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Vitamin D Deficiency

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Meet the editor

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Preface

Vitamin D is one the first hormones, or even the very first hormone that was produced more than 500 million years ago. During evolution, compound D progressively participated in several biological processes. It is now widely accepted that Vitamin D is a steroid (more precisely, a secosteroid) hormone. Many preclinical research and clinical studies demonstrated that hormone D plays several physiological key roles in different areas, and that its essential function is not limited to bone growth and maintenance, as believed in the past. It was demonstrated that its deficiency causes several detrimental effects on our health and these problems can be addressed by means of Vitamin D administration. Thanks to the anti-inflammatory, antiproliferative, and antibacterial activities exerted by hormone D, it is very effective in the treatment of several systemic illnesses in the field of endocrinology, immunology, oncology, hematology, neurology, and reproductive medicine.

This book is organized into four sections: Section 1 "Vitamin D Deficiency in Children and Adults"; Section 2 "Vitamin D Deficiency and Brain Functions"; Section 3 "Vitamin D Deficiency and Metabolic Diseases"; and Section 4 "Vitamin D Deficiency and Chronic Diseases". Chapters in these sections cover the earliest investigations of hypovitaminosis D in children and adults, the consequences of Vitamin D deficiency for the cognitive functions of the brain, and the implication of Vitamin D deficiency in the pathogenesis of cardiovascular, metabolic, immune, and renal disorders.

An important theme developed in Section 1 is Vitamin D status in children, adults, and pregnant women. The different ways to improve the status of Vitamin D deficiency and distinct strategies for it in young and old humans are discussed in several chapters of this section. The exciting role of Vitamin D deficiency in brain disturbances is discussed in the chapters of Section 2. An important future direction for study of Vitamin D deficiency implication in the metabolic processes is a section on the beneficial effects of Vitamin D in obesity and diabetes mellitus. Finally, the last section of this book promotes the understanding of implications of Vitamin D deficiency in the pathology of renal, autoimmune, and cardiovascular diseases that may help to find the best strategy of therapy for the patients with such diseases.

The book summarizes the meaning of Vitamin D deficiency in the development of various chronic diseases in humans. However, many questions still remain and will likely fuel ongoing investigation and debate. These questions will likely keep the story open on pleiotropic effects of Vitamin D for the future.

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Section 1

Vitamin D Deficiency in Children and Adults

Chapter 1

Vitamin D and Its Deficiency in Saudi Arabia

Fawzi F. Bokhari and Mai Albaik

Abstract

Vitamin D is a hot topic that has attracted attention over the past 10 years, especially since a large proportion of people suffer from this nutrient deficiency. Vitamin D deficiency is estimated to be about 1 billion people all over the world and 50% in Asia and the Middle East. Saudi Arabia has also demonstrated a high prevalence of vitamin D deficiency among healthy Saudi individuals. This chapter provides, in detail, a clear and understandable identification of vitamin D, its function, source, synthesis, metabolism, status, and deficiency. The chapter also focuses on studying vitamin D deficiency in Saudi Arabia based on PubMed's initial research criteria.

Keywords: vitamin D, function, synthesis, deficiency, Saudi Arabia

1. Introduction

Vitamin D, "sunshine" vitamin, is a vital topic that has attracted great attention of many researchers and the public over the past decades, because a large proportion of the world's population is deficient in this nutritious element [1]. Vitamin D was first discovered at the beginning of the twentieth century in children with rickets [2].

Vitamin D is a prohormone steroid and belongs to the fat-soluble vitamins. It is responsible for endocrine, paracrine, and autocrine functions [1]. Vitamin D is also essential for calcium absorption, bone mineralization, calcium and phosphorus homeostasis, hormonal release, nerve conduction, and neuromuscular function [3, 4].

2. Function of vitamin D

Vitamin D is known for its crucial role in bone health about a century ago. However, it has also demonstrated its role and effectiveness in extra-skeletal roles of vitamin D during the past two decades [5].

2.1 Vitamin D and bone health

Vitamin D regulates physiological functions by controlling the metabolism of calcium and phosphates, stimulates growth, and promotes the necessary remodeling of bones and teeth [6]. Vitamin D deficiency is often associated with bone disorders (such as rickets, osteomalacia, and osteoporosis); when serum calcium decreases, the thyroid gland immediately releases parathyroid hormone (PTH),

which acts by stimulating bone reabsorption and reduction of calcium urinary excretion [7].

Vitamin D levels are positively correlated with bone mineral density (BMD) [4]. Many observational studies have reported relations between chronic lower vitamin D concentrations and poorer lower-extremity function, lower muscle strength, lower contraction speed, and lower appendicular muscle mass [8]. Vitamin D deficiency can put people at risk because of low bone mineral density, osteopenia, osteoporosis, and tooth loss [6].

2.2 Vitamin D and non-skeletal diseases

Observational studies have shown associations between the low concentration of serum vitamin D and increased risk of cancer, cardiovascular diseases, disorders of glucose metabolism, neurodegenerative diseases, and mortality [3]. Vitamin D modulates a variety of processes and regulatory systems including host defense, inflammation, and immunity and repair, especially patients with lung diseases often have low vitamin D serum level [9].

The biological effect of vitamin D on cardiac function is through reduced remodeling and fibrosis secondary to negative regulation of renin by vitamin D receptor (VDR)-linked gene regulation and through reduced cardiac metalloproteinase activities [10]. In addition, many indications support a relation between hypovitaminosis D and slower nerve conduction and poorer executive functions [8]. VDR are also expressed on immune cells (T and B cells, monocytes/macrophages, mast cells, and antigen-presenting cells) [10]. Moreover, vitamin D may exert positive effects on oral health by affecting the production of antimicrobial peptides [6].

Furthermore, many studies have demonstrated that vitamin D supplementation has a beneficial effect in decreasing the mortality rate under multiple factors, by influencing the cardiovascular system, immune system, tumor progression, and others [11].

3. Source of vitamin D

The main sources of vitamin D are our diet, supplementation, and sun exposure [2].

Two dominant forms of dietary vitamin D are vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) [12]. Vitamin D_2 is produced by plants and invertebrates after ultraviolet radiation exposure [13]. Vitamin D_3 is naturally found in many foods (such as oily fish, egg yolks, cod liver oil, cheese, mackerel, salmon, tuna fish, and beef liver), fortified foods (margarine, breakfast cereals, dairy products, orange juice), and vitamin supplements (both vitamins D_2 and D_3 are available) [1, 14, 15]. Dietary vitamin D provides only 10–20% of circulating levels of vitamin D [13].

The chemical structure of these vitamins (D_2 and D_3) is similar but differs only in their side chains (**Figure 1**). This structural difference modifies their binding to carrier protein vitamin D binding protein (DBP) and their metabolism [16]. Vitamin D_3 is significantly demonstrated more effective than D_2 in increasing serum 25-hydroxyvitamin D [25(OH)D] concentrations due to several reasons including reduced vitamin D_2 binding and metabolites to DBP in plasma, a non-physiological metabolism, and a shorter shelf life of vitamin D_2 [13]; therefore vitamin D_3 is considered the preferred choice for supplementation [14].

Sun exposure is the chief source of vitamin D via the synthesis in the skin through the action of ultraviolet B (UVB) radiation on the precursor of vitamin D_3 [4, 17]. The Commission Internationale de l'Eclairage (CIE) described the efficiency of vitamin D

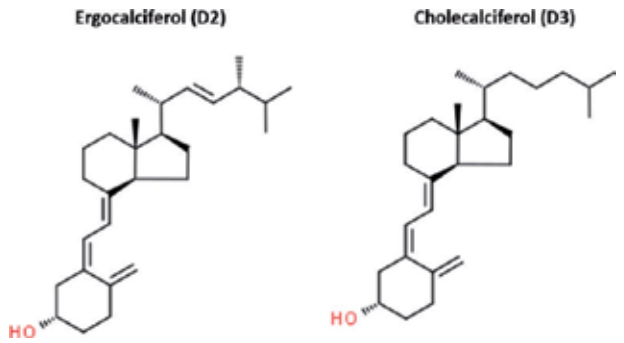

Figure 1.
The structure of vitamins (D2 and D3).

radiation as the efficiency of each wavelength to synthesize vitamin D in the skin. The CIE suggests the efficiency of UVB radiation that covers the spectral range (255–330 nm) with a maximum at about 295 nm [1]. A whole-body exposure to UVB radiation inducing the light pink color of the minimal erythema dose for 15–20 min is able to induce the production of up to 250 μg vitamin D (10,000 IU) [1, 6].

4. Synthesis and metabolism

Vitamin D, either endogenously produced (vitamin D_3) or ingested (vitamin D_2 or vitamin D_3), must be activated in order to produce its effects [5]. This biological activation is performed in a multi-step process (**Figure 2**).

Firstly, UVB radiation penetrates the epidermis and stimulates the conversion of 7-dehydrocholesterol (7-DHC) into pre-vitamin D3 [18] which undergoes thermal isomerization through a sigmatropic hydride shift into vitamin D_3 [13].

Secondly, vitamin D_2 or D_3 is specifically translocated by DBP into circulation and then to the liver for hydroxylation at carbon-25 to form 25-hydroxyvitamin D [25(OH)D] mainly by two cytochrome P-450 enzymes (CYP2R1 and CYP27A1) [5].

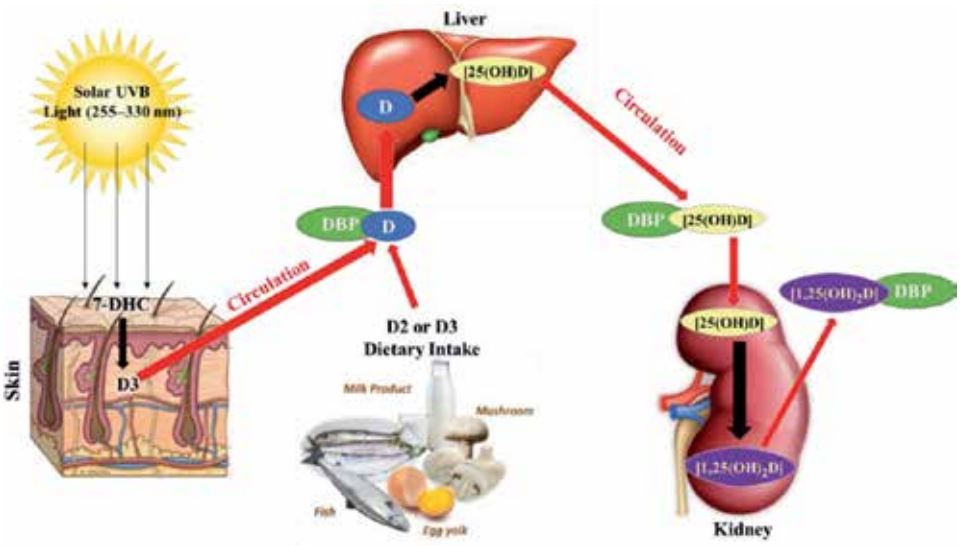

Figure 2.
A diagram illustrating the different sources and synthesis of vitamin D.

[25(OH)D] is an inactive and the most abundant circulating form of vitamin D, and it is generally measured when assessing vitamin D status which has a circulating half-life of about 15 days [2].

Thirdly, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the biologically active form of vitamin D, is generated through second hydroxylation that takes place in the kidney [6] by the enzyme cytochrome P-450 (CYP27B1) monooxygenase 25(OH)D-1-α-hydroxylase [13] [1,25$(OH)_2$D], which serves as a hormone to regulate a variety of cellular functions in other organs or acts inside the kidneys in an autocrine and/or paracrine fashion [5]. Several factors regulate the levels of [1,25$(OH)_2$D], 25(OH) D-1-α-hydroxylase (whose hydroxylation is activated by PTH), calcitonin (which is inhibited by serum levels of calcium), phosphorus, and [1,25$(OH)_2$D] itself [19].

Finally, [1,25$(OH)_2$D] enters the cell by diffusion and binds and activates the VDR [20].

5. Vitamin D receptor (VDR)

VDR is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor as well as its non-genomic actions outside of the nucleus [18, 20]. VDRs are present mostly in body organs such as the colon, small intestine, bone, breast, brain, pancreas, pituitary, and muscles [12].

The widespread distribution of VDRs and production of calcitriol may interpret the increasing number of diseases related to vitamin D deficiency [12]. Binding of calcitriol to VDR prompts the transcription of vitamin D-responsive genes (at least 913 genes) involved in cell proliferation, differentiation, function, and the renin-angiotensin system [2, 21].

VDR forms a heterodimer complex with the retinoid X receptor (RXR) capable of binding to a vitamin D response element (VDRE) in the promoter region of a target gene and thereby regulates transcription of more than thousand genes [20].

6. Factors affecting vitamin D synthesis

Many factors affect vitamin D synthesis and its concentration [1, 3, 6, 11, 17, 19, 20]: aging (age decreases the capacity of the skin to produce vitamin D due to lower availability of 7-DHC), season of the year (autumn and winter), weather conditions (cloudiness), geographical locations (higher latitude), sun exposure, sunscreen (with a protection factor of 30 reduces above 95% of vitamin D synthesis in the skin), skin pigmentation (darker skin needs 3–5 times longer sun exposure to synthesize the same amount of vitamin D than light skin since melanin absorbs UVB radiation), genetic factors (SNPs and mutations), skin damage (burns decrease its production), adiposity (obesity has reduced vitamin D levels), workplace (indoor vs. outdoor), lifestyle, physical activity, clothing habits, air pollution, smoking, diet and calcium intake, vitamin D supplements, and individual height.

7. Vitamin D status

Vitamin D status is best determined by measuring serum 25-hydroxyvitamin D [25(OH)D]; a level higher than 50 nmol/L (30 ng/mL) contributes to the optimal calcium absorption, fall prevention, and prevention of the fracture [4, 22]. Below

this level, PTH levels increase blood circulation, causing secondary hyperparathyroidism, and increase the risk of osteoporosis and fractures leading to bone loss. In addition, moderate increase of PTH may also enhance insulin resistance, weight gain, hypertension, left ventricular hypertrophy, and acute phase response, increasing the risk of ischemic arrhythmias and cardiovascular mortality [4].

According to the Committee of the Institute of Medicine (IOM, USA) and the Endocrine Society [19, 23], vitamin D status defined the values lower than 50 nmol/L (20 ng/mL) as vitamin D deficiency (VDD), while values between 50 and 75 nmol/L are indicated to vitamin D insufficiency (VDI), and values equal or above 75 nmol/L (30 ng/mL) is described as adequate or sufficient vitamin D. The IOM adds extra criterion which is severe VDD with 25 nmol/L (10 ng/mL).

Serum [25(OH)D] test is detected by using high-performance liquid chromatography/mass-spectrometry (LC/MS) methodology [4, 19] which is recommended by the National Diet and Nutrition Survey [19]. The high cost for vitamin D detection hampers the diagnosis of vitamin D deficiency. There is a great need to develop a specific and cheap testing method [4].

8. Vitamin D deficiency

Most studies have identified the vitamin D insufficiency (VDI) at concentrations of [25(OH)D] less than 75 nmol/L (30 ng/mL) and vitamin D deficiency (VDD) at concentrations below 50 nmol/L (20 ng/mL) [1, 4, 10, 17].

Vitamin D deficiency is still a highly prevalent disorder. It is estimated that ~1 billion people are deficient or have insufficient levels of vitamin, in spite of foods fortified with vitamin D and wide supplement intake [2].

VDD is widespread in the whole world as well as predominant in Asia and in the Middle East (more than 50% of the population is VDD and about 75% is VDI) [4]. VDD is found in 30–50% of otherwise healthy middle-aged to elderly adults [11].

Deficiency of vitamin D can result from many reasons such as dietary inadequacy of vitamin D, poor absorption and use, increased requirement, increased excretion and catabolism, limited sunlight exposure, and inefficient production in the skin. Dietary deficiency of vitamin D is associated with milk allergy, lactose intolerance, ovo-vegetarianism, and veganism [1, 4]. In addition, various diseases affect the bioavailability of vitamin D, such as gastrointestinal disorders which limit its absorption; kidney and liver diseases can prevent the activation of the parenteral vitamin D or impair the conversion of vitamin D into its active metabolites [4].

Severe VDD in adults leads to osteomalacia while in children leads to rickets, defective bone mineralization, increased bone turnover, increased risk of fractures [4], impaired reproductive function, and production of gonadal hormone that may affect other organs, e.g., gastrointestinal and renal calcium handling, renal CYP27B1 activity, and bone function [20].

In the critical care condition, VDD has been associated with adverse outcomes such as infections, longer length of stay, acute kidney injury, and higher mortality [10].

9. Vitamin D deficiency in Saudi Arabia

A total of 132 articles studying the deficiency of vitamin D in Saudi Arabia were identified based on the initial PubMed search criteria. About 20 studies have investigated the vitamin D deficiency in healthy individuals living in Saudi Arabia during

Status of women	Prevalence of VDD [25(OH)D] <50 nmol/L	Age group (years)	City of Saudi Arabia	Year of study	Ref.
Premenopausal	80.5%	20–40	Tabuk	2018	[24]
	62%	>21	Riyadh	2017	[25]
	67.8%	19–25	Tabuk	2016	[26]
	74.8%	18–50	Riyadh	2015	[27]
	100%	19–40	Riyadh	2012	[28]
	78.2%	20–50	Jeddah	2011	[29]
	99.03%	18–22	Dammam	2009	[30]
	41.2%	≥18	Riyadh	2008	[23]
Postmenopausal	85%	50–79	Jeddah	2011	[29]
	86.2%	>60	Riyadh	2006–2011	[31]
Pregnant women	88%	>16	Riyadh	2011–2012	[32]
	64.2%	20–40	Al Khafji	2011	[33]
	50%	20–49	Riyadh	2010	[34]

To convert (nmol/L) into (ng/ml), divide the value by 2.496.

Table 1.
Prevalence reports on vitamin D deficiency in Saudi healthy women.

Subjects	Prevalence of VDD [25(OH)D] <50 nmol/L	Age group (years)	City of Saudi Arabia	Year of study	Ref.
Men	66.7%	20–40	Tabuk	2018	[24]
	59%	>21	Riyadh	2017	[25]
	74.4%	18–50	Riyadh	2015	[27]
	92%	20–23	Riyadh	2013	[35]
	87.8%	20–74	Jeddah	2012	[36]
	92.6%	18–22	Dammam	2009	[30]
	32.5%	≥25	Eastern Province	2009	[37]
Newborns	90%	Neonates	Riyadh	2013	[38]
	88%	Neonates	Riyadh	2011–2012	[32]
Children and adolescents	63%	1–6	Makkah	2015	[39]
	92.5% girls 79.3% boys	13–17	Riyadh	2015	[27]
	97.8% girls 92.8% boys	6–15	Western, central, and eastern regions (8 provinces)	2013–2014	[40]
	62.65% girls 40.6% boys	≤15	Different regions	2013	[41]
	86.27%	4–15	Jeddah	2010	[42]

To convert (nmol/L) into (ng/ml), divide the value by 2.496.

Table 2.
Prevalence reports on vitamin D deficiency in healthy men, newborns, children, and adolescents living in Saudi Arabia.

the past 10 years. These studies have demonstrated a noticeably high prevalence of VDD in Saudi women (41.2–100%, **Table 1**) compared to Saudi men (32.5–92.6%, **Table 2**). Deficiency of vitamin D was not only limited to adults but also included newborns (88–90%), children, and adolescents (40.6–97.8%) (**Table 2**).

10. Vitamin D toxicity and safety limits

Excessive oral supplementation and food fortification of vitamin D may lead to toxicity because it raises plasma [25(OH)D] concentrations that exceed DBP binding capacity, and free [25(OH)D] concentrations have direct effects on gene expression once it enters the target cells [13].

Upper safe limit is 5000 IU a day (and some considered it to be 10,000 IU), and the toxicity does not manifest serum levels below 120 ng/mL (300 nmol/L). To reach the latter levels, one must ingest vitamin D in excess of 50,000 IU daily for several months. Thus, the safety of doses to 5000 IU a day is assured [4].

11. Conclusion

In conclusion, this chapter summarizes that vitamin D is a fat-soluble prohormone and has skeletal and extra-skeletal functions. The main sources of vitamin D are sun exposure and diet. Two common types of vitamin D are vitamins D2 (ergocalciferol) and D3 (cholecalciferol). Vitamins D2 or D3 must be activated to produce its effects in a multi-step process. Vitamin D status is determined by serum 25-hydroxyvitamin D [25(OH)D]; the value lower than 50 nmol/L (30 ng/mL) contributes to vitamin D deficiency. Severe vitamin D deficiency leads to osteomalacia in adults, rickets in children, and an increased risk of fractures.

Like other countries in the world, Saudi Arabia suffers from vitamin D deficiency. This chapter illustrates the terrible deficiency of vitamin D for the Saudi population for both genders and for different age groups.

To improve the status of vitamin D deficiency, distinct strategies should be applied to raise the vitamin D stored as a routine measurement through sunlight exposure by increasing daily outdoor activity. Moreover, nutritionists should emphasize increased dairy intake, vitamin D supplementation, calcium supplementation, and vitamin D-fortified foods. Finally, effective educational programs are needed at the Saudi national level to raise public awareness of the serious vitamin D deficiency problem.

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Chapter 2

Vitamin D Deficiency in Children

Teodoro Durá-Travé, Fidel Gallinas-Victoriano, María Urretavizcaya-Martinez, Lotfi Ahmed-Mohamed, María Malumbres-Chacón and Paula Moreno-González

Abstract

In addition to its contribution to bone metabolism, vitamin D seems to fulfill a broad spectrum of biological functions which justifies the interest in monitoring its body content. The aim of this study is to analyze the prevalence of hypovitaminosis D and associated factors in schoolchildren and adolescents living in a region of northern Spain. A cross-sectional clinical and analytical study (calcium, phosphorus, calcidiol, and parathyroid hormone) was accomplished in a group of 602 Caucasian individuals (aged 3.1–15.4 years). Gender, age, body mass index, residence, and season of the year were recorded, and their association with vitamin D deficiency was analyzed by multiple regression. Vitamin D status was defined according to the US Endocrine Society criteria. The prevalence of hypovitaminosis D was 60.4% (insufficiency: 44.6%; deficiency: 15.8%). The female sex, adolescence, season of blood sample collection (autumn, winter, and spring), an urban residence, and severe obesity showed an association with an increased risk of hypovitaminosis D.

Keywords: associated factors, calcidiol, children, deficiency, insufficiency, prevalence, sufficiency, vitamin D

1. Introduction

Vitamin D (cholecalciferol) is a hormone that is basically synthesized in the skin after exposure to sunlight. This substance undergoes initial hepatic (25-OH-D or calcidiol) and subsequent renal [1, 25-(OH)2-D or calcitriol] hydroxylation before the definitive functional activation. The stimulation of ultraviolet radiation (type B) promotes the endogenous synthesis of vitamin D from epidermal 7-dehydrocholesterol, which is the main source of vitamin D, whereas dietary sources account for less than 10% of the total. The function in bone metabolism and calcium homeostasis has been long considered and known. Vitamin D deficiency causes a reduction in the absorption of dietary calcium and an increase in parathyroid hormone (PTH) secretion with the aim to keep steady levels of serum calcium. A deficiency in vitamin D produces osteoclastic activity and causes loss of bone mineral density [1–6].

Recent studies have shown the presence of vitamin D receptors in the majority of organs (blood vessels, B and T lymphocytes, heart, muscles, skin, brain, mammary gland, colon, prostate, gonads, etc.) and the activity of specific enzymes that are not regulated by PTH and induce calcitriol synthesis. All these findings reveal the biological significance of vitamin D. In fact, several observational studies have suggested that vitamin D deficiency could be related to a higher cardiovascular risk,

as well as a risk to present autoimmune, endocrine, infectious, psychiatric, and/or neurological diseases and several types of cancer [1, 4, 7–13]. Additional studies are needed to evaluate the underlying mechanisms.

In other words, in addition to its contribution to bone metabolism, vitamin D seems to fulfill a broad spectrum of biological functions related to cell proliferation, differentiation, and metabolism, which justifies the interest in monitoring its body content. Gender, age, race, season of the year in which serum is collected, sun exposure, and nutritional status have been associated with lower levels of serum calcidiol, but there are disparities among the different authors in the interpretation of these findings [7, 9, 14–20].

The aim of this study is to determine the prevalence of hypovitaminosis D and associated factors among children in northern Spain.

2. Materials and methods

2.1 Participants

The present work is a cross-sectional study conducted in a sample of 602 individuals from ages 3.1 to 15.4 years after the completion of a clinical examination and blood testing in the period January 2014 to December 2014. Tests were carried out in the Pediatric Endocrinology Unit of our hospital. Tanner's criteria were evaluated in every individual and were used in the assignment of individuals in two different groups: school group (Tanner stage I) and adolescent group (Tanner stages II–V). The characteristics of the place of residence were recorded as urban or rural (< or > 10,000 inhabitants, respectively).

All individuals were healthy Caucasian children living in Navarra, Spain. They were selected from the external consultations of the different pediatric subspecialties and were not affected by any chronic pathology with potential interference in growth, body composition, food ingestion, or physical activity. Any participant under treatment with concrete medications (antiepileptic drugs or glucocorticoids), vitamin D, or calcium supplements was put aside from our sample.

2.2 Clinical examination

Body measurements (weight and height) were taken in specific conditions (underwear, barefoot). An Año-Sayol scale (reading interval 0–120 kg and a precision of 100 g) was used for weight registration and a Holtain wall stadiometer (reading interval 60–210 cm, precision 0.1 cm) for height registration. The program Aplicación Nutricional, from the Spanish Society of Pediatric Gastroenterology, Hepatology and Nutrition (Sociedad Española de Gastroenterología, Hepatología y Nutrición Pediátrica, available at http://www.gastroinf.es/nutritional/), provided the estimates of the Z-score values of BMI. The reference charts used for comparison were the graphics from Ferrández et al. (Centro Andrea Prader, Zaragoza 2002).

