary was scanned for variables suitable for analysis. The variables were defined as metabolic parameters and parameters relevant for glucose metabolism. In general, in the prediabetes cohort, a total of 166 participants had a low 25-OH-vitamin D level defined as < 20 ng/ml, and 40 participants had a vitamin D deficiency defined as <12 ng/ml. Next, the population was divided by 25-OH-vitamin D status. Two cohorts were defined. First, "the lower 25-OH-vitamin D cohort" ($n = 308$) is defined as 25-OH-vitamin D < 26.5 ng/ml. Second, "the higher 25-OH-vitamin D cohort" ($n = 309$) is defined as 25-OH-vitamin D ≥ 26.5 ng/ml. The cohorts were split according to the median of 25-OH-vitamin D status (median = 26.5 ng/ml). The range of 25-OH-vitamin D was 6.7. Frequency, mean, and standard deviation were calculated for every parameter. Furthermore, interaction terms between 25-OH-vitamin D and all parameters were explored to determine if the impacts of the variables differed for lower and higher 25-OH-vitamin D groups by a linear and logistic regression model. Then, sex-stratified analysis was performed. The male cohort consists of 375 individuals, and the female cohort consists of 246 individuals. Mean and standard deviation were calculated for every parameter. As the next step, the interaction of 25-OH-vitamin D and sex with all parameters was analysed by linear regression. Lastly, the interaction of only 25-OH-vitamin D with all parameters was explored. For all analyses, statistical significance was defined with a p value <0.05. Analyses were carried out by SPSS Statistics version 26.

3. Results

In total, 621 individuals were included in the present study. In Table 1, 25-OH-vitamin D status and metabolic parameters are presented. The general population was grouped into a lower and a higher 25-OH-vitamin D cohort. In comparison to the lower 25-OH-vitamin D cohort, lower means of total cholesterol (205.67 vs. 214.03 mg/dl), LDL cholesterol (122.45 vs. 132.25 mg/dl), triglyceride levels (105.3 vs. 124.35 mg/dl), cholesterol-HDL quotient (3.46 vs. 4.03 mg/dl), higher systolic and diastolic blood pressures (138.96 mmHg vs. 135.91 mmHg and 85.24 mmHg vs. 82.72 mmHg), and BMI (27.44 kg/m^2 vs. 29.15 kg/m^2) were seen in the higher 25-OH-vitamin D subgroup. Furthermore, the higher 25-OH-vitamin D cohort featured higher means of HDL cholesterol (62.68 vs. 56.65 mg/dl).

Table 2 shows sex-specific data of the prediabetic population. In the female compared to the male cohort, a higher mean concentration of 25-OH-vitamin D (29 vs. 26.96 ng/ml), total cholesterol (216.56 vs. 206.19 mg/dl), LDL cholesterol (129.56 vs. 126.95 mg/dl), and HDL cholesterol (64.81 vs. 55.90 mg/dl) could be observed. Nevertheless, the female cohort had lower mean abdominal circumference (99.85 vs. 102 cm), fatty liver index (50.65 vs. 59.03), and glucose levels (105.25 vs. 107.44 mg/dl). In addition, the male and female cohorts were analysed in respect of 25-OH-vitamin D levels. 25-OH-vitamin D had a negative effect on cholesterol ($r = -0{,}18$, $p < 0.001$), especially in women. 25-OH-vitamin D had a negative effect on cholesterol-HDL quotient ($r = -0.3$, $p < 0.001$) and fatty liver index ($r = -0.27$, $p < 0.001$), particularly in men.

Table 3 reports the correlation of 25-OH-vitamin D with the metabolic parameters by logistic regression models. In

Table 1: Frequency of sex and mean and standard deviation of all parameters in a cohort with lower 25-OH-vitamin D levels and higher 25-OH-vitamin D levels.

