Pathophysiological Implication of Vitamin D in Diabetic Kidney Disease

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Keywords

Diabetes mellitus · Vitamin D · Diabetic kidney disease · Sodium-glucose linked co-transporter 2 inhibitors

Abstract

Background: Vitamin D is a hormone regulating not only calcium and phosphate homeostasis but also, at the same time, exerting many other extraskeletal functions via genomic effects (gene transcription) and probably by non-genomic effects as well. Availability is ensured by dietary intake of its precursors and by de novo production via sunlight. Yet, vitamin D deficiency and insufficiency are very common across the globe and are connected to many pathophysiological states, for example, diabetes mellitus, allergies, autoimmune diseases, pregnancy complications, and recently have also been associated with worse COVID-19 clinical outcomes.

Summary: In this review, we summarize current knowledge about vitamin D metabolism in general, its role in diabetes mellitus (mainly type 2) and diabetic complications (mainly diabetic kidney disease), and potential therapeutic perspectives including vitamin D signalling as a druggable target.

Key Messages: Vitamin D is not only a vitamin but also a hormone involved in many physiological processes. Its insufficiency or deficiency can lead to many pathological states.

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Introduction

Despite the significant effort in the last century to eradicate or minimize vitamin D deficiency among the population, especially children, there is still a high prevalence for vitamin D insufficiency/deficiency worldwide [1, 2]. Vitamin D deficiency and insufficiency are also more common across type 2 diabetes mellitus (T2DM) patients. Furthermore, some authors suggest that vitamin D deficiency may be a prominent element of CKD. Vitamin D has to be metabolically activated in the kidney, and patients with CKD including diabetic kidney disease (DKD) are not able to produce enough of the active form of vitamin D ($1,25(\text{OH})_2\text{D}$). Vice versa, the kidneys are assumed to be a classical $1,25(\text{OH})_2\text{D}$ target. Hence, the vitamin D receptor (VDR) is highly expressed in kidney tissue [3–5]. Impairment of the capability of the kidney to produce and reuptake enough calcitriol and therefore maintain vitamin D, phosphate, and calcium homeostasis is one of the principal pathophysiological components of metabolic bone disease in CKD. Also, urinary loss of both vitamin D and vitamin D binding protein (VDBP) has been reported in these individuals. With vitamin D concentration decreasing over time, clinical outcomes are worsening. DKD, one of the most common forms of CKD, accounts for almost 50% of end-stage kidney disease in developed countries requiring renal replacement

Table 1. Summary of CYP enzymes involved in the metabolism of vitamin D

CYP	Localization	Also known as	Primary substrate	Secondary substrate	Product
CYP2R1	Microsome membrane	Vit. D 25-hydroxylase	Cholecalciferol ergocalciferol		25(OH)D
CYP27A1	Mitochondrial membrane	Sterol 27-hydroxylase	Cholecalciferol	Ergocalciferol	25(OH)D
CYP24A1	Mitochondrial membrane	Vit. D 24-hydroxylase	25(OH)D 1,25(OH) ₂ D		24,25(OH) ₂ D 1,24,25(OH) ₃ D
CYP27B1	Mitochondrial membrane	1 α -Hydroxylase	25(OH)D		1,25(OH) ₂ D

therapy. DKD – by itself and mostly during haemodialysis – also highly increases the risk of cardiovascular diseases such as heart attack, stroke, and others [1, 6].

Vitamin D is a lipid-soluble hormone and micronutrient whose primary function is to regulate calcium and phosphorus homeostasis. Vitamin D deficiency has been linked to rickets over 100 years ago. However, it has much broader effects mediated by genomic (vitamin D directly or indirectly influences expression of up to 2,000 genes) as well as non-genomic mechanisms [1]. Vitamin D deficiency also contributes to many extraskeletal outcomes, including higher risk of type 1 or type 2 diabetes mellitus, allergy, autoimmunity, pregnancy complications, and many other pathologies. A meta-analysis from 2017 connected low levels of vitamin D with a higher risk of respiratory tract infections [7]. A recent retrospective multicentre study statistically significantly associated lower levels of 25(OH)D with mild rather than severe COVID-19 outcomes [8]. The aims of this review are (i) to summarize recent advances in the understanding of vitamin D metabolism, (ii) to highlight its importance for human health, and (iii) to describe its role in the development of T2DM and DKD.