The values of Z-score enabled the assignment of individuals in the following groups:

- Normal: Z-score between −1.0 (15th percentile) and + 1.0 (85th percentile)
- Overweight: Z-score > 1.0 (85th percentile)
- Obesity: Z-score > 2.0 (97th percentile)
- Severe obesity: Z-score > 3.0 (99th percentile)

2.3 Blood testing

The plasma levels of calcium and phosphorous were determined in a fasting sample of blood using colorimetric methods in a cobas 8000 analyzer (Roche Diagnostic, Mannheim, Germany). The determination of calcidiol levels required a high-specific chemiluminescence immunoassay (LIAISON Assay, Diasorin, Dietzenbach, Germany) and the determination of PTH levels a highly specific solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay in an Immulite analyzer (DPC Biermann, Bad Nauheim, Germany).

The distribution of individuals according to Vitamin D plasma levels followed the criteria of the US Endocrine Society [21, 22]. Calcidiol plasma levels lower than 20 ng/ml (<50 nmol/L) corresponded to Vitamin D deficiency, calcidiol levels between 20 and 29 ng/ml (50–75 nmol/L) to Vitamin D insufficiency, and concentrations equal to or higher than 30 ng/ml (>75 nmol/L) to Vitamin D sufficiency. PTH serum levels higher than 65 pg/ml [14, 17] determined secondary hyperparathyroidism.

2.4 Statistical analysis

The figures resulting from the data collection are shown as percentages (%) and means (M) including the respective standard deviations (SD) or confidence intervals (95% CI). The subsequent statistical analysis (descriptive statistics, Student's t-test, analysis of variance, $\chi 2$ test, Pearson's correlation, and multiple logistic regression analysis) was performed using the software package *Statistical Packages for the Social Sciences* version 20.0 (Chicago, IL, USA). A probability value (p-value) <0.05 was established as the level of statistical significance.

Adequate information of the proceedings and potential implications was delivered to the parents and/or legal guardians, and the corresponding consent was required prior to the inclusion in this study in all cases. The study was presented and approved after the evaluation of the Ethics Committee for Human Investigation at our institution (in line with the ethical standards stated in the Declaration of Helsinki, 1964, and later amendments).

3. Results

The average values for calcidiol and PTH plasma levels from the totality of the collections were 27.4 ± 7.7 ng/mL and 33.2 ± 17.3 pg/mL, respectively. Calcidiol levels overtook 30 ng/mL (Vitamin D sufficiency) in 236 individuals (39.6%), oscillated between 20 and 29 ng/mL (Vitamin D insufficiency) in 266 (44.6%), and were lower than 20 ng/mL (Vitamin D deficiency) in 94 (15.8%). The average values for calcium and phosphorus were 9.9 ± 0.5 and 4.6 ± 0.5 mg/dL, respectively. No values for hypo-/hypercalcemia or hypo-/hyperphosphatemia were detected. The frequency of hyperparathyroidism was significantly higher in the deficient vitamin D (11.6%) and insufficient vitamin D (6.6%) groups, whereas the prevalence of hyperparathyroidism was lowest in the sufficient vitamin D (2.3%) group ($p = 0.005$).

Table 1 shows the distribution of the presumed risk factors for hypovitaminosis D (sex, age group, place of residence, season of blood sample collection, and nutrition status).

Table 2 shows and compares the mean values for the clinical characteristics and biochemical determinations according to the risk factors for hypovitaminosis D. Mean PTH values were significantly higher ($p < 0.05$) in females. The average phosphorous values were significantly higher in males ($p < 0.05$). No significant differences were

Item	N (%)
Sex	
Female	340 (43.5%)
Male	262 (56.5%)
Age group	
Child	299 (49.7%)
Adolescent	303 (50.3%)
Residence	
Urban	394 (65.6%)
Rural	207 (34.4%)
Season of study	
Winter	180 (29.9%)
Spring	131 (21.8%)
Summer	106 (17.6%)
Autumn	185 (30.7%)
Nutritional status	
Normal weight	393 (67.3%)
Overweight	69 (11.8%)
Obesity	68 (11.6%)
Severe obesity	54 (9.2%)

Table 1.
Demographics and clinical characteristics of the participants in the study.

detected in age, nutrition situation, calcium, and calcidiol after the comparison between sexes. Calcidiol and phosphorus levels were significantly higher ($p < 0.05$) in the school group, whereas the average values for PTH were significantly higher in adolescents ($p < 0.05$). There were not any significant differences in Calcium levels between the age groups. Those individuals whose residence was in rural areas showed mean values for calcium and calcidiol significantly higher, whereas those individuals living in urban areas had PTH mean values significantly higher. There were no significant differences regarding age, BMI (Z-score), and phosphorous among individuals living in both areas. The lowest calcidiol levels corresponded to spring (26.1 ± 6.2 ng/mL), and they reached a maximum in the summer (34.5 ± 8.0 ng/mL); meanwhile, the lowest parathyroid hormone levels corresponded to summer (26.6 ± 10.1 pg./mL) and reached a maximum value in autumn (37.8 ± 17.2 pg./mL). There were not any significant differences in calcium and phosphorus levels in each season. Mean values of calcidiol and PTH were significantly lower and higher ($p < 0.05$), respectively, in the group of severe obesity than in other groups with different nutritional situations (normal, overweight, and obesity). There were no significant differences in calcidiol and phosphorous mean values among the different groups.

Table 3 exposes and compares the prevalence of the different calcidiol levels in relation to analyzed associated factors in hypovitaminosis D. We did not detect significant differences in the prevalence of the different levels in calcidiol status in relation to sex. However, the prevalence of vitamin D deficiency was significantly higher in the group of adolescents with respect to school children and rural environment. In the same way, individuals with severe obesity showed a prevalence of vitamin D deficiency significantly higher than those in different nutritional situations.

Item	Age (years)	BMI (Z-score)	Calcium (mg/dl)	Phosphorus (mg/dl)	Calcidiol (ng/ml)	PTH (pg/ml)
Sex						
Females	9.88 ± 3.18	0.47 ± 1.69	10.01 ± 0.37	4.52 ± 0.59	26.88 ± 7.75	35.72 ± 18.46
Males	9.98 ± 3.44	0.41 ± 2.10	9.98 ± 0.36	4.71 ± 0.57	27.98 ± 7.69	29.90 ± 15.27
Significance (p)	0.720	0.720	0.348	<0.001	0.083	<0.001
Age group						
School	7.19 ± 2.08	0.17 ± 1.78	10.01 ± 0.38	4.73 ± 0.54	28.50 ± 7.44	30.87 ± 15.42
Adolescent	12.62 ± 1.62	0.71 ± 2.2	9.97 ± 0.34	4.47 ± 0.61	26.22 ± 7.86	35.58 ± 18.91
Significance (p)	<0.001	<0.001	0.157	<0.001	<0.001	<0.001
Residence						
Urban	10.06 ± 3.28	0.44 ± 2.11	9.97 ± 0.35	4.60 ± 0.58	26.46 ± 7.74	34.49 ± 17.64
Rural	9.67 ± 3.31	0.47 ± 1.85	10.05 ± 0.37	4.61 ± 0.60	29.02 ± 7.47	30.89 ± 16.76
Significance (p)	0.171	0.885	0.008	0.807	<0.001	0.019
Season						
Winter	9.78 ± 3.07	0.49 ± 2.5	10.04 ± 0.33	4.57 ± 0.56	26.54 ± 7.39	31.02 ± 18.03
Spring	10.24 ± 3.14	0.55 ± 1.74	10.02 ± 0.37	4.61 ± 0.56	26.11 ± 6.25	32.28 ± 18.05
Summer	10.44 ± 3.28	0.46 ± 2.73	9.97 ± 0.29	4.66 ± 0.64	34.52 ± 8.05	26.65 ± 10.06
Autumn	9.65 ± 3.56	0.33 ± 1.85	9.93 ± 0.40	4.60 ± 0.59	26.26 ± 7.42	37.88 ± 17.21
Significance (p)	0.182	0.806	0.119	0.731	<0.001	<0.001
Nutritional status						
Normal	9.62 ± 3.34	−0.64 ± 0.94	9.98 ± 0.36	4.59 ± 0.57	28.18 ± 7.7	31.11 ± 15.58
Overweight	10.43 ± 2.6	1.37 ± 0.29	10.02 ± 0.38	4.62 ± 0.55	27.65 ± 7.35	33.12 ± 16.25
Obesity	11.09 ± 2.4	2.46 ± 0.28	10.05 ± 0.36	4.66 ± 0.62	26.19 ± 7.01	39.9 ± 20.26
Severe obesity	11.1 ± 3.28	4.66 ± 2.24	9.96 ± 0.36	4.54 ± 0.72	23.09 ± 8.24	45.22 ± 22
Significance (p)	<0.001	<0.001	0.447	0.721	<0.001	<0.001

Table 2.
Clinical and biochemical characteristics according to the presumed risk factors for hypovitaminosis D (M ± DE).

Item	Deficiency N (%)	Insufficiency N (%)	Sufficiency N (%)	Chi^2 (p)
Sex				
Females	56 (16.7%)	161 (47.9%)	119 (35.4%)	5.65 (0.059)
Males	38 (14.6%)	105 (40.4%)	117 (45%)	
Age group				
School	31 (11.1%)	131 (44.3%)	132 (44.6%)	11.696 (0.003)
Adolescent	61 (20.3%)	135 (45%)	104 (34.7%)	
Residence				
Urban	75 (19.3%)	175 (45%)	139 (35.7%)	12.671 (0.002)
Rural	19 (9.2%)	91 (44.2%)	96 (46.6%)	
Season				
Winter	40 (21.1%)	79 (41.6%)	71 (37.4%)	
Spring	17 (13.3%)	76 (59.4%)	35 (27.3%)	69.12 (<0.001)
Summer	4 (5.3%)	11 (14.7%)	60 (80%)	
Autumn	33 (16.3%)	100 (49.3%)	70 (34.5%)	
Nutritional status				
Normal	46 (11.8%)	176 (45.1%)	168 (43.1%)	
Overweight	11 (15.9%)	27 (39.1%)	31 (44.9%)	30.135 (<0.001)
Obesity	12 (18.2%)	33 (50%)	21 (31.8%)	
Severe obesity	20 (37.7%)	23 (43.4%)	10 (18.9%)	

Table 3.
Prevalence of the different calcidiol levels in relation to the presumed risk factors for hypovitaminosis D.

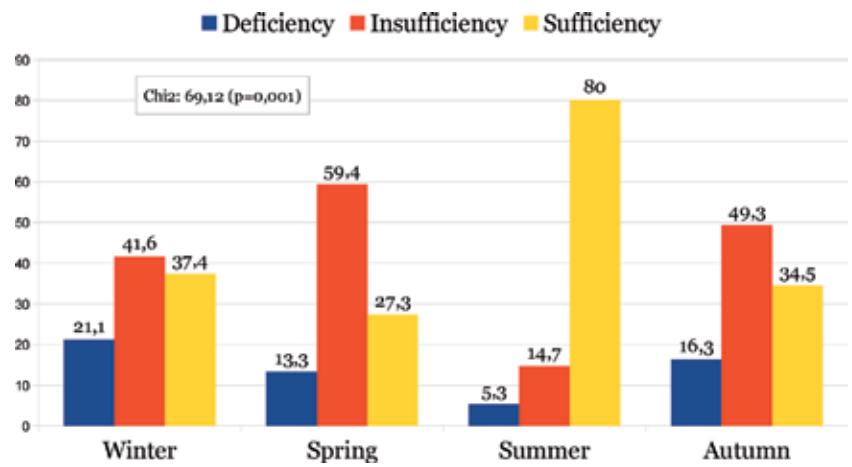

Figure 1.
Prevalence of vitamin D status according to seasons.

Figure 1 presents the prevalence of hypovitaminosis D (deficiency and insufficiency) according to the seasons of the year in healthy pediatric population. The levels of vitamin D during summer was sufficient in 80% of the individuals; this level substantially decreased in autumn and winter (hypovitaminosis was detected in 65.6% and 62.7% during autumn and winter, respectively) and got to the lowest point in spring, a period that revealed a prevalence of hypovitaminosis of 72.7%.

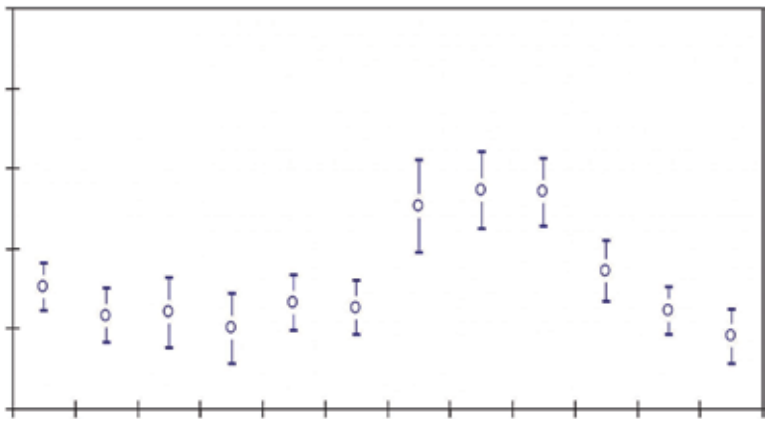

Figure 2.
Calcidiol levels (ng/ml ± CI 95%) throughout the year (ANOVA, p < 0.001).

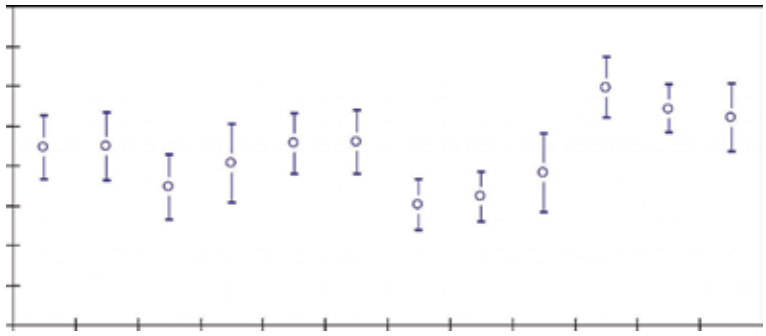

Figure 3.
PTH levels (pg/ml ± CI 95%) in the different months of the year (ANOVA, p < 0.001).

Figure 2 states the average values for calcidiol levels along the months of the year (ANOVA, $p < 0.001$), showing the highest levels during the summer months (July: 32.6 ng/mL, CI 95%: 29.7–35.5; August: 33.6 ng/mL, CI 95%: 31.2–36; and September: 33.5 ng/mL, CI 95%: 31.4–35.6); in contrast, these levels were lower during the winter and spring months.

Figure 3 presents the PTH levels (average) in the different months of the year (ANOVA, $p < 0.001$). The autumn months show the highest PTH levels (October: 39.8 pg/mL, CI 95%: 35.4–43.6; November: 37.1 pg/mL, CI 95%: 34.1–34.1; and December: 36.0, CI 95%: 31.7–40.3) and the end of spring (May: 32.8 pg/mL, CI 95%: 29.0–36.6 and June: 33.0 pg/mL, CI 95%: 29.0–37.0); in contrast, these levels were lower in the summer months (July: 25.1 pg/mL, CI 95%: 21.9–28.3 and August: 26.1 pg/mL, CI 95%: 23.0–29.2).

A significant correlation ($p < 0.001$) between calcidiol and parathyroid hormone levels ($r = -0.24$) was observed; calcium and PTH plasma levels ($r = -0.20$) are also correlated. A significant correlation was detected between age and calcidiol levels ($r = -0.15$) and also between age and PTH levels ($r = 0.18$).

Table 4 illustrates the multiple logistic regression analysis for the presumed predictors of vitamin D status. The female sex, adolescence, season of blood sample collection (autumn, winter, and spring), an urban residence, and severe obesity showed an association with an increased risk of vitamin D insufficiency. Adolescence, season of blood sample collection (autumn and winter), urban residence, and severe obesity revealed an association with an increased risk of vitamin D deficiency.

Items	Deficiency OR (IC 95%) p	Insufficiency OR (CI 95%) p
Sex		
Males	1 (reference)	1 (reference)
Females	1.1 (0.9–1.8) 0.676	1.6 (1.1–2.3) 0.011
Age group		
School	1 (reference)	1 (reference)
Adolescent	2.0 (1.2–3.4) 0.005	1.8 (1.2–2.6) 0.003
Season		
Summer	1 (reference)	1 (reference)
Autumn	3.8 (1.3–11.5) 0.018	9.5 (4.8–18.7) <0.001
Winter	5.8 (1.9–17.4) 0.002	8.8 (4.5–17.5) <0.001
Spring	3.1 (0.9–9.9) 0.058	13.2 (6.4–27.5) <0.001
Residence		
Rural	1 (reference)	1 (reference)
Urban	2.4 (1.4–4.0) 0.020	1.6 (1.1–2.2) 0.01
Nutritional status		
Normal	1 (reference)	1 (reference)
Overweight	1.3 (0.7–1.8) 0.442	0.8 (0.5–1.4) 0.476
Obesity	1.3 (0.7–2.8) 0.443	1.2 (0.7–2.2) 0.498
Severe obesity	4.4 (2.2–8.6) <0.001	4.4 (1.9–10.3) <0.001

Table 4.
Multiple logistic regression analysis for the presumed risk factor for hypovitaminosis (deficiency and insufficiency).

4. Discussion

There is a high prevalence of hypovitaminosis D in the pediatric population in our environment, which potentially represents a serious public health problem. The criteria from the US Endocrine Society have been used for the comparison of the results with the previous published data. According to these criteria, calcidiol has a long half-life (2 to 3 weeks) and is the best indicator of body vitamin D content; they consider normal serum levels when they reach 30 ng/mL or higher and hypovitaminosis D below this level. In this way, hypovitaminosis is classified into insufficiency (between 21 and 29 ng/mL) and deficiency (lower than 20 ng/mL) [4, 21, 22].

The blood sample analysis shows the following prevalence: vitamin D sufficiency in 39.6% of the individuals, insufficiency 44.6% and deficiency 15.8%, respectively. These results might seem to show a high prevalence of hypovitaminosis for a healthy population, but are indeed relatively moderate in comparison to other studies published in different areas or latitudes of our planet (**Table 5**); this may

Authors	Deficiency	Insufficiency	Sufficiency
Cheng et al., 2003 (Jyväskylä, Finland) [23]	32%	46%	22%
Weng et al., 2007 (Philadelphia, USA) [14]	26%	29%	45%
Gordon et al., 2008 (Boston, USA) [24]	12%	40%	48%
Kelly et al., 2011 (Filadelfia, USA) [25]	47%	27%	26%
Andiran et al., 2012 (Ankara, Turkey) [26]	40%	—	—
Tolppanen et al., 2012 (England, UK) [27]	29%	46%	25%
González-Cross et al., 2012 (Europe) [28]	19%	39%	42%
Vierucci et al., 2013 (Toscana, Italy) [17]	46%	34%	20%
Karagüzel et al., 2014 (Trabzon, Turkey) [29]	71%	23%	6%
Durá-Travé et al., 2015 (Navarra, Spain) [30]	13%	45%	42%
Kaddam et al., 2017 (Saudi Arabia) [31]	49%	46%	5%
Fernández-Bustillo et al., 2018 (Galicia, Spain) [32]	5.9%	60.1%	34%
Guo et al., 2018 (China) [33]	10.8%	39%	50.2%

Table 5.
Prevalence of hypovitaminosis D according to different authors.

be due to the fact that in this study their participants were exclusively of Caucasian origin, since, as is well known, the difference in skin pigmentation in different ethnic groups implies a higher risk of hypovitaminosis D [14, 17, 18, 28, 32, 34–37].

Calcidiol measurements—even though they were not significant—were higher in males, whereas PTH levels were significantly higher in females, but there were no significant differences in the prevalence of hypovitaminosis D among sexes. Previously published data are inconsistent [9, 34, 38, 39]. Nevertheless, the logistic regression analysis revealed that hypovitaminosis D, and more specifically the level of insufficiency, was significantly associated with females. Adolescents (pubertal group) had significantly lower calcidiol levels than school children, but the mean values for PTH and the prevalence of hypovitaminosis D were significantly higher in that group, and these results match those provided by other authors [17, 40]. Furthermore, the logistic regression analysis showed that hypovitaminosis D, as insufficiency and deficiency, was significantly associated to the older group. These findings could be somehow disturbing, since adolescence is a key period for growing, development, and bone formation, when vitamin D requirements are increased and deficiency may affect normal bone mass acquisition. Individuals living in urban areas had calcidiol values significantly lower than those living in rural areas, whereas PTH values and prevalence of vitamin D deficiency were significantly higher in those individuals from urban areas. These findings might be related to the different lifestyles inherent to the different environment, since residents in rural areas are likely to experience longer periods of sun exposure, as several authors have highlighted [17, 19].

The characteristics of sun exposure depend considerably on the location. In this way, it has been found how the axial tilt (obliquity) of our planet in the northern hemisphere (beyond 37th parallel—north), mainly in the colder months of the year, causes a change in the density of incident rays and, therefore, the ultraviolet radiation (type B) decreases up to 80–100%. That is the reason why sun radiation is not able to lead to efficient vitamin D synthesis [14, 17, 29, 41]. Hence the main reasons of vitamin D deficiency are usually in direct relation either to any physical agents that obstruct sun radiation (cutaneous pigmentation, sunscreens, etc.) or to

geographical features, such as sunlight exposure, atmospheric pollution, altitude, latitude, and the season of the year [1, 3].

The negative correlation between calcidiol and parathyroid hormone plasma levels has been previously described by different authors [14, 17, 33, 42]; nonetheless, the discussion about the combined oscillations existing among both hormones along a natural year has not been that intense [43, 44]. The analysis of our data has shown simultaneous and asynchronous changes in parathyroid hormone levels with respect to calcidiol, simultaneously with monthly and/or seasonal modifications in calcidiol levels. These adjustments presumably would take place in order to maintain constant calcium levels along the year, as we have noticed in this study. In point of fact, the highest body vitamin D levels are detected in the summer months—there is a more intense sunlight exposure. The levels decrease gradually in the autumn and winter months—except for some biological variability—and they reach the lowest point in springtime. By comparison to the findings of other authors [3, 17, 29], only 18.2% of the 602 individuals classified in the status of vitamin D insufficiency of deficiency presented with PTH levels within the range of hyperparathyroidism; since no diagnosis of hypercalcemia or hypocalcaemia or any bone semiology was previously described, a potential conclusion is that seasonal changes in calcidiol and parathyroid hormone levels would be related to a physiological phenomenon of adaptation to the geographical and climatic conditions endemic to this region. Navarre is a Spanish region located on a high latitude (between 41°55′22 N and 43°16′42 N) with frequent cloudy and rainy days, and this characteristic is important enough to take into consideration that cyclical variation in calcidiol levels in relation to the season of the year could be explained by a possible inefficient vitamin synthesis induced by sun radiation, as several authors have noted [14, 17, 24, 29, 42, 45–47]. Admittedly, the results confirm that vitamin D levels in the summer months were sufficient in 80% of the individuals. They moderately decrease in the months of autumn and winter (the percentage of individual in a situation of hypovitaminosis D gets to 66.6 and 62.7% in autumn and winter, respectively) and fall to the lowest point in spring months, when the prevalence of hypovitaminosis gets to 72.7%.

Because geographical and climatic conditions significantly influence body vitamin D content and, secondarily, PTH plasma levels, a comparison of the different results obtained in the published works from different countries and/or climatic conditions would be unwise and faulty, since the place of residence, latitude, and especially the month of the year when the blood sample is collected always have to be considered. In other words, it is not possible to establish a vitamin D status in a concrete population without considering the seasonal variations because, as this work has shown, a potential condition of hypovitaminosis D is related to the season of the year in which the determination has been made [48, 49].

As BMI (Z-score) increases, calcidiol values decrease and PTH significantly rises, in a way that individuals with severe obesity showed minimum values of calcidiol and maximum values of PTH with respect to the individuals of the remaining nutritional situations. This means, there seems to be a noticeable tendency to present with vitamin D deficiency in the individuals with severe obesity [9, 20, 40, 50]; this eventuality has been outlined as a cardiovascular and/or metabolic risk factor [4, 8–10, 15, 19]. Even though there is not a conclusive explanation in this respect, it has been suggested that this circumstance in obese individuals may be related to environmental factors (decreased sun exposure as a consequence of a sedentary lifestyle, inadequate diet, etc.), although, at present, some authors postulate a hypothetical "sequestration" or excessive accretion of vitamin D in the adipose tissue [2, 4, 7, 8, 16, 18, 21, 42]. Previous reports did not distinguish between obesity and severe obesity, as we did in this work, and this special feature could be of practical relevance, since the logistic regression analysis has verified that hypovitaminosis

D, in levels of insufficiency as well as deficiency, is significantly associated to the nutritional situation of severe obesity.

The development of this study unveiled several limitations. The main weaknesses are the cross-sectional nature of the study and the absence of data on exercise, sun exposure, and the use of sunscreens. There have been some difficulties to get adequate and accurate data, and it restrained us from completing the registration. A nutritional survey (including dietary vitamin D intake, daily supplementation, etc.) was not incorporated in the study. Our experience reflects that dairy product intake in our social context is below the recommended amount and particularly fish intake is quite low in the pediatric population [51]; in this way, only the dietary supplementation of vitamin D could condition the results achieved, but, at present, it is not a widespread practice in our society.

Given the difficulties in maintaining a sufficient body vitamin D content in the pediatric age group throughout the year, the prevention, detection, and, when required, treatment and follow-up of hypovitaminosis should be fully integrated in the programs of health promotion and disease prevention in child and adolescent population corresponding to primary health care. In other words, primary care teams and, more specifically, pediatricians should include a series of preventive measures—in addition to the mandatory vitamin D daily supplementation during the first year of life, 400 UI per day [4, 52, 53], such as promoting adequate sun exposure, in the service portfolio. Around 10–15 minutes of midday sun exposure (between 10 in the morning and 3 in the afternoon) on at least 20% of total body surface (uncovered head and extremities) during spring, summer, and autumn is considered enough to get an adequate vitamin D synthesis [2]. In addition, in case any of the hypovitaminosis D-associated factors is present, especially in those individuals at risk of limited sun exposure (disabled and/or undergoing long stay in the hospital, etc.), the need for additional vitamin D supplementation should be considered, either as pharmacological supplements (600 IU per day), an increase of the ingestion of higher amounts from its natural dietary sources (herring, salmon, sardines, tuna, etc.), or vitamin D fortified foods (dairy products, cereals, etc.) during the months of winter and spring, as several authors have suggested [6, 12, 14, 15, 18, 21, 28, 34, 54].