Variable	Lower vitamin D ($n = 308$)	Higher vitamin D ($n = 313$)	p value
Age	56.02 (±11.24)	60.87 (±9.55)	<0.001
BMI (kg/m^2)	29.16 (±4.8)	27.53 (±4.27)	<0.001
Waist circumference (cm)	102.77 (±11.60)	99.60 (±11.71)	<0.001
Sex			
Male	192 (62.3%)	183 (58.5%)	0.033
Female	116	130	
25-Hydroxy-vitamin D (ng/ml)	18.54 (±5.17)	36.84 (±8.67)	—
Cholesterol (mg/dl)	214.24 (±38.98)	206.37 (±38.83)	0.009
HDL cholesterol (mg/dl)	56.68 (±15.85)	62.11 (±15.91)	<0.001
LDL cholesterol (mg/dl)	132.25 (±35.22)	123.78 (±34.55)	<0.001
Triglycerides (mg/dl)	125.20 (±75.02)	104.63 (±46.08)	<0.001
Cholesterol-HDL quotient	4.03 (±1.32)	3.50 (±0.98)	<0.001
HbA1c (%)	5.57 (±0.36)	5.54 (±0.33)	0.062
Glucose (mg/dl)	106.40 (±9.46)	106.76 (±8.10)	0.539
Glucose tolerance test (fasted)	108.09 (±9.04)	108.00 (±7.92)	0.339
Glucose tolerance test (2 h)	118.28 (±31.46)	120.80 (±31.97)	0.880
Insulin (uU/ml)	14.06 (±8.99)	11.46 (±6.13)	<0.001
Fatty liver index	59.79 (±26.43)	50.58 (±26.61)	<0.001
HOMA-IR	3.85 (±2.48)	3.12 (±1.81)	0.001
Systolic blood pressure (mmHg)	138.96 (±15.89)	135.91 (±16.75)	0.007
Diastolic blood pressure (mmHg)	85.24 (±10.71)	82.72 (±9.94)	<0.001

Lower 25-OH-vitamin D cohort is defined by a 25-OH-vitamin D level <26.5 ng/ml. Higher 25-OH-vitamin D cohort is defined by a 25-OH-vitamin D level ≥ 26.5 ng/ml. HDL: high-density lipoprotein; LDL: low-density lipoprotein; BMI: body mass index; HOMA-IR: homeostasis model assessment.

Table 2: Mean and standard deviation of all parameters in the male and the female cohort.

Variable	Male ($n = 375$)	Female ($n = 246$)	p value
Age	58.17 (±11.12)	58.92 (±10.01)	0.393
BMI (kg/m^2)	28.07 (±3.9)	28.74 (±5.54)	0.078
Waist circumference (cm)	102 (±10.59)	99.85 (±13.28)	0.028
25-Hydroxy-vitamin D (ng/ml)	26.96 (±10.51)	29 (±13.05)	0.033
Cholesterol (mg/dl)	206.19 (±39.69)	216.56 (±37.3)	0.001
HDL cholesterol (mg/dl)	55.90 (±15.05)	64.81 (±16.18)	<0.001
LDL cholesterol (mg/dl)	126.95 (±35.57)	129.56 (±34.41)	0.368
Triglycerides (mg/dl)	117.91 (±72.28)	110.16 (±44.8)	0.135
Cholesterol-HDL quotient	3.93 (±1.3)	3.52 (±0.96)	<0.001
HbA1c (%)	5.5 (±0.34)	5.64 (±0.33)	<0.001
Glucose (mg/dl)	107.44 (±8.35)	105.25 (±9.35)	0.003
Oral glucose tolerance test (oGTT fasted)	108.53 (±8.2)	107.32 (±9.05)	0.138
Oral glucose tolerance test (oGTT 2 h)	118.36 (±31.05)	120.92 (±32.62)	0.399
Insulin (uU/ml)	12.75 (±8.46)	13.19 (±7.17)	0.571
Fatty liver index	59.03 (±26.25)	50.65 (±27.11)	0.001
HOMA-IR	3.57 (±2.33)	3.45 (±2.08)	0.625
Systolic blood pressure (mmHg)	137.81 (±15.48)	136.80 (±17.74)	0.464
Diastolic blood pressure (mmHg)	84.36 (±10.27)	83.35 (±10.58)	0.246

HDL: high-density lipoprotein; LDL: low-density lipoprotein; BMI: body mass index; HOMA-IR: homeostasis model assessment.

the general cohort, a negative correlation was evidenced between the 25-OH-vitamin D level and triglycerides ($r = -0.14$, $p < 0.001$), HDL cholesterol ($r = 0.23$, $p < 0.001$), LDL cholesterol ($r = -0.17$, $p < 0.001$), cholesterol-HDL quotient ($r = -0.26$, $p < 0.001$), total cholesterol ($r = -0.11$, $p = 0.009$), BMI ($r = -0.18$, $p < 0.001$), abdominal circumference ($r = -0.18$, $p < 0.001$), fatty liver index ($r = -0.19$, $p < 0.001$), insulin levels ($r = -0.19$, $p < 0.001$), and HOMA-IR ($r = -0.19$, $p = 0.001$).