Vitamin D Metabolism

General term vitamin D refers to the group of lipid-soluble secosterols, also known as calciferols. Most vitamin D is produced in the skin (approximately 80%) and remaining 20% is from the dietary precursors. In the upper layers of the skin, the vitamin D precursor 7-dehydrocholesterol is converted to previtamin D₃ by UVB irradiation. Previtamin D₃ then undergoes a thermal-induced rearrangement to form cholecalciferol (vitamin D₃) in lower layers of the skin. This thermal process seems to be a rate-limiting step in vitamin D₃ synthesis [9]. Vitamin D is also acquired from the diet in the form of cholecalciferol and ergocalciferol (vita-

min D₂). Vitamin D₃, with half-life approximately 12 h [10], binds to VDBP which enables its transport in the blood. In the liver, carbon 25 is hydroxylated to form 25-hydroxyvitamin D₃ (25(OH)D₃). Several enzymes have 25-hydroxylase activity; however, most important are CYP2R1 and CYP27A1 [11, 12]. CYP2R1 can hydroxylate both vitamin D₂ and D₃ equally, while CYP27A1 hydroxylates vitamin D₃ preferably [13]. 25-Hydroxylation is a highly efficient process; about 75% of vitamin D is hydroxylated during the first pass through the liver [14]. The rate of 25-hydroxylation seems to be less regulated compared to C-1 α hydroxylation and instead depends predominantly on D₂ or D₃ availability [15]. Increasing evidence suggests that CYP2R1 is a major C25 hydroxylation enzyme [15, 16]. Mutation L99P in the *CYP2R1* gene eliminates its C25 hydroxylation activity, and individuals carrying this mutation suffer from vitamin D deficiency. Table 1 summarizes all mentioned CYPs in this review with the respective cell localization, substrate, and final products.

25(OH)D is the main form of vitamin D circulating in the blood with half-life approximately 20 days [10] commonly used as an indicator of vitamin D status. However, it should be noted that 1,25(OH)₂D has approximately 1,000 $\times$ lower plasma concentration than 25(OH)D, and its concentration may be sufficient or elevated even if 25(OH)D is decreased. Nonetheless, an active form of vitamin D is 1,25(OH)₂D, also known as calcitriol, principally formed by CYP27B1 in mitochondria of proximal tubule cells (PTC) in the kidney [17]. Furthermore, 1 α hydroxylation occurs also in other cell types, including parathyroid gland, cells of the immune system, or various epithelial cells [11, 12]. This metabolic activation will be discussed further.

25-Hydroxyvitamin D-24-hydroxylase (CYP24A1) is an enzyme responsible for 25(OH)D and 1,25(OH)₂D catabolism. Both forms can be degraded in two pathways. Mainly via C24 hydroxylation to form 24,25(OH)₂D and 1,24,25(OH)₃D which is then degraded to calcitroic acid.

Another possibility is C23 hydroxylation which produces (1,23,25(OH)₃D and 1,25(OH)₂D-26S,23-lactone as a final product [18]. Both metabolites are preferably excreted through bile into faeces, and only a small portion appears in the urine as 1,25(OH)₂D-26S,23-lactone forms a complex with VDBP [19] which is taken up by the PTC via the megalin/cubilin transporting system [20]. Human CYP24A1 is capable of hydroxylating both C23 and C24 with predominance to the C24 hydroxylation pathway [18]. Prosser et al. [21] showed that A326G mutation in the CYP24A1 gene causes shift of this ratio to the favour of the C23 hydroxylation pathway. Calcitriol acid is rapidly excreted into bile with no known biological activity [22]. The exact reason why two degradation pathways exist is not known. Some authors suggest that the C23 pathway exists as a backup [9] or safety mechanism to rapidly degrade abundant calcitriol [21]. The importance of precise maintenance of calcitriol concentration was demonstrated in a study with CYP24A1 knockout mice and CYP24A1 and VDR double knockout mice [23, 24]. CYP24A1 deletion resulted in hypercalcaemia, impaired bone mineralization, and 50% decreased viability in experimental animals. On the contrary, CYP24A1 and VDR knockout mice did not show these characteristics. These findings suggest that increased 1,25(OH)₂D level, not the absence of C23 or C24 degradation pathways, results in an abnormal phenotype.