5. Conclusion

There is a high prevalence of hypovitaminosis D in the pediatric population in our environment, being female sex, pubertal age, the seasons of autumn, winter and spring, living in urban area, and severe obesity considered as associated factors in hypovitaminosis D. Consideration should be given to the administration of vitamin supplements and/or the increase in the ingestion of natural vitamin D dietary sources.

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Chapter 3

Maternal Vitamin D Status among Different Ethnic Groups and Its Potential Contribution to Adverse Pregnancy and Child Outcomes

Pardis Keshavarz, Parisa Jandaghi, Mojtaba Shafiee, Naorin Islam and Hassan Vatanparast

Abstract

Maternal vitamin D deficiency in pregnancy is a widespread public health concern. Race and ethnicity as biological and cultural factors, respectively, can affect vitamin D status through differences in skin color, sunlight exposure, and dietary intake. Low maternal vitamin D status in pregnancy may affect both mother and fetus adversely. Vitamin D deficiency and insufficiency are linked to a wide variety of adverse pregnancy outcomes such as gestational diabetes, preeclampsia, and preterm delivery. Furthermore, maternal vitamin D deficiency has been linked to several adverse health outcomes in infants and children. The examples include, but not limited to, impaired growth, skeletal problems, and autoimmune diseases such as type 1 diabetes and asthma. This chapter reviews the vitamin D status during pregnancy across different ethnic groups, looking into the adverse pregnancy and child outcomes, followed by a discussion on the association between maternal and child vitamin D status and successful interventions. Strong evidence exists about the association between vitamin D and some health outcomes during pregnancy, while more studies are needed to confirm the other claim. The existing body of evidence justifies the need for well-designed policies and systematic interventions to ensure optimal vitamin D status of pregnant women and their offsprings across different ethnic and racial groups.

Keywords: vitamin D, pregnancy, ethnicity, child, deficiency

1. Introduction

The calciotropic role of vitamin D is well known from the early twentieth century. The recent advances in research opened a new perspective about vitamin D as prohormone with receptors in most tissues of the human body [1, 2]. This indicates additional non-calciotropic effects of vitamin D, such as its role in autoimmunity, chronic disease, infectious diseases, mental health issues, etc. [2]. However, the current dietary recommendations are based on only calciotropic effects of vitamin D although recent studies suggest that higher intakes are required to achieve the optimal vitamin in D status for both calciotropic and non-calciotropic benefits of vitamin D [2].

Pregnancy is a unique stage of life for women when the normal physiology of mother is changing in order to provide the nutritional needs for the growing fetus [3]. Those changes influence the vitamin D hemostasis and availability for the mother and the fetus. In this chapter, we provide an overview of the evidence about vitamin D metabolism during pregnancy, the association between maternal vitamin D status and pregnancy, fetal and postnatal outcomes. Further, we provide insight into the current recommendation for vitamin D intake to achieve optimal vitamin D status and the associated factors, particularly race and ethnicity. Finally, we review the existing policies and practices to assure optimal vitamin status in pregnant women and their offsprings.

2. Vitamin D metabolism during pregnancy

Vitamin D homeostasis is altered during pregnancy in order to provide a successful delivery and optimal environment for the growth of the fetus. This section focuses on adaptive changes of vitamin D during pregnancy as a background for developing pregnancy and fetal related disorders.

Major vitamin D adaptations in pregnancy include: (1) maternal increase of calcitriol; (2) availability of maternal 25(OH)D for optimal neonatal 25(OH)D; (3) increased concentration of maternal vitamin D-binding protein (VDBP) and placental vitamin D receptor (VDR); and (4) increased activity of renal and placental 25(OH)D-1-α-hydroxylase (CYP27B1) [4, 5]. The first change is started in the first trimester, increasing the level of calcitriol in systemic circulation and placenta to 100–200% by the end of the third trimester [6]. It is originated mostly from the kidneys for the purpose of increased intestinal calcium absorption during pregnancy [7]. In fact, the activity of 1α hydroxylase increases while catabolism of calcitriol decreases leading to more intestinal calcium absorption and immune adaptation [8]. The additional contributors of increased maternal and placental calcitriol are prolactin, calcitonin, PTH-related peptide (PTH-rP), estradiol, placental lactogen [9], IGF-1 [10], and FGF23 [11]. Any dysregulation causing activation decrease and catabolism increase of 25(OH)D may lead to preeclamptic mothers, which will be discussed in this chapter [12].

The second adaptation is likely that the levels of 25(OH)D in cord blood are reduced on average 25% in comparison with maternal 25(OH)D [13]. Maternal 25(OH)D concentration remains constant during pregnancy, meaning that the increased level of calcitriol is not related to its precursor synthesis. Maternal 25(OH)D crosses the placenta barrier as the main source of vitamin D in the fetus [14]. Therefore, vitamin D insufficiency in pregnant mothers could affect the fetus. Other factors, including lifestyle, place of living, skin pigmentation, sunshine exposure, and obesity, contribute significantly to maternal vitamin D status during pregnancy [4]. Consequently, a low level of maternal vitamin D leads to impaired fetal 25(OH)D at birth.

The third adaptation is a 40–50% increase in the concentration of VDBP in both systemic circulation and placenta level compared to the non-pregnant woman reaching to a maximum level at the beginning of the third trimester, before starting to decrease by the end of gestation. This leads to a consistent decrease of free 25(OH)D from 15 to 36 weeks, since there is an inverse relationship between free 25(OH)D and VDBP concentration [15]. The mechanism has not fully understood, although studies suggested the high turnover rate of trophoblasts that are in contact with maternal blood directly leads to the increased expression of VDBP on the cell-surface of human placental trophoblasts during normal human pregnancy [16]. Studies conducted by Ma et al. and Liong et al. indicated that VDBP impairment,

either increase or decrease of its concentration, can contribute to the pathogenesis of preeclampsia (PET) and preterm birth [16, 17].

The placenta is an important organ playing a pivotal role in the optimal embryonic improvement and healthy pregnancy. Placenta regulates vitamin D metabolism by its own mechanism [18]. In fact, 1-α-hydroxylase, 24-hydroxylase, VDBP, and VDR have all been detected in trophoblast cultures and in placenta tissue, resulting in local synthesis of $1,25(OH)_2D$ in the maternal-fetal interface as extrarenal calcitriol. Moreover, in the placenta, VDR gene expression is higher within the first and second trimester compared to term placentas [19] and positively associated with transferring of calcium from mother-to-fetal. This indicates that VDR-dependent mechanisms in the placenta can affect fetal skeletal growth [20]. Vitamin D2 is metabolized in the placenta as well [21]. Thus, the placenta has its own mechanism in the regulation of vitamin D metabolism.

Increased 1, 25(OH)D is attributed to the higher activity of CYP27B1 in maternal, kidney, placental trophoblasts, and decidua during pregnancy [22]. The mechanism by which CYP27B1 activity increases in pregnancy remains unclear. However, it has been suggested that PTH analog PTH-related peptide (PTH-rP), synthesized by fetal parathyroid and placenta, could be a potential regulatory factor for CYP27B1 and augments during pregnancy [23].

Vitamin D metabolism manifests significant changes in pregnant women compared to the non-pregnant state. In an optimal ongoing pregnancy, there are three striking alternation within a gestation; two-fold increase of calcitriol in the first trimester, versus about 25% reduction in the levels of 25(OH)D in cord blood as it crosses the placenta barrier and increase the expression of vitamin D receptor and regulatory metabolic enzymes in the placenta. Also, maternal increase of serum calcitriol and VDBP without changes in 25(OH)D and calcium concentration of mother indicates that neonatal vitamin D stores are dependent on maternal vitamin D status.

3. Current vitamin D intake recommendation guideline during pregnancy

The role of vitamin D intake in pregnancy and its consequences for fetal growth is the focus of current attention. The previous Dietary Reference Intake (DRI) review of vitamin D and Institute of Medicine (IOM) workshop on DRI research needs requested for research to evaluate the intake requirements for vitamin D as related to optimal circulating 25(OH)D concentrations across different life cycles and among different ethnic groups of Canadian and US populations [1]. **Table 1** summarizes different recommendations from various agencies which represents in population and individual level.

Health Canada [28] and IOM [1] have recommended dietary allowance of 600 IU/day and tolerable upper intake level of 4000 IU/day for pregnant women in the US and Canada would meet the daily need in 97.5% of the population. There is no consensus on the cut-off point for vitamin D insufficiency. To prevent rickets and osteomalacia, IOM recommended >50 nmol/L concentration of 25(OH)D. While, the Endocrine Society and Osteoporosis Canada suggested a target serum concentration >75 nmol/L based on the available evidence with the daily intake of 1500–2000 IU in order to achieve optimal benefits for skeletal and non-skeletal health [29, 30]. Accordingly, the Canadian Pediatric Society suggested 75 nmol/L as "sufficient" for pregnant and lactating women [31]. Moreover, several pilot studies have recommended that daily intake of 2000 [32], 4000 [33], or even 6400 IU [34] vitamin D would reduce vitamin D inadequacy without any toxicity sign in

Agency	Countries	25(OH)D threshold (nmol/L)			Vitamin D intake (μg/d)		
		Deficiency	Population average	Individual target	EAR	RI	AI
IOM [1]	USA/Canada	<30	40	≥50	10	15	—
NORDEN [24]	Nordic	<30	—	≥50	7.5	10	—
SACN [25]	UK	<25	—	≥25	—	10	—
EFSA [26]	EU	—	—	≥50	—	—	15

25(OH)D, 25-hydroxyvitamin D; EAR, estimated average requirement; RI, recommended (individual) intake; AI, adequate intake; IOM, Institute of Medicine; NORDEN, Nordic Council of Ministers; SACN, Scientific Advisory Committee on Nutrition; EFSA, European Food Safety Authority. Table adapted from Kiely et al. [27].

Table 1.
Summary of the current dietary recommendations for vitamin D in pregnant women.

pregnant women and their infants. The discrepancies in some factors, including different measurement tools of vitamin D levels, various patient populations and different sample sizes that were used in studies, might explain the differences in the recommendations. Because of conflicting evidences, identifying sufficient and upper-limit amount of vitamin D for pregnant women requires further research to be performed.

4. Maternal vitamin D status and adverse pregnancy outcomes

Numerous studies have reported pleiotropic role of vitamin D in pregnancy. Maternal Hypovitaminosis D during pregnancy is related to pregnancy related disorders. Complications caused by low serum measurement of 25(OH)D include gestational hypertension (GHT), PET, gestational diabetes mellitus (GDM), timing and mode of delivery, postpartum depression or anxiety, bacterial vaginosis, and other outcomes such as anemia and lipid disorders, which are discussed in this section.

It should be noted that, there is not enough evidence to support a recommendation for screening all pregnant women for vitamin D deficiency but must be at least 20 ng/ml (50 nmol/L) for bone health [35]. Although some experts suggest vitamin D serum level of at least 32 ng/ml (80 nmol/L) for optimal state in pregnancy [36], some adverse effects have been reported at levels exceeding 70 nmol/L [37]. Consequently, due to the lack of standardized measurement procedures, there is no consensus on the optimal vitamin D status during pregnancy.

4.1 Gestational hypertension and preeclampsia

Elevated blood pressure that appears after 20 weeks without proteinuria or other findings is called gestational hypertension (GHT). This problem is confirmed by systolic blood pressure (SBP) ≥160 or diastolic blood pressure (DBP) ≥110 mmHg for anyone (confirmed over a few minutes) or SBP ≥140 or DBP ≥90 mmHg after 20 weeks (confirmed over 4 hours) observing for the first time [38]. At least 25% of women with GHT is predisposed to PET [38]. PET is diagnosed by high blood pressure (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg) after 20 weeks of gestation along with proteinuria (>300 mg/day) and other organ dysfunction including liver involvement, hematological disturbance, neurological

or renal complications [39]. The pathogenesis of PET involves releasing the angiogenic factors to the maternal circulation which causes insufficient remodeling and trophoblastic invasion of spiral arteries. It leads to shallow implantation and hypoxia, and even release of inflammatory mediators [40]. PET is a multifactorial outcome that has not been fully understood yet; however, maternal calcium status is suggested to be an important factor. Therefore, vitamin D due to its role in calcium hemostasis might have an impact on PET [41]. In addition, vitamin D can be protective of placental vasoconstriction and consequently, PET because of its immunomodulatory effect. Also, vitamin D is a regulator of endothelial and vascular smooth muscle cell proliferation through which regulates blood pressure via Renin-Angiotensin-Aldosterone system (RAAS) [42].

Systematic reviews and meta-analysis, including cross-sectional, longitudinal, cohort, ecological, and observational studies, indicate inconsistent results regarding the association between vitamin D deficiency and PET. Some studies reported an increased risk of PET women with vitamin D deficiency in pregnancy in contrast with others [43–45]. This contradiction of finding can be explained by different vitamin D assessment methods, criteria applied to define vitamin D deficiency, different season and trimester in studies [44]. Therefore, associations are inconclusive, often contradictory, confounded, and lack causality. In addition, supplementation helps to raise the level of maternal vitamin D; however, there is no significant reduction of PET risk with a higher level of vitamin D status [22, 46]. In this regard, more observational and interventional studies with different designs are needed.

4.2 Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is glucose intolerance developed or first diagnosed during pregnancy. Criteria for recognition GDM are controversial [47]; according to World Health Organization (WHO), fasting blood glucose above 92–125 mg/dl and/or 2-h glucose greater than 153–199 mg/dl after glucose intake of 75 g are considered as diabetes mellitus in pregnancy as is a random plasma value of above 200 mg/dl with diabetes symptoms [48, 49]. Vitamin D plays a crucial role in glucose homeostasis through several mechanisms. The first is regulating calcium, which is a regulator for the production and secretion of insulin by the endocrine pancreas. Its second role involves enhancing insulin sensitivity of the target cells in adipose tissues, liver, and skeletal muscles. Moreover, the immune cell regulation role of vitamin D protects β-cells from damaging and improves its function [50, 51]. Although vitamin D deficiency can be associated with the pathogenesis of diabetes mellitus type 1 and type 2, its role in GDM is not conclusive [43]. Conflicting results have been found in case-control, prospective cohort studies, and reviews looking into the risk of GDM with vitamin D status [46]. Some review studies indicate that pregnant women with significant lower 25(OH)D had a higher risk of GDM by 40–60% [45, 52], while in systematic and critical reviews, most studies failed to support the association between vitamin D status and GDM prevention [43, 44]. Thus, more large-scale prospective studies are needed to evaluate this association.

4.3 Postpartum depression (PPD) or anxiety

Depression after delivery is a common psychiatric condition which is called postpartum depression (PPD) [53]. Vitamin D as a neurosteroid suggested having a role in various brain functions and depression by several potential mechanisms. Firstly, Vitamin D plays as a neurotransmitter, neuro-immunomodulation, and neuroprotection in the brain [54]. Secondly, vitamin D has a role in synthesizing norepinephrine and dopamine, which are involved in mood disorders.

Furthermore, vitamin D protects the brain from oxidative stress by preserving the antioxidant glutathione in the brain [55, 56]. Most studies suggested there is an inverse association between vitamin D serum in different stages of gestation and postpartum depression [57–59]. In contrast, some studies indicate no association [60, 61], and even increased risk of PPD with sufficient vitamin D concentrations (≥50 nmol/L) [62]. However, in a systematic review study, a few studies reported the role of vitamin D in this pregnancy outcome [44]. Thus, although postpartum depression might be associated with vitamin D deficiency, inconsistent results need for more extensive studies in this regard.

4.4 Bacterial vaginosis (BV)

Bacterial vaginosis (BV) is a common vaginal infection in women in reproductive ages caused by the replacement of normal vaginal flora for mixed anaerobic bacteria [63]. Women diagnosed with BV are more likely to have preterm delivery [64]. Evidence indicates that vitamin D deficiency is an independent risk factor for bacterial vaginosis in pregnancy [65], and in several systematic reviews and meta-analysis articles, this association has been reported [5, 52, 55, 66]. The risk of BV increases from 3- to 5-fold for serum 25(OH)D values <75 to <30 nmol/L, respectively [67–69]. Therefore, several cross-sectional and observational studies consistently stated the plausible inverse association between vitamin D status and BV during pregnancy. A possible mechanism is related to immune responses regulated by calcitriol. In fact, as mentioned above, vitamin D activates potent antimicrobial peptide hCTD, an active peptide with broad-spectrum antimicrobial activity, in the placenta, macrophages and dendritic cells, inducing the innate immune responses [70]. These findings indicate that adequate vitamin D levels are crucial to enhance immunity, especially in pregnancy.

4.5 Preterm delivery

Preterm delivery is defined as birth completed before 37 weeks gestation [71]. Infection is the most common factor in preterm delivery [72]. Vitamin D plays as an anti-inflammatory and immunomodulatory factor, enabling to reduce the risk of preterm delivery in several plausible ways. One mechanism involves response reduction to microbial pathogens via cancelation of IL-1, IL-6, and TNF-alpha production by macrophages [73]. Uterine immune cells, such as dendritic cells, macrophages, and natural killer cells are modulated by vitamin D [74], and vitamin D receptors are expressed in the ovary, endometrium, and myometrium to maintain reproductive health [75].

There are conflicting findings regarding the effect of Vitamin D on preterm delivery. Some articles including critical and narrative reviews, reported no association between vitamin D deficiency and preterm delivery [43, 46, 76, 77], while single studies, systematic review, and meta-analysis showed significant relationship between them [45, 78–81]. Inconsistent findings can be explained by different designs of each study including the timing of 25(OH)D assessment, and different definition of preterm delivery. For instance, in two studies, ≤35 weeks gestation for preterm delivery was considered [78, 82], while in another study it was <37 weeks gestation [77].

Ethnicity appears to be an important factor regarding preterm birth. Bodnar et al. reported that the risk of preterm delivery increased only in non-white mothers compared to white women [82]. In a cohort study by Wagner et al., Hispanic women with serum 25(OH)D > 40 ng/ml had 79% reduced risk of preterm birth in comparison with those with serum 25(OH)D ≤ 20 ng/ml, while the reduced risk was

45% among black women [83]. Also, an increased risk of preterm birth associated with Vitamin D insufficiency in ethnic minority women in Canada [84] suggests the stratification of women based on their ethnicity in further studies. Another important thing should be considered in study design is the association of vitamin D status in different gestational age with pregnancy outcome. For instance, one study suggested that maternal vitamin D status closest to delivery time was the best indicator for preterm birth because of the more significant reduced risk in the third semester compared to serum concentrations of vitamin D in the first and second semesters [85]. Thus, vitamin D deficiency is likely to be associated with preterm birth, nevertheless, to investigate the explicit relationship between vitamin D status and preterm delivery several factors should be noted including the timing of 25(OH)D assessment (first, second, or third trimester), ethnicity, precise definition of preterm birth, different study design such as human interventional and cohort studies.

4.6 Cesarean delivery

Uterine muscle and skeletal muscle cells have VDR, which is a regulator of the contractile proteins of uterine myometrial cells [86]. Therefore, the strength of the contractile muscles is decreased with vitamin D deficiency as well as malformation of the pelvis, which are indications for C-section [87]. The indication of C-section can be related to vitamin D deficiency, but there is a mixture of results regarding this association. For example, prospective cohort studies suggested there was an inverse association with having a cesarean section and serum 25(OH)D levels [88–90], whereas others did not support this relationship [76, 87, 91]. Regarding ethnicity, in a cohort multi-ethnic Asian study, vitamin D deficiency was related to a higher risk of C-sections in Chinese and Indian women compared to Malay women [86]. Factors such as the different definition of C-section in terms of indication, primary or secondary, emergency or elective might be the reason of inconclusive findings [91]. Although observational studies mentioned above investigated a possible relationship between vitamin D and cesarean delivery, more studies are needed to verify this association along with biological and geographical factors.

4.7 Recurrent pregnancy losses (RPL)

Recurrent pregnancy losses (RPL) and repeated implantation failures (RIF) are two auto- and cellular immune abnormalities, and vitamin D deficiency is prevalent in women with RPL and RIF. Vitamin D plays a pivotal role in the regulation of auto- and cellular immune disorders [92]. Studies reported that 45% of RPL patients had vitamin D deficiency in one study, and increased risk of first-trimester miscarriage and recurrent pregnancy losses (RPL) was found with vitamin D insufficiency [93, 94]. Even this association has been found in early spontaneous pregnancy loss [95]. Studies show an inverse association between vitamin D deficiency and RPL; however, no conclusion can be drawn based on the findings of a few studies and more observational and interventional studies are necessary to confirm such association [43].

4.8 Other related outcomes

Other outcomes including spontaneous abortion [77, 96, 97] and stillbirth [77, 96], short gestational length [98], and low Apgar score [76, 96, 98] were not associated with vitamin D insufficiency, according to a systematic review and meta-analysis of longitudinal studies [99]. However, more studies with different designs such as clinical trials are necessary. Anemia, lipid disorders, periodontal disease,

and HIV-related mortality are other pregnancy implications related to vitamin D deficiency. There is a mixture of results with regard to the vitamin D deficiency and anemia in pregnancy. Some studies indicated this relationship [100], while other studies failed to support it [76, 101]. The findings show a positive correlation between serum vitamin D and atherogenic factors such as total cholesterol and triglycerides [102, 103]. As vitamin D has immunomodulatory effects, it may have a protective role against mother-to-child transmission (MTCT) of the human immunodeficiency virus (HIV). A study on HIV-infected pregnant women conducted in Tanzania found that low maternal vitamin D level (<32 ng/mL) at 12–27 weeks gestation was associated with a 50% higher risk of MTCT of HIV [104]. Moreover, vitamin D insufficiency (serum 25[OH]D < 75 nmol/l) was associated with maternal periodontal disease during pregnancy, as reported by Boggess et al. [105].

Almost all the pregnancy complications related to vitamin D deficiency were discussed in this section. However, the results were contradictory due to small study samples in some studies, cross-sectional design, lack of adjustment for seasonal variation, race and ethnicity, various study design in terms of the trimester (first, second, or third trimester), different definition of some outcomes and even cut points to categorize vitamin D status. These inconsistencies justify the need for more large-scale prospective studies and interventional studies to evaluate these associations comprehensively.

5. Maternal vitamin D status across different ethnic groups

Vitamin D deficiency has been reported among pregnant women globally, particularly among ethnic minorities and white women residents at high latitude [106]. Ethnic variety in vitamin D status of pregnant women can be relevant to large disparities in skin color, vitamin D intake, religion, culture, sun exposure (seasonal variation), and geographical location [107]. In ethnic minorities, the prevention of vitamin D deficiency on a population basis is challenging due to the shortage of clarity surrounding the metabolism and transport of vitamin D [108]. Due to lack of enough evidence regarding the nutritional requirements for vitamin D during pregnancy, there is no pregnancy-specific dietary recommendation for vitamin D. In addition, the question of specific dietary reference setting specifically for each ethnicity (for pregnant women) has not been addressed to date [109].

The best indicator of vitamin D status is the serum 25(OH)D concentration, because it is not regulated, and reflects both dietary intake and synthesis form of vitamin D [110]. However, there is no absolute agreement on normal range of vitamin D, although most authors have consensus that serum 25(OH)D concentration should be ≥50 nmol/L [110–112]. Ethnic differences in vitamin D status are in accordance with the ethnic differences in circulating vitamin D, where a mean value of 57 nmol/L has been reported for Caucasian pregnant women in Canada [113], a median value of 53 nmol/L has been reported for Belgian women [114], 57 nmol/L has been reported for pregnant women in Australia [115]. A cohort study in 2011 indicated that the mean 25(OH)D level in African-Americans was 38.75 nmol/L, Hispanics was 60.25 nmol/L, and Caucasians was 72.5 nmol/L [116]. African-American pregnant women had the lowest serum 25(OH)D in this study. However, in Southern Europe, Morales et al. demonstrated a median plasma value of 25(OH)D in pregnancy of 73.88 nmol/L [117]. Furthermore, a recent systematic review reported that mean 25(OH)D levels in the general populations were higher in America (North America) (75 nmol/L) than in the Middle East (50 nmol /L) or Europe (52 nmol/L) [118].

The absence of race- and pregnancy-specific dietary recommendations places pregnant women of the ethnic minority among the most vulnerable and

under-investigated population groups concerning vitamin D [106]. A review study indicated that pregnant women in Latin America, Asia, the Middle East, and Africa are at the danger of vitamin D deficiency and these are known as the topmost universally locations for occurrence of vitamin D deficiency and reported that incidence of vitamin D deficiency varied from 51.3 to 100% [119]. Recent studies in the United States, Canada, Australia, Iran, Sweden, and Pakistan reported that the range of vitamin D deficiency and insufficiency were 24–95.8% and 54–100% among pregnant women [116, 120–125]. The evidence for racial differences in vitamin D deficiency (**Table 2**) indicated that the global prevalence of 25(OH)D concentrations <50 nmol/L is 54% among pregnant women and 75% among newborns [106]. In other words, one in five pregnant women (18%) and one in three newborns (20%) had serum vitamin D concentration <25 nmol/L, which is a public health concern [106]. Vitamin D status is poorly defined in the South-East Asian, African, and Eastern Mediterranean regions and the non-European population in the Western Pacific region (**Table 2**) [106].