In the sex-segregated analysis, 25-OH-vitamin D was also negatively related to triglycerides ($r = -0.17$, $p = 0.001$, Figures 1(a) and 1(b)), HDL cholesterol ($r = 0.16$, $p = 0.002$), LDL cholesterol ($r = -0.18$, $p < 0.001$), cholesterol-HDL quotient ($r = -0.24$, $p < 0.001$), total cholesterol ($r = -0.17$, $p = 0.001$, Figures 1(a) and 1(b)), BMI ($r = -0.17$, $p = 0.001$), abdominal circumference ($r = -0.16$, $p = 0.002$), fatty liver index ($r = -0.16$, $p = 0.007$), insulin ($r = -0.19$, $p = 0.001$), and HOMA-IR ($r = -0.18$, $p = 0.012$) in the male cohort alone. Additionally, in the male cohort, a negative correlation of 25-OH-vitamin D with glucose ($r = 0.99$, $p = 0.001$) and HbA1c levels ($r = -0.14$, $p = 0.010$) was found. In the female cohort only, a negative correlation of 25-OH-vitamin

Table 3: Correlation of 25-OH-vitamin D on the mentioned variables and all r and p values of the linear regression on 25-OH-vitamin D and sex on all mentioned parameters.

Correlation of 25-OH-vitamin D	General cohort		Male cohort		Female cohort	
Variable	r	p value	r	p value	r	p value
BMI (kg/m^2)	−0.20	<0.001	−0.17	0.001	−0.24	<0.001
Waist circumference (cm)	−0.18	<0.001	−0.16	0.002	−0.19	0.003
Cholesterol (mg/dl)	−0.11	0.009	−0.17	0.001	−0.05	0.48
HDL cholesterol (mg/dl)	0.23	<0.001	0.16	0.002	0.27	<0.001
LDL cholesterol (mg/dl)	−0.17	<0.001	−0.18	<0.001	−0.15	0.017
Triglycerides (mg/dl)	−0.14	<0.001	−0.17	0.001	−0.09	0.18
Cholesterol-HDL quotient	−0.26	<0.001	−0.24	<0.001	−0.28	<0.001
HbA1c (%)	−0.76	0.062	−0.14	0.010	−0.05	0.49
Glucose (mg/dl)	0.25	0.539	0.01	0.99	0.07	0.27
Insulin (uU/ml)	−0.18	<0.001	−0.19	0.001	−0.17	0.026
Fatty liver index	−0.19	<0.001	−0.16	0.007	−0.21	0.005
HOMA-IR	−0.19	0.001	−0.18	0.012	−0.20	0.022
Systolic blood pressure (mmHg)	−0.11	0.007	−0.04	0.450	−0.18	0.005
Diastolic blood pressure (mmHg)	−0.16	<0.001	−0.09	0.078	−0.23	<0.001

HDL: high-density lipoprotein; LDL: low-density lipoprotein.

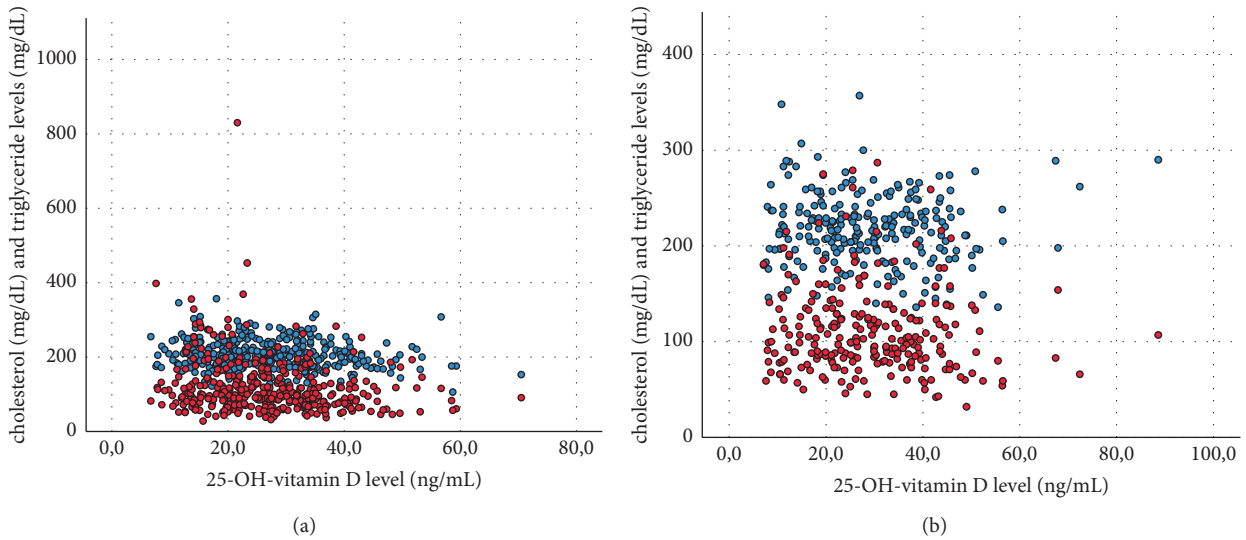

Figure 1: Scatter plots of vitamin D with cholesterol (blue) and triglyceride (red) levels in the male (a) and female (b) cohort.