CYPs can be divided into two main groups, based on localization within the cell: microsomal and mitochondrial (Table 1). Vitamin D metabolism includes both types. Neither microsomal nor mitochondrial CYPs work alone, but the electron transport chain is connected to them and is essential for the whole process. Mitochondrial vitamin D-related CYPs (e.g., CYP27A1, CYP27B1, and CYP24A1) require two additional electron-transporting proteins: ferredoxin reductase and ferredoxin. On the other hand, microsomal CYPs (e.g., CYP2R1) require only one additional protein, NADPH-cytochrome P450 reductase. All of the vitamin D-related CYPs include heme-bound structure involved in single or multiple hydroxylation process [25, 26].

Vitamin D Metabolism in the Kidney

Vitamin D present in the glomerular filtrate (bound to VDBP) is taken up by the kidney proximal tubular epithelial cells via the megalin/cubilin endocytic system (see Fig. 1) [27]. Degradation of VDBP in lysosome releases free 25(OH)D that is subsequently converted to

Fig. 1. Vitamin D and glucose-related transporting mechanisms in the PTC. Vitamin D binds to VDBP in the tubular lumen, and the complex is transported by the megalin/cubilin system into the PTC. Inside the cell, vitamin D is released and metabolized (activated or degraded) in mitochondria while the megalin/cubilin complex is degraded in lysosomes. Vitamin D diffuses into interstitial space where it binds to VDBP. Glucose is transported to the PTC via SGLT2. Inhibition of SGLT2 by SGLT2i increases the amount of Na⁺ in the tubular lumen, which can increase resorption of phosphate and further alters the PTH-vitamin D-FGF23 axis. PTC, proximal tubule cell; VDBP, vitamin D binding protein; SGLT2, sodium-glucose co-transporter 2; NaPi-IIa/c, sodium-phosphate co-transporter IIa/c; GLUT2, glucose transporter 2.

1,25(OH)₂D by CYP27B1 located in the inner mitochondrial membrane. 1,25(OH)₂D is then released to the circulation by passive diffusion across the cytoplasmic membrane. Most of 1,25(OH)₂D in plasma is bound to VDBP and albumin [28, 29]. Human CYP27B1 is a 507-amino acid protein (55 kDa) encoded by the gene localized on the 12q14.1 chromosome with a length of 4.8 kb [14, 26].

As previously mentioned, proximal tubular epithelial cells are major players in 1α hydroxylation catalyzed by mitochondrial enzyme CYP27B1 [17]. Kidneys are able to ensure sufficient production of 1,25(OH)₂D. However, a number of other cell types also expresses CYP27B1 (e.g., the brain, placenta, colon, pancreas, prostate, intestine, lung, macrophages, lymphocytes, parathyroid gland, osteoblasts, and chondrocytes, whose physiological significance is not clear in many cases) [30, 31].

Fig. 2. Physiological regulation of calcium and phosphate metabolism. 1,25(OH)₂D activated by CYP27B1 increases resorption of bones, therefore increasing sCa²⁺ and sP_i concentration. It also increases calcium and phosphate reabsorption in the kidneys and absorption from the gut. PTH can increase bone and kidney mineral resorption as well as CYP27B1 levels and activity, establishing a feedback loop. All these mechanisms increase sCa²⁺ and sP_i. Excess of sCa²⁺ and 1,25(OH)₂D inhibits PTH production. On the

contrary, increased serum phosphate increases the production of FGF23, which inhibits resorption of phosphate from the kidneys and CYP27B1 activity. The excess of 1,25(OH)₂D activates CYP24A1 and inhibits CYP27B1, establishing a negative feedback loop. sCa²⁺, serum calcium; sP_i, serum phosphate; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; PTC, proximal tubule cell.

Regulation of CYP27B1 Activity

As 1,25(OH)₂D half-life is approximately 14 h [32], therefore, renal CYP27B1 activity must be tightly regulated. The main regulators include Ca²⁺, PO₄³⁻, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23). A decrease in Ca²⁺ stimulates secretion of PTH, stimulating production and activity of CYP27B1 [33]. During normocalcaemia, circulating levels of PTH are insufficient to induce CYP27B1 expression. Hence, calcitonin regulation takes place [34]. Hyperphosphataemia stimulates FGF23 production in the bone. FGF23 inhibits CYP27B1 activity and indirectly decreases serum PO₄³⁻ by downregulation of transcription, translation, and translocation of sodium-phosphate co-transporters in the proximal tubule [35, 36]. 1,25(OH)₂D itself inhibits CYP27B1 transcription, since CYP27B1, same as the CYP24A1 gene, contains vitamin D responsive element (VDRE) in a promoter region, establishing a negative feedback loop [26]. In fact, CYP24A1 regulates 1,25(OH)₂D production independently on Ca²⁺ or PO₄³⁻. Excess of 1,25(OH)₂D downregulates CYP27B1 and up-regulates CYP24A1 leading to its degradation. Depend-