Maternal vitamin D deficiency is also investigated for each trimester across different regions and ethnicities. For instance, in Thai pregnant women, high prevalence of vitamin D deficiency and inadequacy were reported in the first trimester: 26.7% (<50 nmol/l) and 56.7% (<75 nmol/l), respectively. Then, vitamin D inadequacy decreased to 30.9% (1.8% deficiency) in the second trimester and 27.4% (2.8% deficiency) in the third trimester [120]. Pregnant women were studied in the first trimester of pregnancy (gestational week 11–14) living in Mediterranean seacoast at latitude 36°N, therefore at a high sun exposure area, were classified as Spanish Caucasians and Arab immigrants [121]. The median serum 25(OH)D concentration for the whole sample was 68.39 nmol/L. Only 35.9% of the participants had adequate serum 25(OH)D concentrations (75 nmol/L), while these concentrations were found to be inadequate (50–75 nmol/L) in 41.4% and deficient (<50 nmol/L) in 22.7% of respondents. Vitamin D status was lower in Arab women in comparison to Caucasian women [121]. Another study reported that in second trimester 40.7% (<50 nmol/L) of Chinese pregnant women who lived at their urban locations had vitamin D deficiency [122]. According to a cross-sectional study in China, 74.9% of Chinese pregnant women had vitamin D shortage (25-hydroxyvitamin D < 50 nmol/L) [123]. In one of the National Health and Nutrition Examination Survey (NHANES) studies reported that among different factors, the largest magnitude of association was observed between race and 25(OH)D status. Those who were non-Hispanic whites in third trimester had a higher vitamin D levels (93 nmol/L) than non-Hispanic black (45 nmol/L) or Hispanic (69 nmol/L).

WHO regions	Percentage of 25-hydroxyvitamin D deficiency	
	% < 25* (nmol/L)	% <50** (nmol/L)
Americas	64	9
European	57	23
Eastern Mediterranean	46	79
South-East Asian	87	N/A***
Western pacific	83	13

Adopted by Saraf et al. [106].
**Vitamin D deficiency.*
***Severe vitamin D deficiency.*
****Not available.*

Table 2.
Prevalence of maternal vitamin D deficiency classified by WHO region.

Vitamin D insufficiency in this study was 13%, 54% among whites, 80%, 95% among blacks, and 45%, 83% among Hispanics for 25(OH)D concentrations <50 and <75 nmol/L, respectively [124]. A recent cohort study reported that 44% of non-European pregnant women in the Netherland had vitamin D deficiency compared to European ones either with maternal serum or cord blood [125]. While from one of the Persian Gulf countries (United Arab Emirates (UAE)), 69% of vitamin D deficiency was reported among pregnant women [126].

Generally, previous recommendations have indicated that winter, higher latitude, dark pigmentation, season, limited sun exposure, and dressing style as risk factors for vitamin D deficiency. It can be difficult to describe the prevalence of pregnant women at risk to vitamin D deficiency because vitamin D status varies according to how deficiency is defined, as well as aforementioned risk factors [127–129]. These studies indicated that despite the geographical location of each region, vitamin D deficiency is highly prevalent among pregnant women because of the lifestyle and nutrition status of mothers. Future studies are required to investigate vitamin D deficiency of pregnant women among different ethnic groups based on similar cut-off values for vitamin D deficiency.

As we discussed in Section 4, vitamin D deficiency has a key role in the development of PET and GDM. In this section, we elaborate on the differences that the occurrence of these two diseases might have among different ethnic groups.

5.1 PET and GDM among ethnic disparities

Pregnant minorities have been the least studied in vitamin D-related diseases such as PET and GDM. According to a meta-analysis, the occurrence of PET is seen at lower vitamin D levels due to the immunomodulation properties of vitamin D [52]. A recent study examined the evidence linking vitamin D deficiency in pregnancy to PET among different ethnic groups. Although prevalence of vitamin D deficiency differed significantly among African-Americans (72.3%), Hispanics (10.6%), and Caucasians (2.1%), there were no direct associations between low 25(OH)D levels and risk for PET among different ethnic groups [130]. While another recent study in UK indicated a greater prevalence of white British women developed GHT (12.0%) and PET (3.4%), compared to Pakistani women (the proportion of GHT and PET were 5.4 and 1.7%, respectively) [131]. The heterogeneity that exists among studies in terms of sun exposure (naturally occurring vitamin D synthesized from), geographical locations, considering different ethnic groups with divergent cultures, may lead to the differences in results.

It has been shown that maternal vitamin D concentration has inversely been linked to impaired glucose metabolism in pregnancy [132]. A few studies considered the effect of ethnicity on this relationship in pregnancy such as Clifton et al. study in Australia, which examined the association between serum 25(OH)D with GDM among five different ethnic groups including European (European Australian, English, German, Spanish), South-East Asian (Chinese, Japanese, Korean, Indonesian, Thai, Malaysian), Asian (Indian, Pakistani, Bangladeshi, Sri Lankan), Middle Eastern (Iranian, Iraqi, Lebanese, Syrian), and Other (Aboriginal Australian, Samoan, Papua New Guinean). They found no significant association between 25(OH)D and GDM in any ethnic group [132]. Generally, ethnicity was not an independent predictor of insulin resistance.

Overall, well-designed prospective cohort studies are needed to inform and update our knowledge regarding vitamin D deficiency and its association with PET and GDM among different ethnic minorities.

5.2 Latitude

Sun exposure decreases dramatically, with increasing latitude [129]. It seems logical that higher latitudes would report a higher proportion of vitamin D deficiency like Northern European countries, in comparison to the European South. In addition, almost all European Mediterranean countries are located at a high latitude (37–38°N), and previtamin D3 photosynthesis is lower during winter. Similar or even smaller proportion of vitamin D deficiency among pregnant women was reported from countries with higher latitudes in Western and Central Europe (latitudes 46–81°N) [133]; Slovenia with 46.5°N latitude, showed a high prevalence of severe vitamin D deficiency (23.6% with the threshold of <25 nmol/l) among pregnant women [134]. Even if in lower latitudes, the prevalence of maternal vitamin D deficiency is still high such as a study in China demonstrated that over 90% of pregnant women in urban northern China (39.9°N), had vitamin D deficiency (<50 nmol/l) and none had normal 25(OH)D concentrations (≥75 nmol/L) [135]. Although the Goulburn Valley in Northern Victoria (Australia) experiences abundant sunshine and located at 36° South of the equator, a place where vitamin D synthesis is possible during the year, 49.4% of pregnant women (<75 nmol/l) had vitamin D inadequacy and 12.2% (25–50 nmol/L) of pregnant women had a mild deficiency and 3.2% (<25 nmol/l) suffered a moderate/severe deficiency in summer [136]. A recent study from Bangkok, Thailand also revealed that 23.3% of Thai pregnant women had vitamin D deficiency in fall and 44.6% in winter (<50 nmol/L), despite the fact that this city is located at 13.45 N latitude and benefits sunlight throughout the year [137].

Over the past few decades, several studies have reported a high prevalence of maternal vitamin D deficiency in countries where women wear concealing clothing such as India (31%) [138], Saudi Arabia (14.2%) [139], and Iran (5.7%) [140] and countries in northern latitudes such as the United Kingdom (21.2%) [141] and Norway (33% deficiency and 18% severe deficiency across three ethnic groups) [142]. Due to less sunlight exposure, these pregnant women are at high risk of vitamin D deficiency. The duration of sun exposure is directly associated with the concentration of 25(OH)D [135, 138]. Mothers with less than 0.5 h/day of sun exposure had a higher prevalence of vitamin D deficiency (38.4%) in China, which would worsen the situation in winter that the tendency toward outdoor physical activity decrease among pregnant women [135]. However, in sunny regions like Turkey, vitamin D deficiency among pregnant women is still a serious problem with the prevalence of 90% [143]. In addition, the prevalence of vitamin D deficiency (48%) was noted among pregnant women in South Carolina at latitude 32°N, where women lived in a sun-rich climate for most of the year [144]. Despite the longer duration of sunlight in UAE (ranges from 9 to 11 h), 69% of pregnant women suffer from vitamin D deficiency. It can be due to the decrease in time spent on outdoor activities and to the dressing style, which limits the surface area exposed to the sun [126].

Overall, latitude is an important factor but the other factors such as culture, belief, indoor lifestyle, etc., would play key roles in vitamin D deficiency in sun-rich countries.

5.3 Skin pigmentation

Skin pigmentation is the main determinant of ultraviolet B-rays (UV-B) absorption in humans [129]. The absorption of UV-B would be more when the skin is pale compared with the darker skin. In other words, the probability of vitamin D deficiency would be higher when skin pigmentation increases in critical periods

like pregnancy [126]. Pregnant women who live in southern Europe have more pigmented skin, probably with less efficient vitamin D synthesis [145]. In Italy, vitamin D deficiency of pregnant women (with different ethnic groups) was examined based on their skin color (fair, black, light brown). It was found that 22% had severe vitamin D deficiency (<25 nmol/L) in total and fair-skinned mothers had higher 25(OH)D concentrations [146]. Another study in Netherland observed several ethnic backgrounds who reside in The Hague in the Netherlands (52°N), that vitamin D deficiency is more common among most non-Western participants (dark-skinned) (59–84%) compare to western women (fair-skinned) (8%) [147]. In southwestern Sweden, vitamin D deficiency was examined among pregnant women from different backgrounds with various skin colors. The prevalence of vitamin D deficiency was 10% overall, and 2% among mothers born in north Europe, while vitamin D deficiency among women born in Africa was 50% (<30 nmol/L) and deficiency was common among Asian mothers as well [148]. A recent US study reported in the multi-ethnic population of pregnant women in the southeastern USA, non-Hispanic black pregnant women are most at risk for vitamin D insufficiency (91%) compare to non-Hispanic white women (47%) and Hispanic women (75%) [149]. The concentration of melanin in the skin determines vitamin D production. Melanin, which absorbs UV-B in the 290–320 nm range, functions as a light filter and therefore regulates the proportion of the vitamin D production [150]. Thus, skin pigmentation is a dominant variable controlling the production of vitamin D under circumstances of low levels of sun exposures because melanin absorbs UV photons in competition with 7-dehydrocholesterol [151, 152]. Overall, darker-skinned pregnant women need a greater duration of sun exposure (4–5 times) (they are more at risk in higher latitudes) in comparison to light-skinned ones to provide a comparable amount of vitamin D [153].

6. Maternal vitamin D status and fetal and postnatal outcomes

A growing body of evidence suggests that maternal vitamin D deficiency is associated with not only pregnancy outcomes, but also later physical and mental health of the offspring. In this section, we aim to review the existing literature investigating the impact of maternal vitamin D status during pregnancy on a range of fetal and postnatal outcomes.

6.1 Fetal skeletal development

During pregnancy, the fetus relies on maternal supply and placental delivery of vitamin D and calcium for optimal development and function, particularly of the skeletal system [154]. Maternal vitamin D deficiency during pregnancy predisposes breast-fed infants to neonatal hypocalcemia and infantile rickets [155, 156]. It has been reported that maternal UV-B exposure in pregnancy is related to bone mineral content (BMC) and bone mineral density (BMD) at age 9.9 years independently of height and lean mass [157]. Further, winter newborns in Korea were found to have 6% lower total body BMC, lower cord serum 25(OH)D and 1,25-dihydroxyvitamin D than summer newborns [158]. These results suggest that low maternal vitamin D concentrations due to limited UV-B exposure may exert direct effects on offspring bone mineral accrual.

The influence of maternal vitamin D status on bone outcomes has been investigated in several observational studies at fetal [159–161], postnatal [162–164], and adult stages [115, 165–167]. Using data from a prospective longitudinal study, Mahon et al. observed that lower maternal 25(OH)D concentration was associated with the

greater femoral metaphyseal cross-sectional area at 19 weeks gestation and 34 weeks gestation. However, lower maternal 25(OH)D concentration was not related to fetal femur length [159]. In another prospective longitudinal study, Young et al. found a significant positive association between maternal 25(OH)D levels and fetal femur and humerus z-scores only when maternal calcium intake was <1050 mg/d [160]. It has also been reported that maternal serum 25(OH)D concentration, together with maternal height and adiposity, was significant predictors of femoral size [161]. In a cross-sectional study with a longitudinal follow-up, tibia BMC was 0.047 g/cm higher, and cross-sectional area was 12.3 mm^2 larger in newborns with the first trimester and cord serum 25(OH)D above the median (42.6 nmol/L) compared with below median newborns [162]. In another study, Morley et al. found smaller knee-heel length in infants of mothers with low 25(OH)D levels (<28 nmol/L) in late pregnancy (28–32 weeks of gestation) compared to babies whose mothers had higher concentrations [163]. Dror et al. reported no association between feto-maternal vitamin D status and early infant whole-body BMC. However, a large percentage of mothers in their study had adequate vitamin D status, which may have affected the results [164]. These observations suggest that maternal vitamin D status during pregnancy can influence fetal bone growth as early as the second trimester of pregnancy.

Emerging evidence suggests that the relationships between maternal vitamin D status and bone outcomes persist into childhood and beyond [115, 165, 166]. In a longitudinal study by Javaid et al., it was found that reduced concentration of maternal 25(OH)D during late pregnancy was associated with reduced whole-body and lumbar-spine BMC in children at age 9 years. Besides, childhood bone mass was predicted by maternal use of vitamin D supplements and the estimated exposure to UV-B radiation during late pregnancy [165]. Zhu et al., in a study of 341 mother and offspring pairs, found a positive association between maternal 25(OH)D concentration and total body BMC and BMD in offspring at 20 years of age [115]. These results were confirmed in another study where maternal vitamin D deficiency (25(OH)D < 50 nmol/L) during pregnancy was associated with lower peak bone mass at 20 years [166]. In contrast to these studies, Lawlor and colleagues observed no relevant association between vitamin D deficiency in pregnant women and offspring's BMC in late childhood [167]. In another study, compared to children born to mothers with high vitamin D status, tibial BMC was lower at birth in children with low maternal vitamin D status, while BMC gain was greater, resulting in similar BMC at 14 months [168]. The evidence derived from observational studies, although conflicting, tends to suggest an association between vitamin D deficiency in pregnant women and reduced bone mineral accrual in the offspring, which may increase fracture risk in later life.

6.2 Birth anthropometry, small-for-gestational-age (SGA), and childhood growth

There is controversy regarding the relationship between maternal vitamin D status and neonatal birth weight, with some studies reporting an association between reduced maternal vitamin D levels and lower neonatal birth weight [169–174], and others showing no association [175–178]. While some studies found no association between maternal vitamin D levels and other anthropometric birth outcomes such as length and head circumference [176–179], others suggested a nonlinear relation between 25(OH)D levels and head circumference [171, 174].

Inconsistencies in the literature have led several researchers to conduct meta-analyses to further clarify the association between maternal vitamin D concentrations and anthropometric outcomes in offspring. Four meta-analyses of observational studies have been published in recent years [45, 52, 180, 181]. In

the first study, Wei et al. carried out a meta-analysis on six studies and found that pregnant women with circulating 25(OH)D levels less than 50 nmol/L experienced an about 1.5-fold increased risk of SGA [45]. These results were confirmed in another meta-analysis of 13 prospective cohort studies, in which a significant positive association was found between maternal vitamin D deficiency and risk of SGA infants (pooled odds ratio 1.58; 95% CI 1.14–2.22) in the random effects model [180]. Similar results were obtained in subgroup analyses by study quality (high vs. low), gestational week for blood sampling (first trimester vs. second trimester vs. mixed), cut-off vitamin D levels (<10 ng/mL vs. <15 ng/mL vs. <20 ng/mL), sample size (N > 1000 vs. N < 1000), adjustment for critical confounders (Yes vs. No), and method for measuring vitamin D (liquid chromatography with tandem mass spectrometry vs. others) [180]. There results suggest that maternal vitamin D deficiency may be associated with an increased risk of SGA infants.

A meta-analysis conducted in 2013 showed that insufficient serum levels of 25(OH)D in pregnant women was associated with increased risk of having SGA infants (pooled odds ratio 1.85; 95% CI 1.52–2.26) [52]. In terms of birth weight, the authors used data from four observational studies and found infants of mothers with low 25(OH)D concentrations during pregnancy (<37.5 nmol/L) had lower birth weight (random weighted mean difference −130.9 g). However, no significant difference was observed between maternal vitamin D status and other anthropometric outcomes such as birth length and head circumference [52]. The most recent meta-analysis conducted by Tous et al. included 54 eligible studies and reported that vitamin D-deficient mothers (<30 nmol/L) had offspring with lower birth-weight (mean difference −87.82 g), head circumference (mean difference −0.19 cm), and a higher risk of SGA infants (odds ratio 1.59; 95% CI 1.24–2.03) compared to mothers with concentrations ≥30 nmol/L. However, no difference was observed in terms of infants' length [181]. The results also revealed a significant association between vitamin D insufficiency (<50 nmol/L) and increased risk of SGA (odds ratio 1.43; 95% CI 1.08–1.91). The authors found no significant differences in birth-weight and SGA between offspring born to mothers with 25(OH)D concentrations <75 nmol/L and those born to mothers with 25(OH)D concentrations ≥75 nmol/L [181]. Further, a meta-analysis of 13 interventional studies revealed that vitamin D supplementation during pregnancy was associated with significantly higher circulating 25(OH)D levels (mean difference: 66.5 nmol/L), birth weight (mean difference: 107.6 g), and birth length (mean difference: 0.3 cm), when compared to the control group [182]. Neonates exposed to low levels of vitamin D *in utero* (<30 nmol/L) showed accelerated growth in length and weight during the first year of life to compensate for their small initial size [183, 184]. There results suggest that maternal vitamin D deficiency is associated not only with increased risk of SGA infants but also with lower birth-weight. However, more studies are required to draw a firm conclusion on the relationship between maternal vitamin D status and other anthropometric outcomes.

6.3 Offspring soft tissue body composition

A growing body of evidence suggests that maternal vitamin D status in pregnancy may play a part in the offspring adipogenesis [185–189]; however, not all studies are in agreement [190, 191]. Tint et al. conducted a study to examine the association between maternal 25(OH)D status at mid-gestation and neonatal abdominal adipose tissue (AT) compartments. The findings indicated an inverse liner correlation between maternal 25(OH)D and both superficial and deep subcutaneous AT compartments measured by magnetic resonance imaging (MRI). In addition, compared to neonates born to mothers with 25(OH)D sufficiency

(>75.0 nmol/L), neonates with maternal 25(OH)D inadequacy had higher superficial and deep subcutaneous AT volumes, despite similar birth weight [185]. However, in a prospective observational study, Godang et al. reported a positive association between neonatal total body fat mass (FM) and umbilical cord plasma, but not maternal, 25(OH)D [190]. This was confirmed in another study where a positive correlation between cord blood 25(OH)D levels and neonatal percentage body fat was observed [192]. Using data from the Southampton Women's Survey, Crozier et al. found that lower maternal vitamin D status at 34-week gestation was associated with lower FM in the offspring at birth but with greater FM at ages 4 and 6 years [186]. Similarly, a prospective pregnancy cohort conducted by Daraki et al. showed that offspring of mothers in the low 25(OH)D tertile (<37.7 nmol/L) had higher BMI and waist circumference at preschool age, compared with the offspring of women with higher 25(OH)D concentrations, and this relationship persisted at age 6 years [187]. Results from a recent prospective cohort conducted in 476 mother/infant dyads also demonstrated that reduced maternal 25(OH)D (first quartile compared to the fourth quartile) was associated with lower birth weight for gestational age z-scores (−0.43 units) but higher 1-year weight-for-length (0.78 units) and 3-year BMI z-scores (0.83 units) in offspring [188]. In another study, maternal deficit of 25(OH)D (<50 nmol/L) was reported to be associated with increased risk of fetal overweight defined as abdominal circumference ≥90th percentile or either as the estimated fetal weight ≥90th percentile. Moreover, a significant association was found between deficit of 25(OH)D in pregnancy and increased risk of overweight in offspring at age 1 year. However, this association was attenuated at age 4 years [189]. In contrast to these studies, Ong and colleagues, in a mother-offspring cohort in Singapore, observed no significant associations between maternal vitamin D status and any of the adiposity outcomes (i.e., BMI and skinfold thickness) at birth or postnatally. This was partly explained by the low prevalence of severe maternal vitamin D deficiency in the studied population (mean maternal vitamin D concentration of 81.3 nmol/L) [191]. These findings suggest that intrauterine exposure to low 25(OH)D concentrations may be linked to lower FM at birth but greater FM during childhood. A higher FM may also contribute to decreased 25(OH)D levels in obese individuals, partly due to the sequestration of vitamin D by AT [193].

A limited number of studies have investigated the influence of maternal vitamin D status on offspring lean mass or muscle strength [194, 195]. Using data from Mysore Parthenon Study, Krishnaveni et al. reported that Indian children born to vitamin D-deficient mothers (serum 25(OH)D < 50 nmol/L) had a smaller arm-muscle area at ages 5 and 9.5 years in comparison with children born to mothers without deficiency. However, no difference in grip strength was observed between the offspring of mothers with and without vitamin D deficiency [194]. In contrast, a study conducted in 678 mother-child pairs showed that maternal serum 25(OH)D concentration in pregnancy was positively associated with height-adjusted hand grip strength but not muscle mass in offspring at 4 years of age [195]. These results suggest that low maternal vitamin D status may be associated with the smaller arm-muscle area and lower muscle strength in the offspring. However, more observations are required to draw a firm conclusion on this matter.

6.4 Respiratory health

In humans, lung development starts *in utero* with the formation of two endodermally derived lung buds and continues through childhood, adolescence, and early adulthood [196]. A growing body of evidence suggests that the origins of respiratory disorders such as asthma can be traced back to the fetal period when the lung is undergoing rapid development [197, 198]. It has been shown that vitamin D

is involved in the process of maturation of the fetal lung including type II alveolar cells maturation and the alveolarization [199]. Currently, there are four meta-analyses published within a 3-year period from 2016 to 2018, which examined the association between maternal 25(OH)D levels during pregnancy and the offspring's respiratory conditions [200–203]. The first meta-analysis included eight studies on the association between maternal vitamin D status and childhood asthma or wheeze. This meta-analysis showed no statistical association between maternal vitamin D during pregnancy and risk of childhood asthma or childhood wheeze [200]. In contrast, the second meta-analysis included 15 prospective studies with 12,758 participants and found a U-shaped relationship between 25(OH)D levels during pregnancy and risk of childhood asthma, with the lowest risk at approximately 70 nmol/L [201]. The third meta-analysis assessed the association of both cord blood and maternal 25(OH)D levels with the risk of offspring's asthma, wheeze, and respiratory tract infections. The results revealed borderline significant inverse associations between *in utero* exposure to vitamin D and risk of asthma and wheeze, but not the risk of respiratory tract infections in offspring [202]. In the final and most recent meta-analysis, Pacheco-González et al. found an inverse association between prenatal exposure to 25(OH)D and the risk of respiratory tract infections. The authors also observed a positive borderline association between maternal or cord blood 25(OH)D levels and lung function at school age. However, no associations were found for asthma and wheeze [203]. The apparently conflicting results of meta-analyses may be partly explained by differences in inclusion criteria, the number of studies, the characteristics of participants and the methodology used. Further, the results of two other meta-analyses revealed that higher maternal intake of vitamin D was associated with lower odds of wheeze during childhood [204, 205]. Taken together, these results suggest the role of maternal vitamin D status as a protective factor for the development of offspring respiratory disorders.

6.5 Immunity and allergies

Some studies are suggesting that low maternal or cord blood 25(OH)D concentrations are associated with an increased risk of developing atopic disorders, including atopic dermatitis or eczema, allergic rhinitis, asthma, and food allergy [206–209]. A study conducted in 270 mother-child pairs showed that a higher cord blood 25(OH)D concentration was associated with reduced risk of eczema in children at 1 and 3 years of age. However, no significant associations were found between cord blood 25(OH)D concentration and the development of allergic rhinitis, allergic sensitization, or asthma [206]. In another study, cord serum 25(OH)D levels were found to be inversely associated with the risk of atopic dermatitis by the age of 5 years, but no association was reported with allergic rhinitis and asthma [207]. Chiu et al. observed a significant association between low cord blood 25(OH)D levels and increased risk of milk sensitization but not eczema, allergic rhinitis, or asthma in early childhood [208]. In another study with the same authors, it was revealed that lower maternal 25(OH)D levels (<20 ng/ml) were associated with a higher prevalence of allergen sensitization before age 2, and higher maternal 25(OH)D levels were associated with lower risk of eczema and asthma at age 4 [209]. Wei et al. conducted a meta-analysis of prospective cohort studies to examine the association between maternal vitamin D status and childhood allergic diseases. They found an inverse association between maternal vitamin D status during pregnancy and risk of childhood eczema but not childhood asthma or wheeze [200]. However, a recent meta-analysis of observational studies reported no significant associations between 25(OH)D levels in cord blood at birth or maternal blood in

pregnancy and the risk of atopic disorders [203]. Taken together, these studies indicate that lower maternal 25(OH)D concentration may be associated with an increased risk of developing atopic disorders.

Here, we discuss the role of intrauterine vitamin D exposure on the risk of two common autoimmune diseases—type 1 diabetes and multiple sclerosis. Type 1 diabetes mellitus is an autoimmune disease characterized by the destruction of the insulin-producing pancreatic β-cells of the Langerhans islets [210]. Staples et al. found an inverse association between annual ambient UV radiation exposure and prevalence of type 1 diabetes in Australia, which was suggested to be due to the role of UV radiation in vitamin D synthesis [211]. In a case-control study conducted in 109 cases and 219 control women, it was observed that lower levels of vitamin D during pregnancy were associated with a higher risk of developing type 1 diabetes in offspring before 15 years of age [212]. In contrast, in another case-control study conducted in Finnish women, Miettinen et al. found no difference between serum 25(OH)D levels during early pregnancy between mothers whose children later on developed type 1 diabetes, and mothers of healthy children [213]. Thorsen and colleagues showed that normal variation in maternal or neonatal 25(OH)D levels has no significant effect on the risk of childhood type 1 diabetes [214]. Jacobsen et al. also failed to find an association between 25(OH)D levels around the time of birth and the risk of developing type 1 diabetes before the age of 18 years [215]. As these studies found inconsistent results, more research is still needed before any conclusions can be drawn on the relationship between maternal vitamin D status and risk of developing type 1 diabetes in offspring.