D with HDL cholesterol ($r = 0.27$, $p < 0.001$), LDL cholesterol ($r = -0.15$, $p = 0.017$), cholesterol-HDL quotient ($r = -0.28$, $p < 0.001$), BMI ($r = -0.24$, $p < 0.001$), abdominal circumference ($r = -0.19$, $p = 0.003$), fatty liver index ($r = -0.21$, $p = 0.005$), insulin levels ($r = -0.17$, $p = 0.026$), HOMA-IR ($r = -0.20$, $p = 0.022$), systolic blood pressure ($r = -0.18$, $p = 0.005$, Figures 2(a) and 2(b)), and diastolic blood pressure ($r = -0.23$, $p < 0.001$, Figures 2(a) and 2(b)) was evaluated.

4. Discussion

To summarize, our study confirmed that higher 25-OH-vitamin D levels are related to a more favourable metabolic profile in patients with prediabetes. 25-OH-vitamin D was negatively related to total cholesterol, LDL cholesterol, triglyceride levels, cholesterol-HDL quotient, BMI, abdominal circumference, fatty liver index, blood pressure values, insulin levels, and HOMA-IR. Furthermore, we observed sex differences regarding the relationship of 25-OH-vitamin D with total cholesterol, triglycerides, HbA1c levels, and systolic and diastolic blood pressures.

Bearing in mind that our study population consists of subjects with prediabetes, the effect of 25-OH-vitamin D on glucose metabolism is of importance, expanding the knowledge on possible vitamin D effects to a group at a high risk of progression to diabetes. More specifically, we could show that higher levels of 25-OH-vitamin D were related to a lower HOMA-IR and lower levels of insulin and HbA1c in the whole study population. Further analysing sex-specific differences, we could observe higher HbA1c values in the female population and a negative relationship of 25-OH-vitamin D with HbA1c values in the female prediabetes cohort. Previous studies support our findings [17–19]. These studies investigated the general population or patients with overt diabetes mellitus. The aforementioned studies claimed

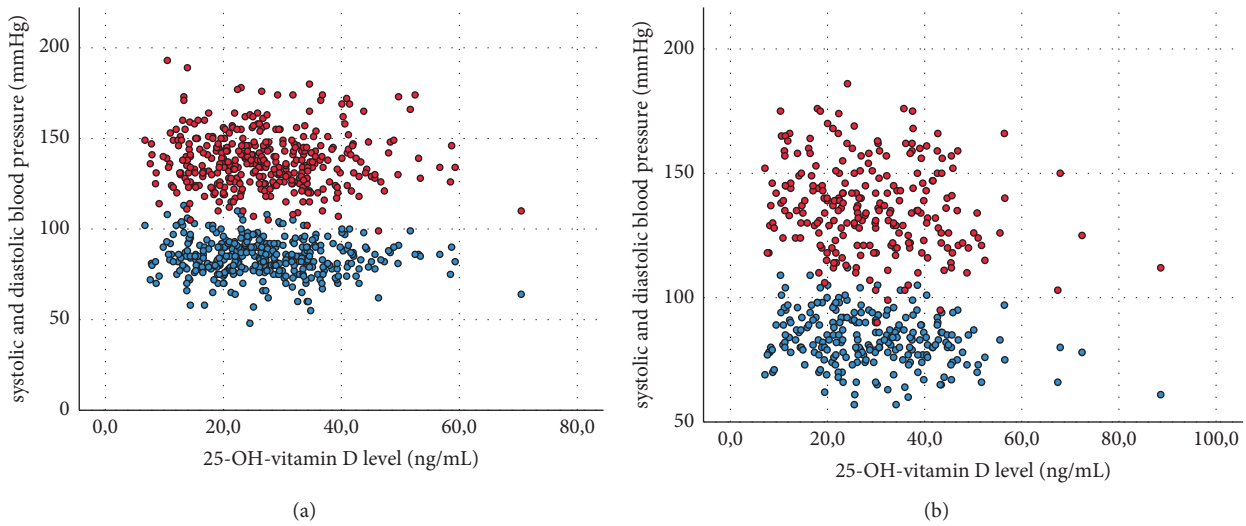

Figure 2: Scatter plots of vitamin D with systolic (red) and diastolic (blue) blood pressures in the male (a) and female (b) cohort.