ing on increased FGF23 level, PTH stimulates degradation of its mRNA. Vitamin D metabolism is strictly controlled to avoid an excessive amount of calcitriol [26, 37] which could lead to hypercalcaemia and hyperphosphataemia which in turn can be responsible for soft-tissue calcification and formation of kidney stones in the long term [1]. Renal regulation of CYP27B1 is shown in Figure 2. Extrarenal CYP27B1 are regulated by different mechanisms, including tissue-specific factors like cytokines [26].

Transport of Vitamin D

VDBP and albumin are the primary transporters of vitamin D metabolites in plasma. VDBP plasma concentrations are 2 orders of magnitude higher (5 × 10⁻⁶M) compared to its major circulating ligand 25(OH)D (5 × 10⁻⁸M), suggesting that only about 2% of VDBP is saturated. Under normal condition, <0.1% of 25(OH)D and <1% 1,25(OH)₂D are present in free form [12, 29, 38]. Free vitamin D metabolites are crucial for most of the vi-

Table 2. Recommendations on reference values of vitamin D

Deficiency, ng/mL	Insufficiency, ng/mL	Sufficiency, ng/mL	Institute
<10		>10	SACN
<20	21–29	>30	NIH, Endocrine Society, KDOQI
<25	<40	>40	Vitamin D Society
<12	12–20	>20	IoM

SACN, Scientific Advisory Committee on Nutrition; NIH, National Institute of Health; KDOQI, Kidney Disease Outcomes Quality Initiative; IoM, Institute of Medicine.

tamin D effects besides metabolites bound to DBP are not available for most of the cells. Later research found that several tissues can transport vitamin D metabolites bound to VDBP into cells due to the megalin/cubilin transporting system. This system is present in kidneys PTC where it reabsorbs VDBP from primary urine, and this mechanism helps to maintain VDBP plasma concentration (Fig. 1) [27]. First finding of the crucial importance of the megalin transporter has been demonstrated by Nykjaer et al. [39] when they knocked out the gene for the megalin transporter in the mouse. Only 2% of megalin^{-/-} mice survived to adulthood. They were significantly retarded in growth and exhibited a severe reduction in bone density caused by high VDBP and 25(OH)D urine excretion due to the missing megalin transporter compared to control animals. A study published 2 years later demonstrated that cubilin and megalin are physiologically important transporters working together. Cubilin-deficient dogs show 45 and 70% reduction in plasma 25(OH)D and 1,25(OH)₂D, respectively, compared to healthy controls [40].

Megaline/cubilin transporting system also occurs in other tissues like the intestinal cells, placenta, and visceral yolk sac cells. Megalin is widely expressed across the body. It has also been found in the epithelium of the eye, thyroid and parathyroid glands, endometrium and lung alveoli, and many others [41].

Mechanism of Vitamin D Action

1,25(OH)₂D as a steroid hormone enters the cells via passive diffusion across the cytoplasmic membrane and enters the cell core in the same way where it binds to VDR [9, 42]. VDR is a member of a large protein family that

includes steroid hormones, vitamin A family metabolites, isoprenoids, fatty acid, thyroid hormones, and eicosanoids. Many receptors of this protein family have not known ligands and are called orphan receptors [12]. VDR acts in concert with other nuclear hormone receptors, mainly the retinoid X receptor. The heterodimer vitamin D-VDR binds to the VDRE and acts as a transcription factor. VDREs are commonly distributed across the genome and encode proteins that determine detoxification, bone metabolism (growth and remodelling), cell proliferation and differentiation, apoptosis, mammalian hair cycle, lipid metabolism, mineral homeostasis, immune and central nervous system function, and longevity [43]. Up to 10% of human genes are directly or indirectly responsive to VDR and 1,25(OH)₂D [1, 44].