Multiple sclerosis (MS) is an autoimmune disease, in which the immune system attacks the myelin sheaths surrounding nerve cells [216]. Accumulating evidence suggests an association between the month of birth and risk of MS [217–219], possibly due to variation in UV exposure, which in turn determines maternal vitamin D levels during pregnancy. In support of this hypothesis, Munger et al. reported that maternal vitamin D deficiency (<30.0 nmol/L) during early pregnancy was associated with increased risk of MS (almost 2-fold) in the offspring compared with women with normal 25(OH)D levels [220]. A nested case-control study demonstrated that exposure to high 25(OH)D levels during the years preceding disease onset was associated with decreased risk of MS in the offspring. However, no decrease in risk of MS was observed in the offspring exposed to high levels of 25(OH)D *in utero* (≥75 vs. <75 nmol/L) [221]. Nielsen et al. also carried out a matched case-control study and found an association between lower levels of 25(OH)D in neonates and increased risk of MS [222]. However, in another population-based case-control study conducted in Sweden, Ueda et al. failed to find such as association [223]. These results suggest that exposure to low levels of 25(OH)D prenatal and in early postnatal life may act as a risk factor for developing MS.

6.6 Offspring brain development and function

A growing body of evidence suggests that low maternal 25(OH)D levels during pregnancy are associated with impaired neurodevelopmental and neurocognitive outcomes during infancy and childhood [117, 224–228]; however, not all studies are in agreement [229, 230]. In this regard, several studies have shown a significant association between suboptimal maternal vitamin D status and reduced language developmental outcomes in the offspring [224–226].

Accumulating evidence also suggests a link between maternal vitamin D levels and the risk of developing neuropsychiatric disorders such as attention deficit hyperactivity disorder (ADHD) [231, 232], autism spectrum disorder (ASD) [233–236], and schizophrenia [237]. Results from a prospective pregnancy cohort

conducted in 487 mother-child pairs revealed that children born to mothers in the high 25(OH)D tertile (>50.7 nmol/l) had decreased risk of developing ADHD-like symptoms at 4 years of age, compared to children of women in the low 25(OH)D tertile (<38.4 nmol/l) [231]. In another prospective study conducted in 1650 mother-child pairs, Morales et al. found an inverse association between maternal circulating levels of 25(OH)D in pregnancy and risk of developing ADHD-like at ages 4–5 years [232]. Using a case-control design study, Chen and colleagues reported that low maternal first-trimester serum levels of 25(OH)D was associated with increased odds of ASD diagnosis at age 3–7 years in the offspring [233]. Another study also found an increased risk of ASD in children who were born to mothers with vitamin D deficiency at mid-gestation [234]. In a large population-based cohort of mothers and their children, gestational vitamin D deficiency was associated with a continuous measure of autism-related traits at 6 years [235]. Magnusson et al. also observed a relationship between maternal vitamin D deficiency and risk of ASD with, but not without, intellectual disability [236]. Further, it has been demonstrated that low maternal vitamin D levels are associated with increased risk of schizophrenia within the subgroup of black but not white individuals [237]. Although more robust studies needed, these results highlight the importance of maternal vitamin D status in offspring brain development and function.

7. The association of maternal vitamin D status and child vitamin D status

Several lines of evidence suggest that there is a strong association between maternal 25(OH)D levels during pregnancy and newborn 25(OH)D concentrations at birth or in the early neonatal period [238–242]. Novakovic et al. reported that maternal circulating 25(OH)D levels were the most significant regulator of neonatal circulating vitamin D concentrations, even over the impact of genetic factors [243]. In another study, maternal characteristics explained 12.2%, and maternal 25(OH)D concentrations explained 32.1% of the neonatal vitamin D variance [13]. These results were confirmed in a systematic review reporting the range of correlation coefficients between maternal and newborn 25(OH)D concentrations, by region: European 0.42–0.95, America 0.68–0.97, Western Pacific 0.19–0.85, South-East Asian 0.78–0.81, and Mediterranean 0.03–0.88 [106]. Therefore, since maternal vitamin D status in pregnancy is an important determinant of neonatal 25(OH)D concentrations, attention should be given not only to vitamin D-deficient pregnant women, but also to their newborns, especially if they are exclusively breast-fed [244].

8. Policy and best practices (supplementation/education)

Vitamin D deficiency is very prevalent across the globe among pregnant women [245]. The range of recommended vitamin D from during pregnancy varies from 200 to 4000 IU/d worldwide. The American Pregnancy Association stated that pregnant women are recommended to have (100 μg/d) of vitamin D intake to reduce the risk of premature birth and infections which is a considerably higher amount of vitamin D compared to the recommended intake of 10 μg/d for women [246]. A daily intake of 600 IU is suggested during pregnancy in China to have healthy and balanced fetal growth [247]. In the United Kingdom, it is advised to have a maternal vitamin D intake of 400 IU/d. Switzerland follows the IOM dietary recommended nutrient intake. For pregnant/lactating women who are at risk of vitamin D deficiency, the advised vitamin D is 1500–2000 IU/d, and for women,

without deficiency, the recommended intake is 600 IU [248]. The ministry of health of New Zealand recommended 200 IU/d dietary intake of vitamin D [249]. Vitamin D requirements are higher among pregnant women (average 400 IU/d), and it is very important to maintain optimum serum level of vitamin D during maternity and for the fetus growth.

WHO encourages receiving vitamin D from a healthy and balanced diet [250]. Some recent studies suggest vitamin D food fortification can work as a means to improve vitamin D status among the overall population, which can benefit pregnant women as well [251]. Currently, Canada, United States, India, and Finland had a vitamin D food fortification policy. In North America, the foods that are naturally enriched by vitamin D, such as fatty fish, are quite expensive and are not readily available to the general population. A majority of vitamin D intake comes from fortified food in North America. The United States has a voluntary fortification policy, and Canada has both voluntary and mandatory fortification policies for specific foods. In the United States, milk including fluid, acidified, cultured, skimmed powder, evaporated milk (1.05 μg/100 g), soy-based beverages (1.25 μg/100 g), soy products (2.23 μg/100 g), margarine (8.3 μg/100 g), butter alternatives spread (8.25 μg/100 g), cheese alternatives spread (6.25 μg/100 g), yogurt (2.22 μg/100 g), fortified fruit juice (2.5 μg/240 mL), meal replacement products (2.5 μg/40 g), cheese products (2.02 μg/30 g), and enriched ready to eat cereal (8.75 μg/100 g), rice, cornmeal, noodle, macaroni (2.25 μg/100 g), farina (8.75 μg/100 g), and instant formula (1–25 μg/100 kcal) are vitamin D fortified [252]. Mandatory fortified products in Canada are margarine (1.5 μg/10 g), infant formulas (10 μg /L), milk (powder, sterilized, flavored, skim, evaporated) (2.5 μg/250 mL), meal replacements (5% of DV/55 g), soy beverages, and soy beverage products (1.5–3 μg/250 mL) [253]. Some recent studies have shown a high prevalence of vitamin D inadequacies despite the mandatory fortification. The Department of Health, the Government of Canada, recently announced that vitamin D fortification levels need to be increased to alleviate the risk of rickets in children and osteomalacia in adults. It proposed that by the end of December 2022, vitamin D level in cow's milk, and goat's milk will be increased to 2 μg/100 ml (current range 0.9–1.2 μg/100 ml) and in margarine, it will be increased 26 μg/100 g (current range 13.3–17.5 μg/100 g) [254]. Fluid milk (2.5 μg/250 mL) and margarine (2 μg/10 g) are also fortified in Finland that had helped the general Finish population to improve vitamin D status. Similar to the United States, Canada fortification policy, edible oil, margarine (0.55 μg/10 g), cow's milk (1.25 μg/250 mL), and ready to eat cereals (5 μg/1/2-¾ cups) are fortified in Australia [255]. Similar approaches can be adopted by other countries to improve vitamin D inadequacy among pregnant women as well as the general population.

Supplement intake can also play an important role in improving vitamin D status among pregnant and lactating women. A recent study assessing maternal vitamin D inadequacies showed that vitamin D supplementation of ≤2000 IU/d minimizes the chance of neonatal mortality [256]. Taking a vitamin D supplement may also reduce the risk of PET, GDM, and low birth weight during pregnancy [257]. The current WHO guideline recommends 200 IU/d of vitamin D supplement intake among pregnant women with vitamin D deficiency to reduce the risk of PET, low birth weight, and preterm birth [258]. In Turkey, free supplementation of vitamin D (1200 IU/d) is provided to all women from early pregnancy to 6 months after delivery [259]. In Canada, pregnant women are suggested to take a vitamin D supplement of 400–600 IU/d [260]. A similar vitamin D supplementation intake approach (400 IU/d) is followed in New Zealand as well during pregnancy The United Kingdom Health Department provides free vitamin D supplementation to pregnant women and newborn children and recommends taking 10 μg (400 IU) of

vitamin D supplements during pregnancy and lactation [261]. Taking vitamin D enriched food and supplement can be advised to maintain optimum serum levels during pregnancy.

9. Conclusion

The existing evidence reveals the importance of adequate vitamin D status during pregnancy for the mother, fetus, and child, although more studies are needed to clarify the exact mechanisms. In a situation where optimal vitamin D status cannot be achieved through diet and sun exposure, food fortification and supplementation seem to be proper approaches. Policies for vitamin D fortification vary across the globe, while more countries are recognizing the importance of vitamin D. The current evidence indicates the need for vitamin D supplementation in pregnant women in a situation that fortification alone cannot address the needs [262]. To our knowledge, only a few countries have free supplementation policies during pregnancy and early life. Effective systematic approaches by relevant agencies and governments are required to synthesize evidence-based recommendations for vitamin D supplementation during pregnancy and customize interventions considering cultural factors to ensure the optimal vitamin D status for pregnant women and their newborns.

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Chapter 4

Nutritional Considerations of Vitamin D Deficiency and Strategies of Food Fortification

Sami El Khatib and Malak Abou Shahine

Abstract

Vitamins and minerals are crucial for human health. Any deficiency can lead to major diseases; however, the most prevalent one is the vitamin D deficiency. Due to its high risk in the Middle East and Lebanon, besides its major effects, solutions to decrease this deficiency are taken nowadays. Vitamin D food fortification is the most popular solution taken now. Liposomes showed highest efficiency in vitamin D fortification. However, a study must be done in order to deduce the amounts needed in the targeted population. Therefore, before fortification starts, FDA regulations must be reviewed. Several foods succeeded in fortification with vitamin D and increasing its levels such as milk and cheddar cheese. Stability and flavors showed good results over fortification, while according to the odor, water sources showed more aroma depth than oil sources. The AOAC methods for vitamin D amount in fortified foods must be applied. Dietary 25(OH)D3 was 7.14-fold more effective at raising serum 25(OH)D than dietary vitamin D3.

Keywords: vitamin D, food fortification, health, FDA, Middle East and Lebanon

1. Introduction

Human body is a complex matrix that requires various components for its healthy functioning. Vitamins and minerals are crucial elements that are often obtained from one's diet; these are referred to as micronutrients. Most of the latter are not produced by the human body and an unbalanced diet could inevitably lead to various nutrient deficiencies that could pose dramatic health risk for the human body that if severe enough could lead to death. Nutrient deficiency could be a direct effect of the changing eating patterns, but mostly due to poverty and lack of access to proper food especially in low- and middle-income societies. For this reason, studies and research were directed toward fortifying consumed food with vitamins that are most likely to be deficient and their deficiency could pose tremendous health problems. One of the most popular nutritional deficiencies in Lebanon and the Middle East is vitamin D deficiency. Due to the high frequency of vitamin D deficiency in Middle East and Lebanon, it is essential to find a product that is widely consumed among the population and provides a good source of vitamin D regarding its stability. Dairy products are one of the most famous consumed foods in our society, which is why it was chosen to be fortified with vitamin D.

2. Nutrition and vitamins

2.1 Factors contributing to individual wellness

Healthy lifestyle improves individual strength, prevents certain illnesses, and maintains an advisable weight. Wellness incorporates physical, emotional, and spiritual well-being (**Figure 1**). The concept of wellness is not an end point in a person's life but rather an ongoing lifestyle. Diet and exercise are so linked to each other that they can be considered opposite sides of a coin. An individual's nourishment is impacted by how much energy is used to perform daily exercises; meanwhile, physical activity directly affects the utilization of supplements in food. Consuming a nutritious diet allows one to progressively perform strenuous exercises for longer durations and prompts an overall better feeling. On the other hand, an imbalanced food intake could lead to inactivity or, in extreme cases, cause severe medical issues [1].

2.2 Essential nutrients for health

Our daily consumed foods include bread, rice, dairy products, fruits, vegetables, and meats. Each of them is composed of various nutrients, which are each characterized by its unique chemical composition. Each has its own function; however, they work together to complete body's functions. Food nutrients are carbohydrates, proteins, fats, vitamins and minerals, and water. Fiber is likewise a fundamental part of our eating routine [2].

Macronutrients (carbohydrates, lipids, and proteins) are those that the body requires in huge quantities to give energy and maintain growth, whereas micronutrients including vitamins and minerals are those nutrients required in lesser quantities to help normal health and body functions [1].

Figure 1.
Factors contributing to individual wellness [1].

2.2.1 Macronutrients

Macronutrients (carbohydrates, lipids, and proteins) are energy-providing nutrients [1].

- Carbohydrates: consist of the three main constituents: carbon, hydrogen, and oxygen. They are made of 3 groups: monosaccharides, disaccharides, and polysaccharides. Monosaccharides are single unit carbs such as glucose. Disaccharides are composed of two sugars connected together. An example of this class is lactose, which is known as milk sugar. Polysaccharides are those formed by more than two sugars and are generally more complex chains such as starch and dextrin [2].

- Fats: is an essential part of our body. Fat serves a vital role in protecting the cells and tissues of the essential organs in our body such as brain and heart. Food fats are composed of solid fats, fluid oils, fat-soluble vitamins, and cholesterol. Fats contain oxygen less than carbohydrates. Hence, 1 g of lipids gives the body more energy than carbohydrates (9 Cal/g of fat compared to 4 Cal/g of carbohydrates) [2].

- Proteins: are huge natural compounds. Proteins, similar to starches and fats, contain carbon, hydrogen, and oxygen. Likewise, proteins contain around 16% nitrogen, which differentiates them from starches and fats. Proteins are used mainly for growth and development purposes [2].

2.2.2 Micronutrients

Nutrients required in little quantities to have healthy and normal body functions are "micronutrients" [1].

- Minerals: are inorganic elements that are not broken down by digestion, absorption, or heat. Minerals support with the regulation of body functions and are classified into major and trace minerals [1].

Minerals are needed for body-building; enhancing bones, teeth, and structural parts of soft tissues; muscle contraction; clotting of blood; and nerve stimuli [2].

- Vitamins: are organic compounds that assist in controlling physiological processes [1]. They engage in various activities throughout the entire body and help in the release of energy from the macronutrients [3].

2.2.3 Water

Water represents around 60 percent of the body weight. It is necessary for the use of food materials in the body and for disposal of food excess later on [2].

2.3 Vitamins

Vitamins (natural carbon-containing composites) adjust many of the body's processes [1]. They are available in small amounts; however, they are essential for physiological functions as growth and development. Vitamins can work as antioxidants, cofactors in metabolic oxidation-reduction responses, and hormones [4].

2.3.1 Fat-soluble vitamins

Vitamins A, D, E, and K are fat-soluble. They remain inside the fatty parts of foods and are absorbed along with dietary fat. Fat-soluble vitamins have good storage in the body since they are kept in the adipose tissues. Fat-soluble vitamin toxicity symptoms include hair, skin, bones, eye injuries, and anorexia nervosa [1].

2.3.2 Water-soluble vitamins

Fat-soluble vitamins include the B vitamin and ascorbic acid (vitamin C). They are distributed in many foods. Water-soluble vitamins are absorbed easily via the enteral tract straight into the blood and then into the cells. Water-soluble vitamins are not stored in large amounts, except vitamin B12. Thus, they should be eaten daily [1].

2.4 Vitamin deficiencies

Vitamin deficiency is the condition of a long-term lack of a certain vitamin. Most common and serious vitamin deficiencies are as follows [1]:

- Vitamin A, which leads to vision defect, impaired growth, and immunity
- Vitamin D, which leads to rickets, osteomalacia, or osteoporosis
- Vitamin K, which leads to coagulopathy and bone health impacts
- Vitamin B12, which leads to pernicious anemia, nerve damage, memory loss, and dementia
- Vitamin C, which mainly leads to Scurvy disease, fractures, and depression

3. Vitamin D

Vitamin D, known as the sunshine vitamin, is a real hormone delivered from body's sterols by the photolytic activity of UV light on the skin [4]. It is a fat-soluble seco-steroid that may be either created within the skin from its precursor (7-dehydrocholesterol) by exposure to sunlight or offered from diet [5, 6]. Vitamin D with calcium, magnesium, and phosphorus plays a crucial role to support bones and teeth health [4].

3.1 Vitamin D sources

Vitamin D can be attained from foods as vitamin D3 (cholecalciferol) or as nutrient D2 (ergocalciferol). D3 is acquired from animal sources, while D2 is available in parasites and mushrooms irradiated with UVB [7]. Vitamin D approximate content in various foods is shown in **Table 1** [8].

Since most foods contain low measurements of vitamin D (**Table 1**), fortifying common foods is becoming a trend and common practice nowadays. In addition, nutritional supplements are a reliable solution for compensation of the vitamin deficiency [4].

Food	Approximate vitamin D content (μg/100 g)
Fortified	
Milk	0.8–1.3
Margarine	8–10
Nonfortified	
Butter	0.3–2
Milk	<1
Cheese	< 1
Liver	0.5–4
Fish	5–40

Table 1.
Major food sources of vitamin D [8].

3.2 Vitamin D needs and normal levels

The Food and Nutrition Board has set an adequate intake (AI) for vitamin D due to the inability to set a more precise RDA level because of the variability in sun exposure among individuals [9]. Recommendations for vitamin D instructed an AI for vitamin D of at least 500 IU/day (12.5 μg) and more than 1000 IU/day (25 μg) for those not exposed to enough sunlight [8].

International units are used to quantify vitamin D. 1 IU is equal to 25 metric weight unit of cholecalciferol, and 1 g of cholecalciferol is equal to 40 IU [7].

3.3 Metabolism and regulation

Metabolism of vitamin D occurs through different stages that includes hydroxylation. Through these stages, vitamin ingested is transformed into its active form (**Figure 2**) [4].

Vitamin D synthesis starts when 7-dehydrocholesterol (cholesterol precursor found on skin) is visible to sunlight and then transformed to previtamin D [9, 10]. 7-Dehydrocholesterol is an intermediate precursor for vitamin D, it is found throughout the epidermis and dermis, and thus has the most elevated limit with respect to cholecalciferol synthesis [11].

The first step occurs when 7-dehydrocholesterol absorbs the UVB photons to convert them into previtamin D3. Then, photoisomerization occurs in order to covert previtamin D3 into vitamin D3 (cholecalciferol). However, it is not a problem since during delayed sun exposure previtamin D3 is converted into its inactive forms (lumisterol and tachysterol) [12].

Large portion of vitamin D reaches liver from lipoproteins or vitamin D–binding protein and is then transformed by hydroxylation to yield 25-OH-D3 [4]. 25-OH-D3 is the major circulating type of vitamin D used by clinicians to evaluate vitamin D level, though it is latent and must be transformed to its active form (1α,25-dihydroxyvitamin D) in the kidneys [13].

When calcium decreases in the body, parathyroid hormone is released leading to calcitriol synthesis increase (vitamin D active form: 1α,25(OH)2D) (**Figure 3**). Because of the hypocalcemia (declined blood calcium), PTH is released from parathyroid gland resulting in an increase in the hydroxylation of 25(OH)D3 to calcitriol. Then, calcitriol plays its role with PTH or by itself on its target tissues leading to an increase in serum calcium levels. Kidneys, bones, and intestines are the primary target tissues [8]:

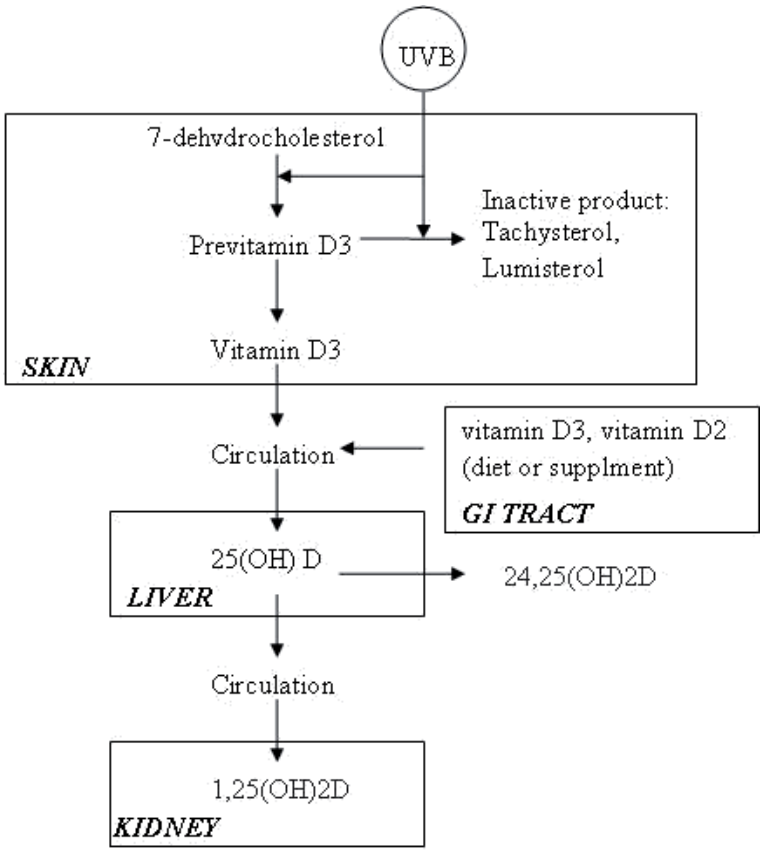

Figure 2.
Sources, sites, and processing of vitamin D metabolites [10].

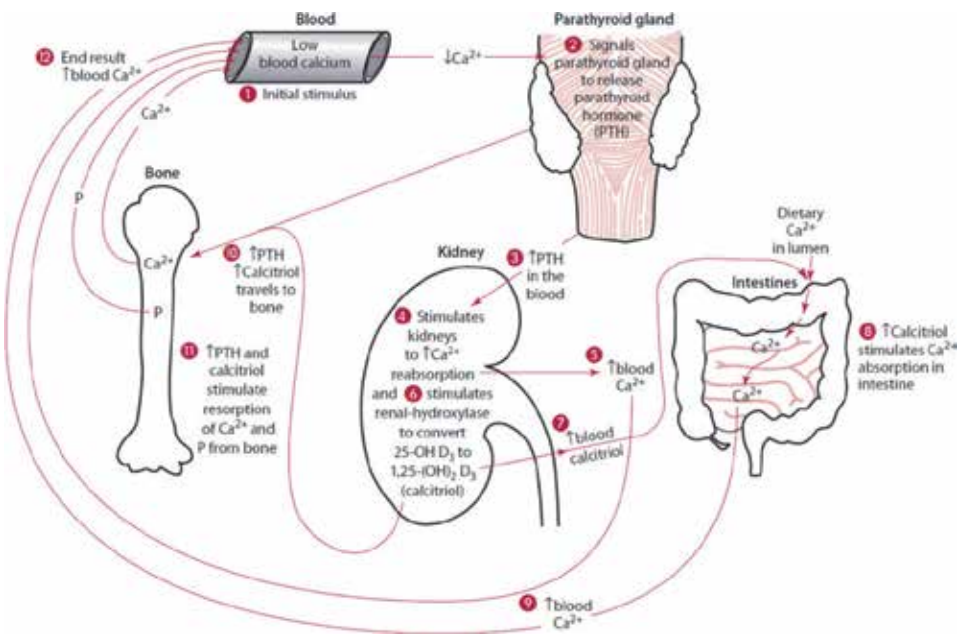

Figure 3.
Calcitriol, 1,25 (OH)2 D3, synthesis and actions with parathyroid hormone (PTH) [8].

3.3.1 Intestine

Increasing calcium and phosphorus absorption is the major role of calcitriol in the intestines. It works by the participation of calcitriol with cell membrane receptors to improve calcium absorption [8].

3.3.2 Kidney

Calcitriol with the help of PTH increases calcium reabsorption in the distal renal tubule into the blood. Phosphorus released by the kidney is boosted and can result in lower serum phosphorus levels [8].

3.3.3 Bone

Regarding the bone, PTH plays its role with calcitriol for reabsorption increase of calcium and phosphorus from bone to reach normal blood calcium level. Osteoclasts catalyze calcium and phosphorus from bone. The net impact of this is to raise blood calcium and phosphorus levels [8].

3.4 Factors affecting vitamin D production

Vitamin D skin synthesis is negatively influenced by factors such as the following [6]:

- Season: winter season decreases the quantity of ultraviolet light reaching the skin, while summer season increases it.
- Skin pigmentation: dark pigments interfere with UV light entering the needed skin layer.
- Clothing: covering the skin leads to inadequate sunlight skin exposure.

3.5 Clinical manifestation of vitamin D levels

Calcidiol (25(OH)D) is the vitamin D metabolite that is estimated to identify a patient's vitamin D status [14]:

- Vitamin D deficiency: 25(OH)D between 21 and 29 ng/mL
- Vitamin D lack: 25(OH)D < 20 ng/mL
- Normal vitamin D status: 25(OH)D > 30 ng/mL
- Vitamin D overproduction: 25(OH)D > 40–60 ng/mL
- Vitamin D toxicity: 25(OH)D > 150 ng/mL

However, females are at 3 times higher risk of having vitamin D deficiency compared to males. There is no relationship between the age and vitamin D levels in males, but in females, those having an age between 30 and 40 are at higher risk for deficiency. But, children (10–20 years) are at highest risk for deficiency [15].

3.6 Vitamin D transport

3.6.1 Transfer from chylomicrons to plasma

Practically, all consumed vitamin D is held in a nonesterified structure, which is believed to be associated with the outside of chylomicrons (lipoprotein particles). Vitamin D that is not moved in the plasma is taken up with chylomicron remainders by the liver, where it is then transferred to the same binding protein and discharged to the plasma [4].

3.6.2 Vitamin D–binding protein

Vitamin D is transferred in the plasma to a great extent in association with protein, as other sterols, which is the vitamin D–binding protein (DBP) [4].