that vitamin D may decrease insulin resistance, fasting glucose, insulin levels, and HOMA-IR [17–19]. However, in the general population, no effect of vitamin D on HbA1c levels could be found [19]. Vitamin D's effect on insulin, HbA1c, and HOMA-IR can be explained by the ability of 25-OH-vitamin D to bind on pancreatic β-cells and therefore alter their function in a positive way [2]. A prior study showed that daily vitamin D supplementation in participants with prediabetes could reduce their risk of developing overt diabetes [14]. A new finding of our study is that we studied sex differences in the association of 25-OH-vitamin D status and metabolic parameters in a cohort with prediabetes.

Various studies analysed the effect of vitamin D on metabolic parameters. Vitamin D in combination with probiotics increased HDL cholesterol levels in patients with diabetes mellitus [17]. Other studies reported that vitamin D alone may increase HDL cholesterol levels in hypertensive patients [20]. In our study, we found similar results in a population with prediabetes, especially in women. Concerning total cholesterol and LDL cholesterol, previous studies had divergent results. An earlier study reported higher levels of total cholesterol and LDL cholesterol related to higher vitamin D levels in a population diagnosed with arterial hypertension [20]. On the contrary, lower levels of total cholesterol and LDL cholesterol were described following vitamin D supplementation in an older Lebanese population [19]. In our study, 25-OH-vitamin D was negatively related to total cholesterol and LDL cholesterol levels in a population with prediabetes. Particularly in the female population, we could see a decrease in total cholesterol with higher 25-OH-vitamin D. Vitamin D might be able to alter the gene expression of apolipoproteins [21] and thus affect cholesterol levels. Previous studies investigated that higher cholesterol levels are associated with higher fat mass. Notably in women, we confirmed such an association potentially because women generally have a higher percentage of subcutaneous fat [22]. Further previous studies investigated a positive correlation of fat mass on LDL and HDL cholesterol levels [23, 24]. Moreover, former studies provided knowledge that vitamin D can inversely affect obesity and BMI [18]. In our study, lower BMI values were also related to higher 25-OH-vitamin D levels. The reason why vitamin D is lower in individuals with higher BMI levels could be that it is stored in the excess fat mass of obese subjects [25]. Another hypothesis is that individuals with higher BMI values might have a higher distribution volume and therefore require greater dietary intake of vitamin D as compared to lean individuals [26]. Previously, it could be observed that vitamin D intake lowers the risk for nonalcoholic fatty liver disease [27]. In addition, men have a higher risk of fatty liver disease as oestrogen might exert protective effects [28]. Therefore, it is not surprising that we observed a negative association of 25-OH-vitamin D on the fatty liver index, particularly in men.

In the present study, we have to report some limitations. First, we lack a control group without prediabetes. Secondly, we only measure vitamin D levels at one time point in a cross-sectional design. Thirdly, the database did not ask for menopausal status. Strength of the present study is the rather large number of subjects with documented prediabetes and that, to the best of our knowledge, it is the first study investigating the association of vitamin D with a variety of metabolic parameters and glucose metabolism in this specific population, including analysis of potential sex differences. Various studies investigated vitamin D levels and their impact on patients with diabetes mellitus. A previous study could show that vitamin D supplementation in a population with prediabetes does not lead to a lower risk of diabetes mellitus [14]. Still, the findings of our study could lead to greater awareness of sex differences in the association of 25-OH-vitamin D levels with metabolic parameters in a cohort of patients with prediabetes. The results may emphasize the importance of vitamin D measurements and possibly supplementation in case of deficiency in prediabetic patients. In the current COVID-19 pandemic, patients with impaired glucose metabolism are even a more important

target group of research as patients with diabetes mellitus not only have a higher incidence of COVID-19 but also a higher mortality rate, especially males [29]. Furthermore, studies suggested that vitamin D supplementation might be able to reduce risk for COVID-19 and progression to severe disease and mortality [30]. Therefore, the present study may contribute to further understand the impact of vitamin D on metabolism in this vulnerable population. Additionally, our sex-specific results of vitamin D's association with metabolism could help to improve gender-sensitive care for the population with prediabetes.

Data Availability

The data used to support the findings of this study may be released upon application to the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

EWK, SN, RW, and GE contributed to data curation. TG, ML, and AKW conceptualized, investigated, supervised, validated, and visualized the study, contributed to funding acquisition, provided the methodology, resources, and software, and administered the project. TG performed formal analysis and wrote the initial draft of the manuscript. All other authors contributed substantially to the discussion and reviewing/editing. All authors approved the final manuscript.

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