Most of the VDRE (>98%) are located around 400–500 base pairs upstream or downstream of the transcriptional start site of the vitamin D responsive gene [9, 43]. A typical sequence of VDRE is a tandem repeating oligonucleotide of 6 base pairs including a 3-nucleotide spacer. This repetition can slightly differ between genes within the genome or species (for more details, see [9, 43]).

As mentioned above, vitamin D has non-genomic effects as well. They include the activation of signalling molecules (e.g., phospholipase C, phospholipase A, and phosphatidylinositol-3 kinase) as well as quick generation of secondary messengers (e.g., Ca²⁺ and phosphatidylinositol 3,4,5 trisphosphate). Ca²⁺ and Cl⁻ channels are vitamin D responsive as well [45].

Vitamin D Deficiency and Insufficiency

Ross et al. [46] published a recommendation for vitamin D plasma levels in 2011 concluding that vitamin D (measured as total 25(OH)D) at plasma concentration 16 ng/mL meets the needs of approximately 50% of the population, and 20 ng/mL covers 97.5% of the population. Moreover, the concentration above 50 ng/mL can cause adverse effects. Historically, vitamin D deficiency and insufficiency were not clearly defined (Table 2). The authors usually suggest vitamin D deficiency as 25(OH)D plasma concentration <20 ng/mL, insufficiency as 21–29 ng/mL, and optimal level of 25(OH)D above 30 ng/mL [47–49]. Previous research found that the ideal physiological concentration of PTH in plasma is achieved when 25(OH)D is above 32 ng/mL [50].

Several factors affect vitamin D status, such as sun exposure, skin pigmentation (less pigment allowing to synthesize more previtamin D), time of day, season, area of

skin exposed to sunlight, body weight (only 1% of body fat increase was associated with 0.46 ± 0.22 ng/mL reduction in circulating 25(OH)D [51]), latitude, food-related vitamin D intake, and age (older people synthesizing less previtamin D) [48, 52]. Recommended daily intake and tolerable upper intake of vitamin D for adults are 600 IU/day and 4,000 IU/day, respectively [53]. Nevertheless, even doses around 10,000 IU/day are usually not associated with any adverse side effects. Overdose with vitamin D can cause hypercalcaemia and acute kidney injury, and it is usually caused by dosage equal or above 50,000 IU/day for a couple of weeks or months [46]. The recent study documented 19 people overdosed with vitamin D [54] with the average daily intake 100,000 IU/day orally or intramuscularly for 5–14 weeks.

Most popular supplements of vitamin D are cholecalciferol and ergocalciferol. In the 1930s, they were both assumed to be equal for humans, but in the second half of last century, pieces of evidence about cholecalciferol being a better option started to accumulate. Recently, cholecalciferol is preferred as a dietary vitamin D supplement. Trang et al. [55] showed that cholecalciferol could increase plasma 25(OH)D more effectively than ergocalciferol. Vitamin D₃ is also maintaining 25(OH)D levels higher for a more extended period of time [56]. Recent review summarizing current knowledge about 25(OH)D supplementation suggests even higher efficiency of cholecalciferol in increasing plasma 25(OH)D levels compared to ergocalciferol with 1.7 to 8 higher potency depending on dosage, different pharmacokinetics, and basal 25(OH)D levels [52].

Vitamin D, T2DM, and DKD

T2DM is considered to induce and maintain a low-grade chronic inflammation as a consequence of metabolic derangements related to decreased insulin sensitivity and impaired insulin secretion. Monocyte chemoattractant protein 1 is a chemokine produced by mesangial cells and renal tubular cells that play a crucial role in the recruitment of macrophages into the kidney, causing low-grade inflammation [57]. Together with many other pathways, vitamin D is implicated in the development of tissue and organ diabetic complications. Observational studies and some meta-analysis suggest that vitamin D deficiency increases the risk of T2DM development and the incidence of its complication [58, 59]. Vitamin D can stimulate transcription of the insulin receptor, insulin itself, and its concomitant