3.7 Vitamin D storage

Vitamin D storage in the liver is minimal, in contrary to other fat-soluble vitamins. Vitamin D levels do not go above 25 nmol per kg in the liver. Plasma calcidiol (25-hydroxyvitamin D) is the storage form of vitamin D, which has a half-life of 3 weeks [11]. Long-standing admission of vitamin D inside the physiological range induces storage in fat tissues and most likely in other tissues of clinical importance [16].

3.8 Vitamin D toxicity

Vitamin D toxicity is incredibly uncommon; however, it can occur by unplanned or purposeful ingestion of unreasonably high portions. Portions of more than 50,000 IU every day raise levels of 25-hydroxyvitamin D to more than 150 ng for each milliliter (374 nmol/L) as well as hypercalcemia and hyperphosphatemia. Portions of 10,000 IU of vitamin D3 every day for as long as 5 months do not cause toxicity [13]. Excessive exposure to sunlight represents no danger of toxicity through overproduction of endogenous cholecalciferol [8].

When calcium plasma concentrations reach 2.75–4.5 mmol/L, vitamin D toxicity causes several symptoms such as nausea, appetite loss, cramps, and diarrhea, and in more severe cases, it causes hypercalcemia. When plasma calcium levels exceed 3.75 mmol/L, hypertensive encephalopathy occurs due to the contraction of smooth muscles. Hypercalcemia and expanded vitamin D levels lead to soft tissue calcification (kidneys, heart, and lungs) [11].

4. Vitamin D deficiency

The most popular and worldwide deficiency currently is vitamin D deficiency [17]. It is a worry for public health and has several acute and chronic impacts. It results from wrong lifestyle starting in predominance obesity and inadequate sun exposure [18].

4.1 Vitamin deficiency

Living with a lifestyle that is inadequate in any food group will result in a vitamin deficiency. A vitamin deficiency results in different diseases and disorders. The clinical signs and symptoms are the final stage in hypovitaminosis [4].

Stages of vitamin deficiency are as follows:

1. Marginal deficiency
    - Stage I—depletion of vitamin stores
    - Stage II—cellular metabolic changes
2. Observable deficiency
    - Stage III—clinical defects
    - Stage IV—morphological changes

During marginal deficiency, there is only depletion of vitamin stores and its effect on cells. This depletion cannot be recognized without chemical or biochemical testing, which shows the stores' concentrations. However, in observational deficiency, the signs and symptoms appear and morphological changes take place [4].

4.2 Vitamin deficiency causes and effects

Vitamin D deficiency is caused by the following [13]:

1. Decreased synthesis in the skin: due to creams, aging, and skin pigment, the vitamin D3 synthesis will be reduced.
2. Decreased bioavailability: obesity and malabsorption diminish the availability of vitamin D3.
3. Breastfeeding: decreased amount of vitamin D in human milk can lead to vitamin D deficiency when the child is exclusively breastfed.
4. 25-hydroxyvitamin D diminished synthesis: vitamin D malabsorption caused by liver failure results in 25-hydroxyvitamin D diminished synthesis.
5. 1,25 dihydroxy vitamin D diminished synthesis: decreased phosphorus excretion and decreased serum levels of 1,25-dihydroxyvitamin D are caused by chronic kidney diseases.

4.3 Manifestation of vitamin D deficiency

Vitamin D deficiency's first stages include rickets and osteomalacia, whereas the final and long-term stages include osteoporosis in which there are chronic changes [19].

4.3.1 Rickets

Vitamin D deficiency in children leads to rickets. Rickets is classified by bone mineralization loss [8]. This occurs due to deficiency in both vitamin D and calcium [7]. Consequently, the bones twist due to their inability to hold the body, stand, or walk [3].

4.3.2 Osteomalacia

As rickets occur in children, osteomalacia occurs in adults [4]. Osteomalacia is the result of the demineralization of bones [7]. In the case of osteomalacia, nonmineralized bones are much more than mineralized bones. Consequently, the

patient will have muscular weakness, bone tenderness, and pain and will be at higher risk of fractures [4].

The decreased ability to produce vitamin D3 in elderly due to the decrease in 7-dehydrocholesterol in the skin results in osteomalacia also. Therefore, vitamin D is essential for preventing and treating osteomalacia in elderly [7].

4.3.3 Osteoporosis

Osteoporosis is the result of inability to synthesize or get enough vitamin D from sunlight and food. This results in bone calcium loss and thus fractures [3]. It is due to multiple causes as aging, weakened vitamin D digestion, and decreased estrogen levels. It is widely seen in elderly, postmenopausal women, and those taking steroids [4].

4.4 Vitamin D deficiency prevention steps

Vitamin D deficiency can in fact be prevented by different methods. These can include the following [13]:

- Expose body to sun or ultraviolet radiation for the needed time.
- Ingestion of 400–100 IU/day for kids and 800–1000 IU for grown-ups.
- Control serum phosphate in case of kidney disease.
- Sustaining maintenance level.

4.5 Vitamin D deficiency in Middle East and Lebanon

International Osteoporosis Foundation (IOF) study showed that almost all countries have 25(OH)D3 levels beneath 30 ng/ml, while South Asia and Middle East have levels beneath 10 ng/ml [19].

The most widely seen medical issue in Middle East and North Africa is vitamin D deficiency. This results in diseases and disorders and most widely osteomalacia. Results showed 50–90% 25(OH)D3 deficiency, and these patients have levels below 20 or 30 nmol/ml [15].

Results showed higher rates of vitamin D deficiency in Lebanon especially Beirut regions as 57.86% were deficient using a cut-off of 20 ng/ml [15].

Females are 3 times more prone to have hypovitaminosis D. An investigation on Lebanese grown-up population has demonstrated that hidden ladies had just about 3 times more incidence of extreme hypovitaminosis D than nonhidden subjects [15].

Results verified that females are at more risk to have vitamin D deficiency. Besides, hidden ladies are 3 times more at risk than nonhidden ladies [15].

5. Vitamin D fortification

5.1 Food fortification basic principles

Fortification is the process of supplementing food with needed nutrients for their health benefits and in order to prevent diseases as defined by the Codex General Principles [20].

Fortification levels differ from one nation to another according to different factors. These factors include the dimension of enrichment, the bioavailability of

the fortificants, and the range of fortified consumed foods. Fortification can be achieved by adding a single nutrient (such as the addition of iodine to the salt), or by adding a blend of nutrients [20].

The general medical advantages of fortification include the following [20]:

- Prevention of micronutrient deficiency
- Correction of a micronutrient deficiency
- Achieving the health benefits of a nutrient (for example, there is some proof to recommend that a diet full in selected antioxidants may aid in preventing cancer and different disorders.)

5.2 Vitamin D fortification history

In order to prevent or defend rickets, humans usually used one teaspoon of cod liver oil daily (1 teaspoon has 10 μg = 400 IU of vitamin D). Vitamin D food fortification started in the 1940s in the US and other nations such as Britain. In the beginning, vitamin D was supplemented to milk and then to other foods and beverages. However, in the 1950s, events of hypercalcemia appeared, and an adjustment was done for fortifying foods with vitamin D. As a result of that, few side effects of hypercalcemia had been seen in newborn children in the former German Democratic Republic, where babies were enriched with discontinuous portions of 15 mg (600,000 IU) of vitamin D as a push to defend rickets. In these newborns, serum 25(OH)D levels increased to a few hundred nmol/L [17].

5.3 Vitamin D3 fortification forms

Various techniques for fortification of foods with vitamin D were accomplished. In a study done for the evaluation of fortifying cheddar cheese with vitamin D methods, vitamin D3 was supplemented by: addition of a water-soluble emulsion, crystalline liposoluble vitamin D, or water-soluble vitamin D multilamellar liposomes [21]. Results showed better recovery of vitamin D3 in liposomes than that in homogenized cream and water-dissolved emulsions. Similar results were shown in commercial water and fat-soluble types of vitamin D3 in delivering an evenly dispersed concentration of vitamin D3 in prepared cheddar [22].

Vitamin D3 was fortified into a cheddar-like matrix, yogurt, or dessert in either a crystalline or an emulsified structure. The emulsified structure was more stable in cheddar over a three-month storage period at 4°C, with roughly 6% of the crystalline vitamin D3 lost under these conditions, while the two types of vitamin D3 were stable in yogurt and dessert with storage for the normal shelf life of the items [23].

5.4 Data required for determination of vitamin D fortification levels

Vitamin D fortification levels determination requires the following [24]:

1. Survey about the quality of the diet and amount of ingested nutrient in a target population
2. Average sun exposure adequacy in accomplishing vitamin D adequate levels
3. The recent vitamin D levels in the country

When the fortification program is achieved, supervising and assessments should continue to get better data and higher effectiveness [24].

5.5 Factors affecting vitamin D food fortification

Vitamin D food fortification is affected by factors such as availability of fortificants and suitable vehicles for the fortificant [24]:

1. Availability of mechanical experience in producing D2 and D3.

2. Having a good capacity for raising 25-hydroxyvitamin D blood concentration.

3. Having different formulas that suit all foods: the fat-rich items and fat-poor foods. For example, a formula of dry preserved vitamin D is found and has benefits as it contains antioxidant that defends the strength of vitamin D for extended time, yet within the appearance of minerals.

4. Having the proper vehicle for fortification:

    a. Widely consumed food must be used as fortification vehicle, financially available, and consumed all through the year.

    b. The fortification vehicle must ensure an even scattering by economical technologies.

    c. Vitamin D bioavailability in fortified items, for example, milk, milk powder, and cheddar.

    d. Vitamin D fortification vehicle must have negligible olfactory impact.

5.6 FDA regulations

FDA authorizes the use of nutrients as supplements and additives after enough scientific safety reviews. The additive must be used under the law to stay in the safe side. Several studies were done to set a law for the use of vitamin D and its safety [25].

Vitamin D is available in different structures. The two main structures are vitamins D2 and D3. Vitamin D is certified and commonly identified as safe (GRAS) to be used in food, with the following exact conditions [25].

FDA authorized adding vitamin D to infant formulas, calcium-fortified foods and beverages, breakfast cereals, certain cheese and cheese products, soy beverages, and milk and milk products [25] (**Table 2**).

Category of food	Maximum levels in food (as served)
Breakfast cereals	350 IU/100 g
Grain products and pasta	90 IU/100 g
Milk	42 IU/100 g
Milk products	89 IU/100 g

Table 2.
Maxim levels of vitamin D in foods [25].

The IOM (Institute of Medicine) specified the maximum allowed daily intake as UL of a supplement that represents safety of conflicting impacts when the supplement is ingested over delayed time [25].

5.7 Foods fortified with vitamin D

The USA began vitamin D milk fortification in 1930s. In the USA, vitamin D food fortification is optional. They focus on food vehicle and the practical and dimensional use in order to avoid fortification above what is recommended by the FDA. The addition of vitamin D commonly in conjunction with calcium is usually to several foods including orange juice, breakfast grains, rice, and soy milk [24].

Vitamin D widely used concentration is 100 IU per serving. The consumption of foods that have been fortified with vitamin D significantly increased 25(OH)D blood levels. The average personal intake of almost 11 μg/day (440 IU/day) from fortified foods (range 120–1000 IU/day) raised 25(OH)D levels by 7.7 ng/mL. Said relation correlates to a 0.5 ng/mL rise in 25(OH)D for every 40 IU (1 μg) ingested/day calories [24].

5.7.1 Milk

A couple of studies have researched the long impact of additional vitamin D3 on the initial vitamin D load of milk. Hollis in his article "Vitamin D and Its Metabolites in Human and Bovine Milk" demonstrated a 10-fold rise of vitamin D3 consumption from 100 to 1000 μg/d and contributed a 7·5-fold rise in vitamin D3 levels of the milk and a 2-fold rise in 25(OH)D3 [26].

5.7.2 Eggs

Two frequently used strategies to fortify eggs with vitamin D are: increasing sunlight exposure and supplementing bird diet with vitamin D. It is proved that eggs that were exposed to light have higher vitamin D3 content. Browning and Cowieson verified the effectiveness of diet enrichment with vitamin in increasing both vitamin D3 and 25(OH)D3. However, Browning, Cowieson, and Duffy verified that addition of 25(OH)D3 had positive impact on 25(OH)D3 level of the egg yolk. Thus, within a diet, 25(OH)D3 could be consumed straightforwardly by hens with no exchange to vitamin D3 within the circulation [26].

5.7.3 Cheddar cheese

It is verified that vitamin D is stable during pasteurization, manufacturing, and maturing. It is evenly distributed in the cheddar and does not influence the flavor. The positive part is that vitamin D has the same effect in supplements and in cheddar matrix. The disadvantage in fortifying cheddar with vitamin D is the loss in the wheying off step. However, it is solved by decreasing the whey load before manufacturing and the use of ultrafiltration (UF) [27]. Ganesan proved vitamin D stability over a 9-month maturity period while using typical types of vitamins. The use of UF to cheddar milk results in little yet considerable reductions in vitamin D losses in whey [28].

5.7.4 Bread

Bread is a widely consumed food item; it is prepared from flour and water by baking, as well as nongrain material such as nuts, fats, raisins, and nutrients to improve the nutritional quality of the meal eaten. Vitamin D can be added during different stages (fermentation, baking, and storage). It is verified that 60 min is the

reasonable time for dough fermentation, since when raising the fermentation time, oxidation of vitamin occurs [5].

5.8 Stability, odors, and off-flavors

In the USA, milk is commonly enriched with vitamin D with an amount of 400 IU per quart. Vitamin D fortification occurs before pasteurization step, and the liquid milk shelf life is usually 1 year at room temperature and in dark [29].

5.8.1 Stability

The stability of vitamin D throughout production and storage in dairy and milk products is verified, as well as its stability in high temperature short time handling and storage without light and acid [29].

Vitamin D loses its stability in some conditions. It decreases in acidic media as it transforms into its inactive form isotachysterol, or by heating to a temperature above 150°C in the presence of air [27]. However, it is stable at temperatures below zero, at 4–8°C, at 25°C, and during cooking at 200°C [24].

In the study of elevating vitamin D3 under two different conditions, high temperature processed decreased fat milks (increased temperature/decreased time, pasteurized at 73°C for 15 s, and ultra-heat treated, purified at 138°C for 2 s) and reduced fat yogurt (purified at a temperature of 85°C for a half hour), it is verified that the amount of vitamin D does not decrease during preparation [30]. In addition to that, it was stable during storage and has no effect on sensory qualities [23].

Banville found that vitamin D3 fortification in cheddar in the liposome form decreased after 3–5 months of maturing and lost its stability [21]. It is shown also that vitamin D3 has stability in nonfat foods such as in squeezed orange (storage for 30 days at 4°C) [31]. Both vitamin D3 and vitamin D2 showed their stability in fortified squeezed orange [32].

5.8.2 Flavor

Even at maximal level of fortification (1200 IU), there were no flavor differences in vitamin D–fortified milk. This shows that increasing the concentration of vitamin D is better to improve the nutritional quality and increase vitamin D amount ingested and do not influence the liquid milk flavors [29].

5.8.3 Odors

Odors were affected by vitamin D fortification, as they give rancid oil and painty odor. However, it is shown that the aroma depth is way more in water source than that in oil source. In oil sources, the aroma depth is lesser due to the oil matrices found that defend oxidation. After vitamin D oxidation occurs, aldehydes are found in the product [29].

5.9 Measurement of vitamins D2 and D3 in foods

The Association of Official Analytical Chemists (AOAC) International, which set up authority and "legally defensible analytic" strategies in the United States, has approved the accompanying chemical techniques for the examination of vitamin D in foods [23]:

Method 980.26: vitamin D in multivitamin preparations

Method 981.17: vitamin D in fortified milk and milk powder

Method 982.29: vitamin D in mixed feeds, premixes, and pet foods
Method 992.26: vitamin D3 in ready-to-feed milk-based infant formula
Method 995.05: vitamin D in infant formulas and enteral products (for tube feeding)
Method 2002.05: cholecalciferol (vitamin D3) in selected foods (fortified milk, infant formula, gruel, margarine, and cooking and fish oil)

The AOAC strategies recorded above are alike on a basic level. Every strategy includes four key advances [23]:

1. A digestion step to break down the food matrix (alkaline saponification)

2. An extraction step for the separation of vitamin D from the food matrix

3. A clean-up step, to isolate vitamin D from other food components

4. Quantitative detection by HPLC with UV

However, these strategies are time-, work-, and expert-consuming. Besides that, they are allowed only for restricted items (generally dairy), and not suitable for nonfat items in certain countries. The AOAC techniques target vitamin D3, and not vitamin D2, that might be available either normally or as a fortificant [23].

Instrumental techniques for the analysis of vitamin D in foods incorporate [23]:

- partition by high-pressure liquid chromatography (HPLC),

- detection by ultraviolet absorption (UV), and

- diode array detection (DAD) or mass spectrometry.

The accompanying approach was created, approved, and used to survey vitamin D3 levels in Danish pork and dairy items [33, 34]:

1. Homogenization and addition of vitamin D2 internal standard

2. Digestion by alkali saponification for 45 min in a boiling water bath

3. Extraction with petroleum ether: diethyl ether

4. Clean-up with silica solid-phase extraction columns

5. Purification with semipreparative HPLC (amino + silica columns, normal phase)

6. Detection by reverse-phase HPLC with DAD

5.10 Vitamin D fortification efficacy

It is verified that the oral use of 25(OH)D3 is efficient in rising plasma 25(OH) D levels [26]. The common level of vitamin intake range is between 10 and 20 μg/d. Cashman in his study gave adults (mean age of 57 years) 20 μg vitamin D3 or 20 μg 25(OH)D3 for those having with serum 25(OH)D of 28·9 nmol/l in winter. The results showed after 2.5 months treatment an increase in both vitamin D3 and 25(OH)D3. Jetter in his study provided fit postmenopausal ladies with 20 μg 25(OH)D3 or 20 μg vitamin D3 for about 4 months over the winter. The results showed better effectiveness of 25(OH)D3 than that of dietary vitamin D3 [26].

Results showed better effectiveness for 25(OH)D3 in absorption and increasing the plasma levels. In addition to that, 25(OH)D3 is better for human health as Bischoff Ferrari et al. proved a decline in systolic blood pressure and enhancements in a few markers of immunity in healthy postmenopausal ladies. Also, 25(OH)D3 supplementation corrected the excess bone turnover, improved plasma lipid level rise in HDL cholesterol, and diminished LDL-cholesterol in osteopenic and dyslipidemic postmenopausal ladies [26].

6. Conclusion

After referring to various studies considering fortifying foods and dairy products with vitamin D, it is proven that dairy product vitamin D fortification is an effective strategy to diminish the prevalence and incidence of vitamin D deficiency among populations. This is because this method is cost-effective regarding its positive effect on public health and its impacts with minimal sensorial changes including flavor and order. Dairy products have been shown to be a suitable food type to be fortified with vitamin D. Milk and many cheese products, and yogurt are fortified with vitamin D and have shown to be stable and an effective source of vitamin D, and this is why we can consider other types of dairy products to be fortified with vitamin D like Labneh, in which further research will be done in order to test for stability of vitamin D and the most appropriate form to be used in it. According to the appropriate form of vitamin D used in dairy products, liposomes of vitamin D are effective for cheddar cheese fortification. This method proves the effectiveness and liposomes of food fortification that has been followed for years, a strategy that tremendously leads to decreasing the burden of nutrition deficiency among populations especially in developing countries. In fact, public awareness stays a must in promoting education about vitamin D deficiency, its symptoms, risk factors, and causes.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

An Evidence-Based Review of Efficacy and Safety of Dietary, Natural Supplements and Sunlight in Vitamin D Deficiency

Jenson Mak

Abstract

There have been recent concerns about the propensity of calcium and vitamin D supplementation to cause cancer. In osteoporotic patients, this has led to increasing recommendations advocating the replacement of calcium supplementation with dietary or other means. Around the world, the problem of vitamin D deficiency remains, being a large contributor of rickets and osteomalacia in the developing world and osteoporosis in post-menopausal women and people dependent on long-term corticosteroid treatment. We review the alternatives of vitamin D supplementation through dietary, other natural supplements as well as sunlight therapy, in an evidence-based manner. We will also review the safety aspect of each modality.

Keywords: vitamin D deficiency, osteoporosis, rickets, bone health, diet, natural supplements

1. Introduction

Vitamin D is a fat-soluble vitamin utilised by human beings for normal bone development; maintenance by increasing the absorption of calcium, magnesium and phosphate; as well as potential pleiotropic effects on the prevention of cancer, heart disease, autoimmune disease, type 2 diabetes and depression [1].

A circulating level of 25-hydroxyvitamin D greater than 30 ng/mL (50 nmol/L) is considered a healthy level of vitamin D to maintain. About 1 billion people in the world have vitamin D deficiency, ranging from children (leading most notably to rickets), pregnant and lactating women, certain racial groups, post-menopausal women and the elderly (leading to osteoporosis) [2].

Vitamin D deficiency arises from multiple causes including inadequate dietary intake and inadequate exposure to sunlight. About 50–90% of vitamin D is absorbed through the skin via sunlight, whilst the rest comes from the diet.

Vitamin D clinical trials have increased over the past decade. Despite these trials, studies (including meta-analyses) have provided inconsistent conclusions. Some meta-analyses make the assumption that vitamin D is a pharmacological agent, and not as a nutrient. Bolland [3, 4] and Zhao's [5] findings suggest that vitamin D supplementation does not prevent fractures or falls (and in fact one study suggested it may contribute to falls in community-dwelling older women at higher dosages [6],

Figure 1.
In many countries, there is an abundance of sunlight throughout the entire year. Ironically, the sun-protective messages for the primary prevention of skin cancers become stronger than those of the moderate benefits of sunlight.

or have clinically meaningful effects on bone mineral density, and suggested 'little justification to use vitamin D supplements to maintain or improve musculoskeletal health'). A recent umbrella review did conclude that most RCTs have been carried out in populations that are not vitamin D deficient and, because of this, possible beneficial effects from vitamin D supplementation cannot be excluded [7].

Further, complementary medicine supplementation receives less stringent regulation for approval to the general public. For example, in Australia, vitamin D or other supplements which claim that it 'may' improve bone health and general well-being are approved on the Therapeutic Goods Administration as an ARTG [8] compared with vitamin D as a medication.

Given the urgent need for the general population, clinicians and consumers will benefit from the latest evidence for vitamin D supplementation (through diet, extraneous supplementation and sunlight therapy), including its efficacy and potential harm (**Figure 1**).

2. Epidemiology

Vitamin D deficiency is a major global public health problem in all aged groups, particularly in the Middle East. There is striking lack of data in infants, children and adolescents worldwide and in most countries of South America and Africa [9].

Of concern, even in countries where there is sun exposure all year-round, reports of deficiency is common in infants (90% in Turkey, 46% in black Americans, 96% in Kuwait and 99% in Indian), in children (58% in Belgium, 72% in Malaysia and 95% in Afghanistan), in adolescents (50% of non-whites in Canada, 46% in China and 45% in the United Arab Emirates) and in adults (52% of women in New Zealand, 97% in 62% in Korea and 77% in Brazil).

3. Pathophysiology

Vitamin D is crucial in calcium and vitamin D metabolism, as its absence around 15% of dietary calcium and 60% of phosphorus is absorbed. 1,25-Dihydroxyvitamin D interacts with the vitamin D receptor which increases

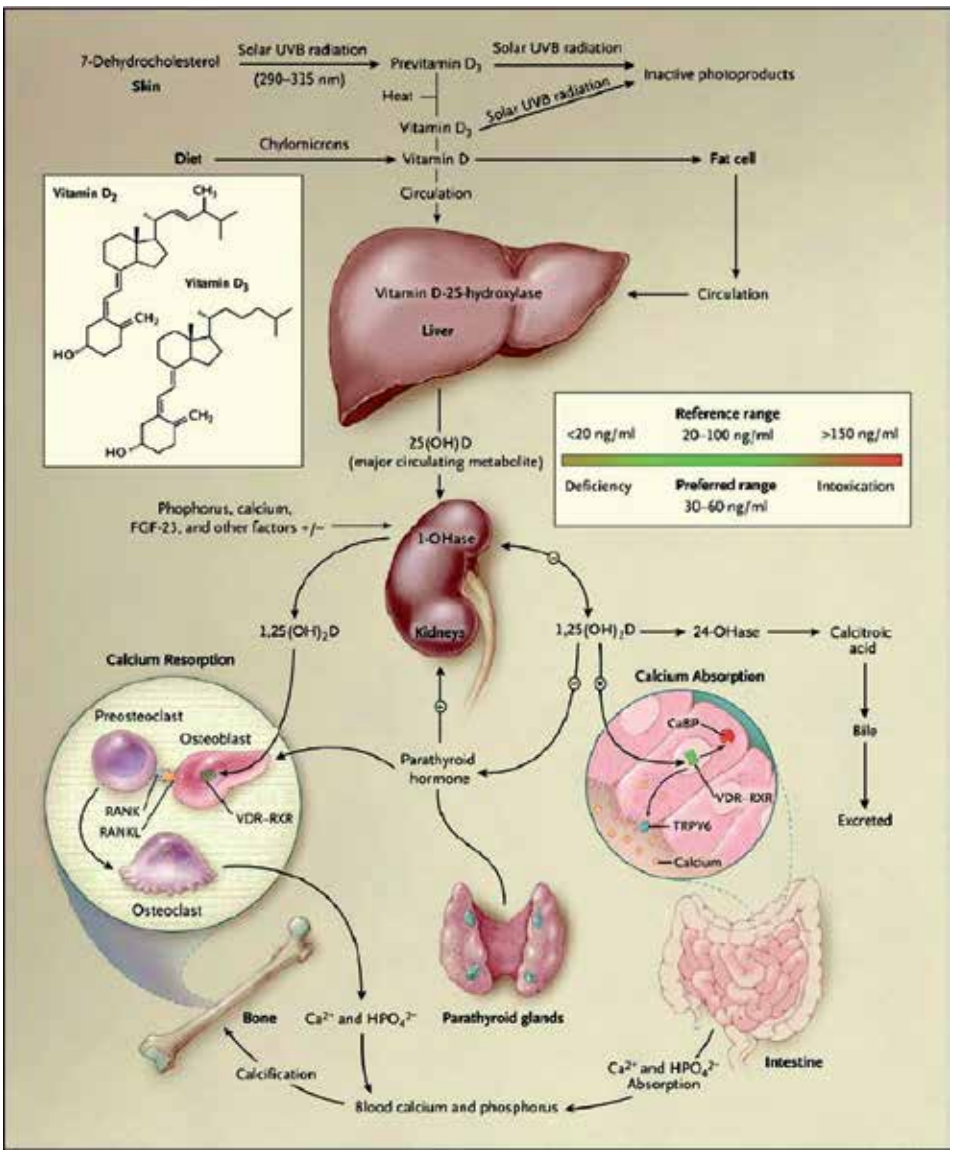

Figure 2.
Vitamin D Metabolism (From Horlick [10]).

the intestinal calcium absorption to 40% and phosphorus to 80% (**Figure 2**, [10]). Vitamin D is transformed for activation through two hydroxylations in the body. Among the metabolic products or modified versions of vitamin include calcitriol (1,25-dihydroxyvitamin D3); the active form of vitamin D, with a 15-hour half-life; and calcifediol (25-hydroxyvitamin D3) with a 15-day half-life [10]. Vitamin D is bounded to specific receptors located throughout the body. Whilst serum 25(OH) D levels do not indicate the amount of vitamin D stored in body tissues, it is the best indicator of vitamin D status.

When the level was 30 ng/mL or less, there was a significant decrease in intestinal calcium absorption that was associated with increased parathyroid hormone [10]. Further, there is strong support from reports that maternal vitamin D deficiency leads to overt bone disease (congenital rickets) from before birth to post-natal life [11]. Throughout the world, there has been widespread debate as to the serum 25(OH)D concentrations associated with deficiency (e.g. rickets), for optimal bone health and optimal overall health, and most cut points have not been developed by an agreed mutual scientific process. The US Institute of Medicine concluded that individuals are at risk of vitamin D deficiency at serum 25(OH)D concentrations <30 nmol/L (<12 ng/mL). However, some individuals are potentially at risk for inadequacy even at levels ranging from 30 to 50 nmol/L (12–20 ng/mL). The committee agreed with sufficient levels at ≥50 nmol/L (≥20 ng/mL) and that 50 nmol/L is the serum 25(OH)D level that covers the majority of needs of the population (97.5%) [12].

Further, hypovitaminosis D in its mild but especially in severe forms can exacerbate symptomatic hypocalcaemia following intravenous bisphosphonate (zoledronic acid) [13]. Even though this is rare, associated hypocalcaemia may be life-threatening and require immediate resuscitation and evaluation, often requiring hospitalisation to prevent additional morbidity and mortality risk from tetany, refractory hypotension, seizures or arrhythmias. Therefore it makes good sense to optimise vitamin D levels prior to administration of these agents [14].

Vitamin D toxicity can occur when blood 25(OH)D levels are 88 ng/mL or greater [15]. Symptoms may include sleepiness, vomiting, weakness, headache, nausea and constipation, and acute toxicity may cause hypercalcaemia and hypercalciuria.

4. Evidence-based review

Whilst there have been several treatment guidelines published, they are consensus-based rather than evidence-based [16–19]. This review seeks to address this issue and identify any gaps in research for vitamin D replacement, listing the types available including dietary, other supplementation and sunlight, for bone health (rickets, osteoporosis) as well as to address safety and efficacy in an evidence-based manner.

Systematic search of MEDLINE, CINAHL and EMBASE for articles published rom September 2015 to August 2019 and the Cochrane Database of Systematic Reviews (most recent issue searched—Issue 2, 2019) was conducted by the author. Randomised controlled trials, meta-analyses and reviews of all aspects of vitamin D supplementation in humans were reviewed. Articles pertaining to osteoporosis in a specific condition (e.g. epilepsy) were excluded.

All studies were reviewed independently by the author, who recorded individual study results, and an assessment of study quality and treatment conclusions was made according to evidence-based protocols.

Out of a total of 211 articles from PUBMED for vitamin D supplementation and osteoporosis, we found 22 articles satisfying the research criteria. Out of a total of 54 articles from PUBMED for vitamin D supplementation and rickets, we found 22 articles satisfying the research criteria. Of the 76 reviews from Cochrane Database of Systematic Reviews, there were 4 suitable review articles [20–23].

5. Vitamin D replacement in diet

Human beings require and source vitamin D from diet, dietary supplements and exposure to sunlight.

Whilst in particular for older people and those with restriction in diet, a diet rich in oily fish (which can theoretically improve 25-OHD levels and prevent vitamin D deficiency), in reality this is a challenge to achieve [24]. Only a few foods are a good source of vitamin D. These include fortified dairy products and breakfast cereals, fatty fish, beef liver and egg yolks.

Apart from the flesh of fatty fish (such as salmon, tuna and mackerel) and fish liver oils, very little foods contain vitamin D. Trace amounts of vitamin D can be found in egg yolks, cheese and beef liver. These foods contain vitamin D3 and its metabolite 25(OH)D3 [25]. Vitamin D2 is contained in some mushrooms [26, 27].

In the standard Western diet, fortified foods provide most of the vitamin D [26]. For example, US milk is fortified with 100 IU/cup. (In Canada, by law its milk is fortified with 35–40 IU/100 mL and its margarine at ≥530 IU/100 g.) However, other dairy products derived from milk, such as ice-cream and cheese, are generally not fortified. Some ready-to-eat cereals for breakfast often contain small amounts of added vitamin D, as do some brands of orange juice and yogurt. Some plant-based alternatives to mild (such as drinks made from oats, almond or soy) are sometimes fortified with vitamin D to the amount (about 100 IU/cup) found in fortified cow's milk. Several countries of the developed world (e.g. United States, Canada and Australia) mandate the fortification of infant formula with vitamin D, 40–100 IU/100 kcal in the United States and 40–80 IU/100 kcal in Canada; however, this is not practical especially in those who breastfeed and those countries which do not mandate fortification of their milk formulas.

6. Sunlight for vitamin D replacement

Most people meet at least some of their vitamin D needs through exposure to sunlight [28]. Solar ultraviolet B radiation crosses the skin and converts 7-dehydrocholesterol to previtamin D3, which is rapidly converted to vitamin D3. Excessive sunlight exposure does not cause intoxication because any excess previtamin D3 or vitamin D3 is destroyed by sunlight, and in the skin there is reversible conversion to inactive sterols [24].

In residential aged care, practical attempts for therapeutic sunlight therapy have produced only mild 25(OH)D3 improvements and depended on the season of exposure [29]. On a practical level, excessive exposure to ultraviolet is the primary cause of skin cancer, including squamous cell carcinoma, basal cell carcinoma and cutaneous malignant melanoma [30], so this is not a pragmatic approach from a public health perspective. Essentially, people with limited sun exposure require good dietary sources of vitamin D or take a supplement to achieve recommended intake levels.

Besides increasing sun exposure, the best way to get additional vitamin D is through supplementation.

7. Natural supplements for vitamin D

In fortified foods and supplements, vitamin D is also available in two forms, vitamins D2 (ergocalciferol) and D3 (cholecalciferol), that differ in their side-chain structure only. Vitamin D2 is manufactured by the UV irradiation of ergosterol in yeast, and vitamin D3 is manufactured by the irradiation of 7-dehydrocholesterol from lanolin and the chemical conversion of cholesterol [10]. Both forms effectively raise serum 25(OH)D levels [28, 31]. It appears that at nutritional doses vitamins D2 and D3 are equivalent, but at high doses vitamin D2 is less potent.

The recent increase in vitamin D interest by the general public has fuelled a big rise in sales of over-the-counter vitamin D preparations. Additionally, products with progressively increasing content of over-the-counter vitamin D preparations are becoming increasingly prevalent. Many types of pharmaceutical preparations for vitamin D supplementation are commercially available, including oily drops, capsules and tablets [31].

Individuals at high risk for deficiency should have a vitamin D blood test first; a dosage of up to 3000–4000 IU may be required to restore blood concentrations. In the Middle East and African countries, vitamin D doses ≥2000 IU/day may be needed to reach the target 25(OH)D level ≥20 ng/ml [32].

Whilst for pregnant women and children (1000 IU daily is safe) [33], there are very few concerns regarding vitamin D complications; in the elderly and especially those who are vitamin D replete, there has been some concerns about increased falling risks (especially at high doses) [34]. A 1-year, double-blind, randomised clinical trial conducted in Switzerland of 200 community-dwelling men and women 70 years and older with a prior fall, randomised to receiving monthly 24,000 IU (low-dose), 60,000 IU of vitamin D3 and 24,000 IU of vitamin D3 plus 300 μg of calcifediol, found that the higher-dose groups increased vitamin D levels more effectively but did not improve lower extremity function (over 12 months) and indeed increased fall incidence slightly [35]. Whilst this is not a cause for alarm, the study does confirm the popular scientific idiom 'Too much of a good thing is bad thing'. Bischoff-Ferrari [36] advocates against regular high-dose bolus or monthly moderate doses of vitamin D for fracture prevention. Indeed Zhao [5] in a meta-analysis of 33 randomised trials involving 51,145 participants found no benefit of supplements containing calcium, vitamin D or both compared with places in fracture prevention among community-dwelling older adults (over 50 years); however, it is noted that the research is limited by the lack of reporting of whether participants had osteoporosis, osteopenia or low bone mass, as well as participants mainly from Europe and the United States.

In contrast, research on vitamin D replenishment in the mild to moderate vitamin D-deficient elderly population following a hip fracture showed that a single loading dose of cholecalciferol (250,000 IU vitamin D3, the REVITAHIP (Replenishment of Vitamin D in Hip Fracture) strategy) or placebo, both receiving daily vitamin D (800 IU) and calcium (500 mg), was able to improve vitamin D levels and reduce falls (**Figure 3**) and pain levels [14]. The REVITAHIP investigators adopted a similar loading dose vitamin D followed by maintenance vitamin D at 800 IU (and calcium) daily supplied to the participants, due to the very high rates of vitamin D deficiency noted in the HORIZON Recurrent Fracture study (observed in the first 385 patients [37]). The investigators found that virtually all participants in the active treatment group reached target vitamin D (>50 nmol/L) at weeks 2 and 4 compared with the placebo group (**Figure 4**).

Interestingly, Smith [38], in a group of elderly women (mean age 66 years) with hypovitaminosis D (<50 nmol/L), demonstrated that vitamin D followed

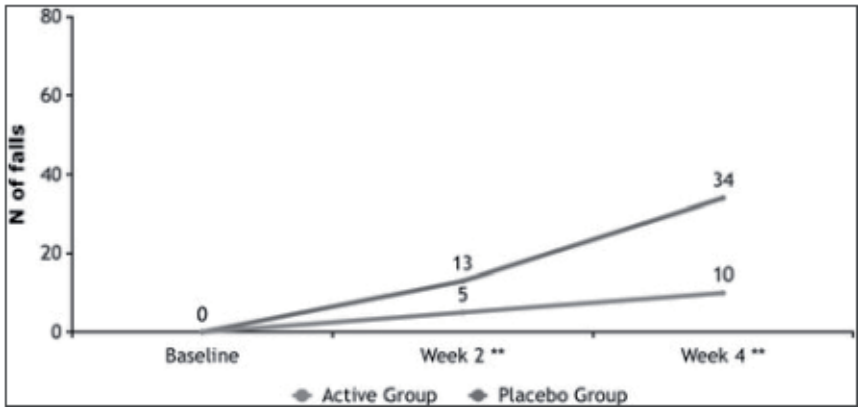

Figure 3.
REVITAHIP Active Vitamin D Protocol (initial 250,000 IU followed by 800 IU Vitamin D3 and 500 mg Calcium Daily at 4 weeks shows significance in reducing falls.

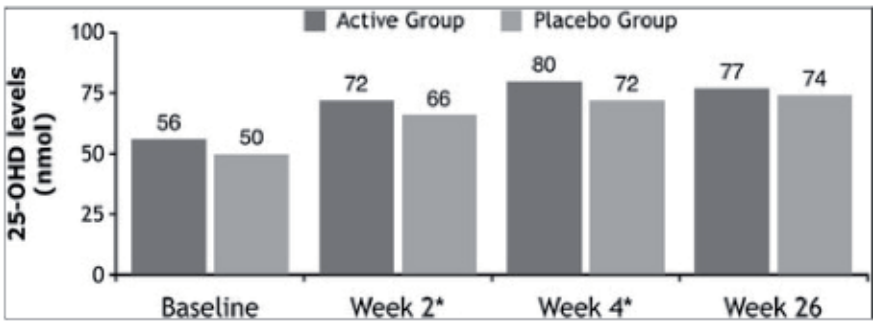

Figure 4.
REVITAHIP Active Vitamin D Protocol (initial 250,000 IU followed by 800 IU Vitamin D3 and 500 mg Calcium Daily at 2, 4 and 26 weeks showed significant 25-OHD levels improvements compared with the placebo group.

a U-shaped curve on falls whether analysed by serum dose or serum 25(OH)D3 levels. The investigators found no decrease in the incidence of falls on low vitamin D doses (400, 800 IU), a significant decrease in falls on medium doses (1600, 2400, 3200 IU) ($p = 0.020$). Counterintuitively, Smith found no decrease on high doses (4000, 4800 IU) compared to placebo ($p = 0.55$). The rate of falls was 60% in the lowest quintile <25 ng/ml (<50 nmol/L), 21% in the low middle quintile 32–38 ng/ml (80–95 nmol/L), 72% in the high middle quintile 38–46 ng/ml (95–115 nmol/L) and 45% in the highest quintile 46–66 ng/ml (115–165 nmol/L). A similar U-shaped pattern in the subgroup with a previous fall history was noted among the quintiles of 25(OH)D3 levels (**Figure 5**, [38]).

Conglomerating the results from Bischoff-Ferrari, Lyles, Smith and Mak, there does not appear to be any justification in having regular high-dose vitamin D supplements at monthly intervals, due to the risk of vitamin D toxicity, hypercalcaemia and perhaps oversaturation of vitamin D receptors on skeletal muscles, leading to a propensity to falls. The author would recommend the REVITAHIP strategy of initial single loading dose bolus (250,000 IU) followed by regular 800 IU daily [14, 37], or 24,000 IU monthly [35], supplementation but would recommend to be cautious with the Smith approach of medium daily dosages, probably up to 1600 IU daily without a bolus [38], for high-risk populations, and would support that larger monthly doses of 100,000 IU need further evaluation with respect to efficacy and safety.

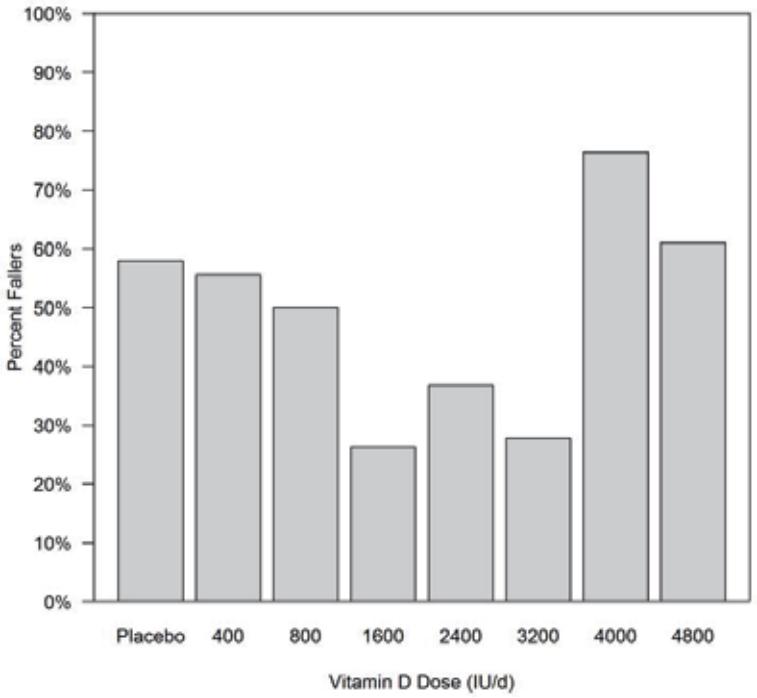

Figure 5.
The rate of falls was 60% in the lowest quintile <25 ng/ml (<50 nmol/L), 21% in the low-middle quintile 32-38 ng/ml (80-95 nmo/L), 72% in the high middle quintile 38-46 ng/ml (95-115 nmo/L) and 45% in the highest quintile 46-66 ng/ml (115-165 nmol/L). A similar U-shaped pattern in the subgroup with a previous falls history was noted amongst the quintiles of 25(OH)D3 levels [38].

8. Conclusions

Vitamin D is an essential fat-soluble hormone with multiple positive pleiotropic effects on the human bodies besides the optimisation on bone health. The evidence for ensuring high-risk groups such as pregnant women, children and those from the Middle East and African countries is vitamin D replete with either dietary, sunlight or combination with appropriate vitamin D supplements at moderate doses. For community-dwelling older adults with a history of falls, osteoporosis and/or fractures, there is still evidence for a good safety profile for moderate dosages (either through an initial single-dose bolus followed by daily low-dose or regular moderate daily or monthly dosages). The author does not recommend regular high-dose bolus (>500,000 IU bolus) or greater than 50,000 IU monthly given its possible increased risk of falls and associated fractures.

Author Note

The author dedicates this chapter to his eldest son Johann, for his constant bravery, courage, diligence and dedication to his sport, studies and in life, and to his mother Demi, for her constant reminder to strive for perfection.

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Section 2

Vitamin D Deficiency and Brain Functions

Chapter 6

Association of Vitamin D Deficiency and Mood Disorders: A Systematic Review

Jigna Shah and Sakshi Gurbani

Abstract

The cells of our body comprise calcitriol (1,25(OH) vitamin D_2), the active form of vitamin D, an integral biological substance that has an impact on a large number of biological processes. While high prevalence of vitamin D deficiency is detected in population worldwide, the reports from sun-soaked countries like India are also alarming to note that the deficiency of vitamin D as high as 70 to 90% is observed leading to several chronic diseases in the majority of people. Deficiency of vitamin D is observed not only because of low levels of vitamin D in the diet, less exposure to sunlight, reduced cutaneous vitamin D synthesis, but also due to consumption of particular medicines, undue alcohol intake, and tobacco smoking. Vitamin D is known to affect estradiol, dopamine, and pro-inflammatory cytokine levels, besides being involved in the regulation of mechanisms pertaining to hormones like glucocorticoids. When vitamin D binds to vitamin D receptors (VDR) present in the central nervous system, it is noted to be responsible for the regulation of brain neuronal functions. Low 25-hydroxy vitamin D levels are found to have a higher incidence of various mood disorders. This review focusses on vitamin D receptors, VDR gene mutations, and pathophysiology causing vitamin D deficiency disorders.

Keywords: vitamin D, VDR receptors, mood disorders, deficiency, insufficiency, cognition

1. Introduction

Vitamin D is an integral biological substance used to maintain bone health primarily, but it also plays its importance in several other biochemical pathways within the human body. Vitamin D, at the end of its metabolizing physiology, gets converted into an active hormone metabolite of vitamin D, i.e., calcitriol ($1,25(OH)_2$ vitamin D), which binds to vitamin D receptors (VDR). Vitamin D enzymes present in the central nervous system are responsible for the regulation of cellular function in several tissues located in the body including brain neurons. Vitamin D comes in two main forms: the first form is vitamin D_2 also known as ergocalciferol which is obtained from sources like mushrooms grown in UV light, dietary supplements, and fortified food, and the second form of vitamin D is vitamin D_3 also known as cholecalciferol, obtained from oily fish and fish oil, liver, egg yolk, butter, and dietary supplements. Vitamin D_2 and D_3 are not equal when it comes to raising your vitamin D status. Both are effectively absorbed into the bloodstream. However, the liver metabolizes them differently. The liver metabolizes vitamin D_2 into 25-hydroxy

vitamin D_2 and vitamin D_3 into 25-hydroxy vitamin D_3. These two compounds are collectively known as calcifediol. The main circulating form of vitamin D is Calcifediol. The amount of Calcifediol can be determined by checking its blood levels in the body. However, vitamin D_2 seems to yield less calcifediol than an equal amount of vitamin D_3 [1, 2].

Vitamin D deficiency is prevalent worldwide. The knowledge of the causes of vitamin D deficiency and community affected by the same causes are prominent, and hence, differentiation in the therapy and supplementation of these populations is focused upon accordingly. Further, in India, the prevalence of vitamin D deficiency ranges from 40–90% in all age groups and high-risk groups alike, with the majority of study responses reporting 80–90% prevalence as reported by the National Center for Biotechnology Information (NCBI), India [3]. Vitamin D deficiency contributes to a high disturbance in the health/disease ratio and adds to the disease burden of the country [4, 5]. The daily requirement of the human body for vitamin D is not fulfilled by the dietary pattern of the Indian population, and hence, fortification of various foods with vitamin D is emphasized under the initiatives of various national programs [2].

Vitamin D deficiency can be defined as circulating 25(OH) vitamin D levels below 20 ng/ml, while vitamin D insufficiency is defined by circulating levels below 32 ng/ml [3]. Vitamin D receptors are located in the bone, skeletal muscle, immune cells, and several other body tissues (including brain, prostate, breast, and colon). Deficiency of vitamin D hormone at its receptor site or the enzyme metabolizing site causes disturbed cell signalling, further indicating the increased risk of diseases like autoimmune diseases, cancer, tuberculosis, cardiovascular diseases, bone diseases, neurodegenerative diseases, and mood disorders, specifically discussed in this review. Low 25-hydroxyvitamin D levels less than 20 ng/ml are found to have a higher incidence of mood disorders consisting of premenstrual syndrome (PMS), seasonal affective disorder (SAD), non-specified mood disorder, and major depressive disorder (MDD) [1].

The physiology of vitamin D in the human body involves both synthesizing and catabolizing pathways. Vitamin D is either absorbed by dietary intake or is synthesized in the presence of ultraviolet B (UVB) rays ranging from 290 to 310 nm. In the epidermal layer of the skin, 7-dehydrocholesterol gets converted into pre-vitamin D_3 in the presence of UVB rays, which further, under thermal reaction, forms vitamin D_3 (also known as cholecalciferol). Vitamin D-binding proteins bind to vitamin D_3, and by circulatory transport this protein-bound vitamin D_3 reaches the liver, where it is further metabolized into 25(OH) vitamin D (calcifediol) and an inert form of vitamin D. Tightly regulated by parathyroid hormone (PTH), 25(OH) vitamin D converts into 1,25-dihydroxy vitamin D (also known as calcitriol), which is an active hormonal form of vitamin D in the kidneys and other extrarenal tissues. This active metabolite binds to vitamin D receptors to regulate the several tissue and cellular functions. When vitamin D deficiency occurs due to inadequate intake of vitamin D through diet or by application of excessive sun-protective agents, it causes dysfunctional regulation of glucocorticoid signalling which is known to be implicated in major depressive disorders and various other mood disorders, together with other body functioning disorders. It is reported to have elevated levels of glucocorticoid (a type of cortisol) for the patients of MDD [1, 6].

This review discusses sources of vitamin D, its association with different types of mood disorders in a different population, and its disease processes, together with the possible downstream molecular and genetic pathways associated with vitamin D deficiency and mood disorders. Further, this review focusses on the vitamin D deficiency causing mood disorders to the childbearing mothers and premenstrual syndrome to the ladies on the onset of their menses.

2. Vitamin D status

Vitamin D status is determined by assessing serum levels of 25(OH) vitamin D after 3 months of a stable regimen of vitamin D intake. Serum 25(OH) vitamin D is used to measure vitamin D status because it is the major circulating form of vitamin D and the most stable form of vitamin D. The National Health and Nutrition Examination Survey (NHANES) III data 8, which used a conservative measure of vitamin D deficiency {25(OH) vitamin D} levels <15 ng/mL, has reported 42.4% of African American women and 4.2% of white women are deficient in vitamin D during their childbearing years [2, 8, 9].

Toxic states (hypervitaminosis D) may occur when 25(OH) vitamin D levels supersede 100 ng/ml; however, in a study involving individuals diagnosed with multiple sclerosis treated with high doses of vitamin D, there was no evidence of toxicity found in individuals with 25(OH) vitamin D levels above 200 ng/ml [1].

3. Dietary recommendations of vitamin D

The dietary recommendations are largely based on bone health and assuming a minimal sun exposure of an individual under study. While the safe upper limit is set at 4000 international unit/day (IU/d) for healthy adults, for pregnant women, doses are higher than 6000 IU/d. Based on limited data from randomized controlled trials, some authors suggest that pregnant women can be supplemented with 1000–2000 IU/d during the second and third trimesters, and a deficiency during pregnancy can be treated with daily doses of 4000 IU [5].

4. Synthesizing and metabolizing physiology of vitamin D

Vitamin D is a secosteroid hormone that is either absorbed by dietary intake or manufactured by the ultraviolet beam (UVB) rays ranging from 290 to 310 nm reaching the epidermis of the skin. In the presence of epidermal 7-dehydrocholesterol, the absorbed vitamin D gets converted into pre-vitamin D_3. Within the epidermis, a thermal reaction occurs to convert the pre-vitamin D_3 into vitamin D_3 also known as cholecalciferol [10, 11]. Vitamin D_3 further, in process, moves to bind to the vitamin D-binding plasma proteins. Vitamin D_3 is transported via vitamin D-binding proteins to the liver where it is metabolized into 25(OH) vitamin D (calcifediol) and an inert form of vitamin D. Calcifediol is tightly regulated by parathyroid hormone and converts it into 1,25-dihydroxy vitamin D also known as calcitriol [1]. Calcitriol is the active form of vitamin D, which binds to VDRs in the intestines, bones, and kidney and other extrarenal tissues to enhance the absorption of calcium from the intestines, promotes calcium deposition in bones, and decreases parathyroid hormone concentrations (PTH) [3, 6]. In the process, calcitriol binds to vitamin D receptors, the receptors from the nuclear receptor superfamily that regulates the cellular function in several tissues located in the body including brain neurons [1].