secretion but also suppresses transcription of monocyte chemoattractant protein 1, therefore decreases inflammatory load to the kidneys [57]. As described above, low levels of vitamin D can cause hyperparathyroidism leading to dephosphorylation of glucose transporter-4 with subsequent decreased insulin-stimulated glucose transport. Therefore, vitamin D supplementation appears a promising strategy to prevent T2DM development (for more details, see [59]). However, a meta-analysis of published interventional studies with vitamin D supplementation in diabetic patients brings (rather typically) contradictory results. For example, Wu et al. [60] found that vitamin D supplementation significantly decreases HbA_{1c} and fasting blood glucose levels in non-obese patients with T2DM and overt vitamin D deficiency, but not in those without insufficiency. On the contrary, a more recent meta-analysis did not find any statistically significant changes in HbA_{1c} and fasting blood glucose levels [61]. A recent double-blind randomized clinical trial showed decreased insulin sensitivity after 6 months of cholecalciferol supplementation measured as HOMA-IR and also lower insulin levels [62]. These different results are probably due to different meta-analysis approach, leaving space for further research.

DKD is a common microvascular complication of diabetes mellitus with an incidence of approximately 30–40%. DKD is a major cause of the ESRD, renal transplantation, and mortality among patients with diabetes mellitus [63]. DKD is morphologically characterized by both glomerular (thickening of the glomerular basement membrane, expansion of the mesangium, glomerulosclerosis, and podocyte injury) and tubular changes, but the exact pathogenic mechanism – likely multiple and slightly different in T1DM versus T2DM – is still poorly understood [64]. Furthermore, significant inter-individual variability in the extent and type of glomerular/tubular involvement has been documented with genetic factors suspected to play a role [65].

Haemodynamic alterations, glucose and lipid metabolism disorders, non-enzymatic glycation, polyol pathway, proinflammatory cytokines, and oxidative stress were reported to be responsible for DKD onset and progression. However, present research suggests that abnormal inflammatory response is a crucial contributor to this disease [64]. Vila Cuenca et al. [66] recently showed that paricalcitol (a synthetic vitamin D analogue) modulates the endothelial barrier by enforcement of junctional VE-cadherin and the cortical F-actin cytoskeleton thus stabilizing the endothelial barrier in vitro. Vitamin D also acts

as a renin-angiotensin-aldosterone system (RAAS) inhibitor, and its insufficiency can lead to increase of RAAS activity and eventually harm the renal tissues [67]. RAAS system is involved in the pathogenesis of DKD, and its inhibition is commonly used in patients with T2DM. A recent meta-analysis found that active vitamin D analogues in combination with RAAS inhibitors reduced proteinuria by 16% compared to standard RAAS-blocking therapy [68].

Several studies reported significantly decreased 25(OH)D plasma levels in a patient with DKD [69], leading to vitamin D insufficiency or deficiency overtime via several mechanisms: (i) the reduction in the number of functional nephrons. Second, (ii) CYP24A1 is overexpressed in diabetic patients, leading to elevated degradation of the principal vitamin D metabolite. Furthermore, (iii) CYP27B1 activity is decreased probably due to increased FGF23 levels – simultaneously increasing CYP24A1 transcription. Finally, (iv) proteins lost in urine including VDBP, megalin, and cubilin are all essential for vitamin D homeostasis [67]. All these factors result in impaired vitamin D homeostasis and decrease the level of both 25(OH)D and 1,25(OH)₂D and cause secondary hyperparathyroidism.

A recent meta-analysis did not find any significant protecting effect of cholecalciferol or calcitriol supplementation in patients with DKD [3]. In contrast, another meta-analysis found a significant decrease in albuminuria after 24 weeks of paricalcitol supplementation (2 µg/day) [70]. Based on the current knowledge, despite the widespread belief that maintaining an optimal level of vitamin D might help to prevent diabetes mellitus and its complications, current evidence-based knowledge does not support a widespread vitamin D supplementation in diabetic patients [3, 59]. A recent long-term observational study found that T2DM patients with 25(OH)D <12 ng/L are at higher risk of cardiovascular mortality than those with higher 25(OH)D levels [71]. Further research is definitely required to clarify vitamin D effects on T2DM subtypes and various phenotypes of its complications.

Inhibition of Glucose Reabsorption in the Kidney and Vitamin D

Sodium-glucose linked co-transporter 2 (SGLT2) inhibitors (SGLT2i), also called gliflozins, are a relatively new class of drugs used as anti-diabetic treatment (with a realistic potential for repurposing to a non-diabetic pop-

ulation in the future). It acts via the inhibition of SGLT2 expressed in the proximal tubule. SGLT2 is responsible for reabsorption of up to 97% of glucose from primary urine (approximately 180 g/day under healthy condition). SGLT2i decrease glucose reabsorption approximately by 40–60%, thus producing glucosuria and subsequent decrease of glycaemia [72, 73]. Besides, as shown with many recent studies, SGLT2i are also able to decrease the following parameters: HbA_{1c} (–0.79% [95% CI: –0.96% to –0.62%]), weight (–1.74 kg [95% CI: –2.03 to –1.45 kg]) [74], albuminuria (–43% [95% CI: –54% to –31%]) [75], systolic blood pressure (–3.77 mm Hg [95% CI: –4.65 to –2.90 mm Hg]) [74], and plasma uric acid levels (by 10–15% depending on dosage) [76]. By contrast, some adverse effects have been reported, for example, increased LDL cholesterol, increased risk of urinary infections with a hazard ratio (HR) (1.43 [95% CI: 1.05–1.94]) compared to placebo, and genital tract infection with HR (3.48 [95% CI: 2.33–5.20]) compared to placebo [74]. Gliflozins only rarely cause diabetic ketoacidosis (HR 10.80 [95% CI: 1.39–83.65]) [77]. Interestingly, two meta-analyses reported an increased risk of breast cancer in patients using gliflozins [74, 77].

Some authors also suggest the possibility of disrupted bone metabolism; inhibiting co-transport of Na⁺ and glucose might increase the electrochemical gradient for sodium in the proximal tubule. Excess of sodium boosts phosphate reabsorption via SLC34A1 (NaP_i-IIa) and SLC34A3 (NaP_i-IIc) co-transporters, and increased phosphate levels induce FGF23 expression. As mentioned above, FGF23 suppresses CYP27B1 expression and induces CYP24A1 expression. Therefore, circulating levels of 1,25(OH)₂D might decrease. Lower 1,25(OH)₂D levels reduce calcium absorption from the gastrointestinal tract, thereby promote secretion of PTH [35, 78]. Disruption of the PTH-vitamin D-FGF23 axis may lead to bone demineralization. Recent studies showed decreased levels of calcitriol and increased PTH and FGF23 in both diabetic patients and healthy controls [75, 78]. On the other hand, a recent meta-analysis did not find an increased risk for bone fracture [77, 79].

A recent study observed the effect of canagliflozin on bone mineral density (BMD) and bone biomarkers in a patient with type 2 diabetes [80]. A total of 716 patients followed for up to 104 weeks were divided into three groups receiving placebo and 100 and 300 mg of canagliflozin administered once daily. The authors found statistically significant differences in total hip BMD between placebo and both experimental groups with hip BMD decrease by 0.9 and 1.2% in the 100- and 300-mg

canagliflozin group, respectively. No other statistical significances were found in measured parameters related to bone metabolism. The study also measured oestradiol, a hormone causing bone demineralization when its plasma levels are decreased. Compared to the placebo group, oestradiol decreased by 14.2 and 21.0% in 100- and 300-mg canagliflozin groups, respectively, at week 52. Canagliflozin use was also associated with increased β -CTX, a serum bone resorption marker. Interestingly, osteocalcin, a serum bone formation marker, was also increased significantly in both 100- and 300-mg canagliflozin groups compared to placebo at week 52. These results suggest augmented bone turnover in patients treated with canagliflozin, and further research is needed to elucidate the clinical significance of these findings.

Conclusions

Vitamin D and its metabolites play a crucial role in human health. Vitamin D is involved in calcium and phosphate homeostasis, and up to 10% of our genome is responsive to $1,25(\text{OH})_2\text{D}$. Given the vitamin D deficiency/insufficiency and T2DM prevalence growing worldwide in the last decades, health burden for health care systems might be aggravated by their mutual pathophysiological interconnections. It is well documented that patients with DKD are excreting vitamin D and VDBP via urine, suggesting impairment of reuptake in PTC. Despite the

broad range of established beneficial vitamin D effects in diabetes and CKD/DKD settings and thus importance to maintain physiological concentration in plasma, no definite conclusions can be drawn from the current literature on benefits of vitamin D supplementation in these individuals. Despite widespread recognized safety of SGLT2i, recent evidence suggests disruption of the PTH-vitamin D-FGF23 axis, resulting in impaired bone metabolism. Further research is needed to provide evidence about seriousness of metabolic disruption and its consequences.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

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