5. Challenges for estimation of serum vitamin D level

The estimation of calcitriol is very challenging as calcitriol ($1,25(OH)_2$ vitamin D) has very short $t_{1/2}$ and thus does not reflect long-term vitamin D status. Also, it is observed that the total 25(OH) vitamin D is the most reliable marker for vitamin D

status which measures both vitamin D-binding protein (DBP)-bound and free 25(OH) vitamin D. In the light of widespread variation in measured results and divergent results in response to vitamin D supplementation, there is a need for a distinct method for estimation of vitamin D levels. Further, free 25(OH) vitamin D levels may vary according to genotype and single nucleotide polymorphisms (SNPs) in the DBP gene for which the assays are still not well established [5].

6. Prevalence of vitamin D deficiency

Vitamin D receptors are traced throughout the brain explaining the role of vitamin D in psychosomatic disorders, and it was found to have an equivocal call for vitamin D deficiency and depression going hand in hand [12].

Vitamin D insufficiency/deficiency is a worldwide problem, affecting all ages and races. Optimal 25(OH) vitamin D concentrations for skeletal health are >30 ng/ml. Serum 25(OH) vitamin D concentrations are generally lower in blacks than in whites and people who avoid exposing them to the sun. The increased use of sunscreens is hypothesized to increase the prevalence of vitamin D deficiency. Older adults, as a result of hyperparathyroidism related to renal insufficiency, tend to require more vitamin D to achieve adequate levels of 25(OH) vitamin D. As a result of the change in the definition of adequate concentrations, the prevalence of vitamin D deficiency is higher than previously thought. The prevalence of vitamin D deficiency among older men and women living in the United States and Europe ranges from 40–100%. The National Health and Nutrition Examination Survey from 2000 to 2004 found that ~25% of men >50 years of age and 30–35% of women >50 years of age had 25(OH) vitamin D concentrations <0.001. Two studies performed in Colorado and Georgia found that despite reported consumption of more than the required daily intake of vitamin D (400–600 IU/d), the prevalence of vitamin D insufficiency (defined as <32 ng/mL and < 20 ng/ml, respectively) among community-dwelling older adults (mean age, 77.8 and 77.0 years, respectively) ranged from 36.7 to 74.0% [3].

7. Vitamin D receptor

7.1 VDR in the brain

Vitamin D receptors are present on the nervous system tissues and cells especially dopaminergic nerves. In the momentary phase of cerebral development, vitamin D may act like a neurosteroid hormone in the areas of neurotransmission, neuroprotection, and neuroimmunomodulation. Vitamin D receptors belong to a hybrid class of nuclear receptor superfamily, which gets activated by vitamin D, a neurosteroid hormone that plays its major role in the nervous system by following mechanisms of differentiating, regulating Ca^{2+} ions, homeostasis, modulation of neurotrophins, and release and activation of key brain hormones and enzymes for neurotransmitter metabolism. VDR is a large molecular weight protein molecule weighing 50–60 kilodaltons, which consists of several functional binding domains, specifically and typically for all steroid hormones responsible for ligand binding, DNA binding, heterodimerization, nuclear localization, and ligand activation of transcriptional factors [13].

VDR detection in the brain tissue has been studied by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry to understand the localization of nuclear ligand binding sites for the transcription of phenotypic characters [14].

7.1.1 VDR gene location

Two important points of consideration for the production of the active form of vitamin D are 1,25(OH)2 vitamin D_3, a vitamin D receptor, and an enzyme 1 α-hydroxylase, which are found in the adult human brain. Identification of both the receptor and the enzyme were done in neuronal and glial cells in a regional and layer-specific pattern. The equivalent distribution of VDR regions and 1α-hydroxylase enzyme regions together with their frequent discrete distribution is found in layers and subregions of brain tissue [15].

7.1.2 Expression of VDR

Expression of VDR has been documented in tissues, including the brain, heart, skeletal muscle, breast, prostate, colon, activated macrophages, skin, and the areas prone to tumor expression with any devised mutation. The age factor is known to dominantly decrease the VDR expression. Many in vitro studies with human and animal cells have observed the expression of not only VDRs but also an enzyme, 1α-hydroxylase, which is expressed in most of the body tissues and cells, specifically in the kidneys. Therefore, it appears that these cells locally produce the active form of vitamin D by a regulated mechanism, in a paracrine fashion, to be used in various cellular and physiological functions. This structures the strong biologic basis for the association between serum vitamin D concentrations and extra-skeletal physiology [15].

7.1.3 VDR gene mutations

VDR gene mutations have been characterized by altered behavior of VDR null mutant mice. A study revealed anxiety-like behavior with decreased exploration when the VDR mutant mice were subjected to anxiety evaluation [16]. Another study focusing on the investigation of anxiety parameters in VDR mutant mice demonstrated unaltered spatial memory, olfaction, gustation, and hedonic responses [17].

VDR gene mutation is considered to influence the working of vitamin D hormone, which is essential for the growth and differentiation of a variety of organs, including the complete central nervous system. Many studies have suggested the crucial role of vitamin D in the brain, inducing many CNS genes. Inhibition of brain neurotransmission can be seen by VDR gene mutation causing modulation of neuroprotection, neurotrophins release, and activity of key neurotransmitter metabolism enzymes [16].

7.1.4 Regulation of normal brain neurotransmitters by normal VDR gene

The active form of vitamin D, i.e., calcitriol has a fast and strong ligand binding to their respective receptors located in the bone, brain, and breast tissues, as well as in immune cells [6]. The upregulation of transient receptor potential (TRP) vanilloid calcium-selective cation channels, such as TRPV5 and TRPV6, is done by positive induction of vitamin D. Vitamin D regulated channels may express the role of the hormone by potential modulation of sensory pathways representing several cellular sensors responding to temperature, touch, pain, osmolarity, taste, and other stimuli [17].

7.1.5 VDR immunoreactivity

The immunohistochemical study depicted the distribution of the VDR in multiple brain regions inclusive of neuronal and glial cells and other regions of the substantia nigra in the normal functioning human brain. Further, it also revealed

the presence of the VDR and 1α- hydroxylase in the human brain and confirmed the cell expression for either the receptor or the activating enzyme in neuronal or glial origin [14, 15].

8. Effect of vitamin D on mood and cognition

Vitamin D receptors and the 1α-hydroxylase enzyme have been isolated and found in the regions of the cerebral cortex and cerebellum, suggesting the conversion of calcifediol into an active form of vitamin D, i.e., calcitriol in the brain for a local cellular response [3, 18]. Several studies discuss the deficiency of vitamin D in the body at its targeted ligand binding sites due to less sunlight exposure or sun blockage, vitamin D receptor mutation causing phenotypic-conformational changes at the ligand binding site, and insufficient vitamin D-fortified diet, all causing major or minor mood disorders and illustrate the effectiveness of vitamin D or sunlight therapy (phototherapy), gene therapy, or supplemented vitamin D diet therapy for the treatment of depression and other mood disorders, demonstrating the associations between 25(OH) vitamin D concentrations and mood alone or mood and cognition in adults of all ages, including pregnant women, older adults, and targeted vitamin D-deficient population globally [3, 10].

9. Mood disorders

Vitamin D receptor mutant gene leads to the translation of mutant mRNA into defective vitamin D receptor proteins. Normal VDR is responsible for the regulation of glucocorticoid signalling, which, in this case, gets dysfunctional due to vitamin D deficiency. Dysfunctional glucocorticoid signalling is majorly implicated in several mood disorders like major depressive disorders, seasonal affective disorders, etc. Glucocorticoid, a type of cortisol, is seen to be increased in MDD and decreased in bone disorders. According to the recent diagnostic and statistical manual of mental disorders (DSM-IV), a major depressive disorder is diagnosed or said to be present when a person exhibits at least five of the following symptoms during 2 weeks, most of the day or nearly every day:

- Depressed mood
- Loss of interest or pleasure in daily activities
- Significant weight loss or gain
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or inappropriate guilt
- Diminished ability to concentrate or make decisions, problem with attention and cognition
- Recurrent suicidal thoughts with or without a plan [1]

Genes are encoding for vitamin D_3, 25-hydroxylase, and 1α-hydroxylase (CYP27B1), where CYP27B1 are the enzymes that metabolize vitamin D_3 into calciferol hormones, which is further involved in brain functions. These are expressed in neurons and glial cells presenting VDR. Calciferol hormone, being a neuroactive compound, regulates the behavioral functions such as anxiety, hyperactivity, and depression. Hypovitaminosis is a deficient condition that is found to be associated with an increased risk of multiple sclerosis, seasonal affective disorder, schizophrenia, Parkinson's disease, and Alzheimer's disease. Vitamin D deficiency could also be associated with autism, explained by a piece of indirectly related evidence. Furthermore, mood and cognitive performance appear to be dependent on plasma vitamin D level to some extent [19].

10. Types of vitamin D-deficient mood disorders

10.1 Seasonal affective disorder

Prevalence of seasonal affective disorder is seen when the vitamin D stored in the body are low with prominent seasonal changes. The disorder occurs during a particular time of a year where the sun exposure to the skin decreases leading to vitamin D deficiency, and the symptoms of the disorder can be resumed spontaneously on sun exposure. The individuals suffering from seasonal affective disorder have typically reported depression-like symptoms mostly in the winter months, where the levels of intensity of sunlight and photoperiod were predominately reduced [19–21]. Studies in the United Kingdom estimated the prevalence of SAD between 2.4 and 3.5%. The etiology of this disorder has not been fully elucidated, but the mechanisms leading to SAD are understood and linked to reduced sunlight exposure and daylight length. SAD is led via an eye-brain-endocrine system pathway or a skin-vitamin D causal pathway. In the mammalian population, the first stage of in vivo vitamin D synthesis necessitates the irradiation of skin by UVB light, dependently showing the lower vitamin D serum levels in the winter months than that in summer months. It has been also postulated that at cellular or subcellular levels, vitamin D can directly influence the endocrine system of our body via ligand binding on vitamin D receptors present in the entire central nervous system of a human body [21]. A prospective, randomized controlled trial was conducted in a group of 15 subjects with SAD to postulate the hypothesis of the association of vitamin D deficiency and seasonal affective disorder in which eight subjects received 100,000 IU of vitamin D and seven subjects received phototherapy. The Hamilton Depression Scale, Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Depression version (SIGH-SAD), and the SAD-8 depression scale were administered for the evaluation at two stages of treatment, i.e., first at onset of treatment and second after one month of the therapy. Also, intervention therapy was used to measure serum levels of 25-hydroxyvitamin D (25-OH D) planned in a gap of 1 week before and after the intervention. Improvement in all subjects was seen with the one's receiving vitamin D. Depression scale measure had no significant results for the phototherapy [1, 22]. Major improvement was seen in both the abovementioned groups, and this improvement in 25(OH)D was intertwiningly associated with the improvement in depression scale score. Hence, it is evident that vitamin D supplementation has an important role in the treatment of SAD [10, 13, 14].

In order to understand the seasonality of mood change, it is integral to understand the seasonal changes in photoperiod to hypothesize the most vitamin D-deficient mood disorders. Many biological techniques are utilized for detecting photoperiod.

For example, initiation of the behavioral changes such as migration patterns and breeding behavior is conserved in many species, primates, and humans which are evident with changes in season and intensity of sunrays. Further, the scientists pointed out that the regulation of circadian phase shift is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN has been shown to be involved in seasonal affective mood disorders. Further it was also presented that the SCN has an inhibitory action to the hypothalamic–pituitary–adrenal (HPA) axis and that this action can be altered by vitamin D dietary and supplementation restrictions [23].

10.2 Major depressive disorders

Major depressive disorder is a type of depressive disorder that is most likely to observe an association between vitamin D deficiency and anxio-depressive disorders. This association can be demonstrated with a parallel comparison between the motor and behavioral disorders observed in animal models of depression and VDR-KO mice [13]. Several theories are suggesting seasonal mood swings in humans. The binding of vitamin D on the ligand binding site of VDR present on the hypothalamic core (which plays a crucial role in mood regulation) can be witnessed to have a link between several seasonal changes in photoperiod and seasonal mood swings. Epidemiological data are coherent with such a cross-linking hypothesis. As an instance, the evidence is suggestive that the established low serum 25(OH) vitamin D_2 concentrations are closely related to the active experience of mood disorders in 80 subjects aging 65 years and older. Many studies have demonstrated that significant lower serum 25(OH) vitamin D_2 and 1,25$(OH)_2$ vitamin D_3 concentrations are observed in depressive sample subjects than healthy controlled subjects. Indirect confirmation was made by studying the association between depression and osteoporosis in around 4000 women aged 67 years [15]. Nevertheless, these results can also be mitigated and potentially related to functional impairment and physical inactivity, both of which reasons to increase with osteoporosis and have an independent correlative associated with depression [16].

Various clinical trials support the theory of the efficacy of vitamin D supplementation on mood disorders by varied sources like vitamin D-fortified diet, sunsoaking, etc. [17]. Improvement in depression scale experiments was noted, and the improvements were associated with vitamin D supplementation technique, while not much improvement was observed with the phototherapy technique. It is specifically prescribed to have at least 800 IU daily dose of vitamin D which plays a major decisive role in mood disorder case studies [4, 22, 24].

10.3 Premenstrual syndrome

Premenopausal women face one of the most common disorders known as premenstrual syndrome. Up to 20% of reproductive-aged women are affected in the range of moderate-to-severe premenstrual syndrome and is associated with significant levels of mood impairment. Irritability, mood swings, anxiety, depression, breast tenderness, bloating, and headaches are some of the most common symptoms included in PMS. Women are reported to have a depressed mood during the last week of the luteal phase which resides within for few days from the onset of menses [9]. Many studies have postulated that blood serum calcium levels and vitamin D levels are lower in women with PMS and that vitamin D supplementation and calcium supplementation may reduce the severity of the symptoms [25].

It is hypothesized that the dysregulation of calciotropic hormone is seen to be a major provocative factor in premenstrual syndrome. The severity of the symptoms of PMS is directly linked to calcium homeostasis, regulated directly by vitamin D

and parathyroid hormone as the key factors. However, low dietary vitamin D intake and inhibited induction of parathyroid hormone have been directly associated with the development of premenstrual symptoms [8].

10.4 Postpartum depression (PPD)

Vitamin D insufficiency is common in its most vulnerable pregnant population, and several studies have demonstrated the association of diminished levels of 25(OH) vitamin D with depressive symptoms [4]. Further, the diagnosis of low levels of 25(OH) vitamin D in maternal serum during pregnancy is associated with a higher incidental risk of postpartum depressive symptoms [26, 27]. Serum 25(OH) vitamin D levels for pregnant and postpartum women with major depressive episode, beginning within the first 4 weeks after childbirth, can be influenced by a multitude of factors like age, race/ethnicity, marital status, type of insurance, educational level, feeding type, and others. In addition, the season that accounts for the amount and strength of UVB exposure, i.e., photoperiod and vitamin D supplementation, also are found to be responsible for the episodes of major depression [28]. Vitamin D supplementation during pregnancy increases maternal serum 25(OH) vitamin D levels and thereby ensures higher availability for the offspring neuronal development. Vitamin D levels can also be inversely associated with infertility parameters, preeclampsia, blood glucose, bacterial vaginosis, primary caesarean section, and postpartum depression, but direct correlation is seen in pregnancy associated with breast cancer [28, 29].

The core symptoms of PPD are similar to that of any major depressive disorder like depressed mood or loss of interest in normal activities, sleep and appetite disturbances, loss of energy, feelings of guilt, and suicidal thoughts. Hence, the diagnosis of PPD becomes challenging as the sleep pattern changes and weight changes are also often observed in the normal postpartum period. It is further also exhibited that the lower maternal 1,25(OH)2 vitamin D levels have been found to be associated with higher levels of postpartum depressive symptoms as per the Edinburgh Postpartum Depression Scale scores. The promising results were observed by only one randomized clinical trial wherein the assessment was done by administration of high-dose vitamin D therapy in depressed subjects [6, 7].

Following birth in the first few days, the lower levels of 25(OH) vitamin D are reported for a greater risk of postnatal depressive symptoms and are also linked to serum vitamin D level in the second trimester of pregnancy. Further, the association of low 25(OH) vitamin D level was established with a continuous enhancing risk of reported level of symptoms that may indicate any one type of mood disturbance. Thus, it is confirmed that adequate intake of vitamin D is essential during pregnancy not only for the positive impact on the health and development of the offspring but also is a way to protect against postpartum mood disturbance in mothers [26]. Also, estrogen supplementation and vitamin D therapies have beneficiary effects on inflammatory response and related factors in women suffering from PPD [6, 29, 30].

When accounting the cortisol levels and hypothalamic–pituitary–adrenal axis reactivity in postpartum women, during the third trimester, maternal cortisol levels reach approximately three times that of nonpregnant levels. While the basal levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone, and cortisol are high, the HPA axis reactivity to stressful stimuli is dampened in late pregnancy. Furthermore, while the baseline cortisol levels return to normal within a couple of days after parturition, the hyporesponsiveness of HPA axis is found to be persistent in breastfeeding women [6].

The HPA axis hyperactivation or hypoactivation has always been associated with depressive states. It has also been hypothesized that depression during pregnancy

and postpartum depression may have different pathogenesis; the first is found to be dejected with hyperactivity in the HPA axis and the second being atypical. The activity of the HPA axis is usually reduced in seasonal affective disorder, atypical depression, and PPD, which could point to a similar pathologic mechanism in all the three conditions mentioned. Furthermore, the physiological excess production of CRH at the end of pregnancy leads to a transient downregulation of hypothalamic CRH postpartum, which could possibly lead to an elevated risk for depression. Indeed, the hypothesis of PPD being related to hypoactivation of the HPA axis has been substantiated by a number of studies where women with PPD display lower baseline or reduced HPA responsiveness than controls, although conflicting data are available. In addition, women with a history of PPD appear to have increased levels of corticotropin-releasing hormone which further stimulates the dependent cortisol response in the experimental conditions of pregnancy. PPD can also be predicted by increased stress-induced cortisol levels or CRH levels [6, 20].

Together with SAD, PPD has also been classified, under the depressive states characterized by hypoactivation of the HPA axis. Increased serum concentrations of biomarkers detecting the inflammatory response, for example, IL-6, a pro-inflammatory cytokine with a variety of endocrine and metabolic actions, have been observed in major depressive conditions. In this, IL-6 interacts with the HPA axis, and the interacted complex has significant higher serum levels in women with postpartum depressive symptomatology. Conclusively, vitamin D affects monoamine functional groups, the HPA axis, and immune responses to stress and symptom production [20, 38].

10.5 Aging depressive symptoms

Aging depressive symptoms are noticed for both hypervitaminosis D_3 and hypovitaminosis D_3, which leads to premature aging of fibroblast growth factor 23 (FGF-23) that is emerging as a significant mediator/hormone for early aging symptoms, and its FGF-23 effects are dominated by vitamin D-mediated excess of calcitriol. The early aging phenotypic features include thin skin, intestinal atrophy, spleen atrophy, muscle atrophy, weight loss, short life prognosis, osteoporosis, and atherosclerosis. There is a tight physiological regulation of 24-hydroxylase, the hormonal form of vitamin D_3, which can be modulated by physiological serum concentrations of calcidiol. This regulation of hormonal form of vitamin D_3 explains the development of aging depressive symptoms [31]. However, some intoxications occur during the early period of synthesis and distribution of vitamin D_3 with its substitution/fortification. After the Second World War, the children in many parts of Europe were administered with extremely high oral doses of vitamin D_3 and suffered from hypercalcemia, nephrocalcinosis, early aging, cardiovascular complications, and early death, supporting the possibility of hypothesizing that the hypervitaminosis D_3 can accelerate aging symptoms [19, 24, 31].

Calciferol hormone insufficiency may accelerate the risk of diseases of CNS. A recent study postulated that the hypovitaminosis D_3, also famously known as vitamin D deficiency, may cause premature or immature aging of cognitive functions. Thus, both a lack and an excess of calciferol hormones enhance aging in major dependency [32]. Initial events affect the genome, causing telomere shortening or accumulation of DNA damages, which are modulated by the tumor suppressor protein, p53. Hormonal forms of vitamin D_3 appear to control the basic mechanisms of aging and related diseases [19].

The hypothesis of the role of vitamin D in aging is considered based on three axial parameters, namely, calciferol hormone serum concentrations, risk of disease,

and onset of aging. The former two parameters are seen based on lower and higher values, and the latter parameter is seen as the typical or premature onset of aging. It is observed that both low and high action of calciferol hormones trigger premature aging, including diseases of CNS. Hence, an optimal serum concentration appears to delay aging [19].

10.6 Suicidal attempts due to vitamin D deficiency

Significant lower levels of vitamin D are seen in patients with suicidal tendencies than both non-suicidal depressive patients and healthy control individuals. Deficiency of vitamin D was found in 58% of cases of all the reported cases of suicides, compared to around 30% cases found for the healthy controlled cases and the non-suicidal depressed patient cases [33–35].

Accumulating studies indicate that a dysregulated immune system could be a contributing factor to depression and possibly specifically to suicidal tendency. Direct evidence of causality comes both from animal models, where induction of peripheral inflammation is known to lead to depressive changes, and from the so-called cytokine-induced depression in humans, where treatment with interferons (IFN) of patients with hepatitis increases the risk for development of both depression and suicidal tendency [32]. Thus, an indirect proportional relationship between serum vitamin D concentration and inflammatory cytokines is seen to be established, i.e., the lower the vitamin, the higher are the levels of the inflammatory cytokines IL-6 and IL-1β in the blood. The future prospective must be seen to compare vitamin D levels between other groups of psychiatric patients and groups of patients with personality disorders [7, 34, 36].

10.7 Schizophrenia

A study conducted in the year 2006 on the psychiatric population to understand the association between lower plasma levels of 25(OH) vitamin D_3 and mood disorders revealed that all 82 subjects were suffering from psychiatric disorders. Further, 53 patients were suffering from mood disorders, and the remaining 29 patients were diagnosed with schizophrenia. All these patients were found to have low vitamin D_3 plasma concentration which confirms the significant association of low vitamin D_3 plasma concentration with mood disorders and related disease symptoms. Additionally, significant hypovitaminosis D was also witnessed in mood disorders like major depression, bipolar disorder, and dysthymia than with schizophrenia [37]. Also, according to one of the neurodevelopmental hypothesis of schizophrenia, it was revealed that the prenatal vitamin D deficiency in a mother could be a high-risk factor for schizophrenia in an offspring [24].

10.8 Attention deficit hyperactivity disorder (ADHD)

Attention deficit hyperactivity disorder is an early-onset, chronic, a neurodevelopmental disease characterized by attention deficit, hyperactivity, and impulsivity mostly in children, affecting nearly 2–18% of children worldwide, and is found to be one of the most common psychiatric disorders in childhood stage [32]. Learning like basic skills can be affected in childhood and can also cause various psychological and social interaction problems in children and the adult population. The neurotransmitters like dopamine (DA) and noradrenaline (NA) play a crucial role in maintaining attention, concentration, motivation, awareness, and cognition. With the major role of vitamin D in cerebral function, it might have a direct role

in the etiopathogenesis of ADHD in children and young adolescence. Further, vitamin D is also responsible for the regulatory synthesis of neurotrophic factors such as neurotrophin (NT), NT 3 and NT 4, nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF), known to be significantly involved in cell differentiation and survival. Thus, ADHD is etiopathologically connected to vitamin D deficiency. In initial years of growth and development of life, vitamin D deficiency or extensive insufficiency can be harmful to neuronal development and function, resulting in stimulation of neurogenesis [7, 10].

11. Conclusion

To summarize, vitamin D deficiency is associated with several types of mood disorders involving various molecular and genetic mechanisms related to vitamin D receptors and VDR gene. The VDR gene mutation alters vitamin D binding capacity to vitamin D receptor, preventing vitamin D activation into calcitriol which regulates synthesis of neurotrophic factors. Failing to maintain these neurotrophic factors in the presence of vitamin D deficiency leads to cerebral dysfunction and thereby contributes to mood disorder symptoms. Further, vitamin D deficiency is also associated with cognitive reasoning and mind disturbance that trigger off mood disorders like major depressive disorders, seasonal affective disorder, suicidal tendency, postpartum depression to the childbearing mothers, premenstrual syndrome to the ladies on the onset of their menses, ADHD, schizophrenia, and aging depressive symptoms. Futures studies and clinical trials can also be structured to establish a better understanding of the effects of the vitamin D deficiency on several mood disorders, behavioral disorders, and cognition.

Conflict of interest

None.

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Chapter 7

The Effects of Vitamin D Deficiency on Neurodegenerative Diseases

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Abstract

Approximately 90% of the elderly population in the western countries has at least a mild to moderate vitamin D hypovitaminosis. Besides the well-known function of vitamin D in calcium homeostasis, it has been recently found that several enzymes and receptors involved in its homeostasis are expressed in the nervous system and brain suggesting also an important role in the brain homeostasis. Interestingly, epidemiological and clinical studies found reduced vitamin D level associated with an increased risk of several neurodegenerative disorders. In this chapter, we focus on a potential link between vitamin D and Alzheimer's disease, Parkinson's disease, multiple sclerosis, prion disease, and motor neuron disease. Epidemiological studies were summarized, an overview of the known potential underlying pathomolecular mechanisms are given, and results from clinical studies dealing with vitamin D supplementation were presented. As an outlook, recent literature suggesting an impact of vitamin D on autism spectrum disease, depression, and schizophrenia are briefly discussed. In conclusion, the identification of an abundant vitamin D metabolism in the brain and the tight link between the increasing number of several neurological and mental disorders emphasize the need of further research making a clear recommendation of the intake and supplementation of vitamin D in a growing elderly population.

Keywords: vitamin D hypovitaminosis, Alzheimer's disease, Parkinson's disease, multiple sclerosis, prion disease, neuropsychiatric diseases

1. Introduction: relationship between vitamin D and neurodegenerative diseases

The secosteroid vitamin D_3 was identified in the year 1928 by Adolf Windaus and colleagues. Its synthesis starts in the skin based on 7-dehydrocholesterol through extraneous cause of ultraviolet B radiation in a spectrum of 290–315 nm wavelengths. Via the vitamin D-binding protein, it is transported in the blood to the liver where the 25-hydroxylase CYP2R1 hydroxylates vitamin D_3 to 25-hydroxyvitamin D_3 ($25(OH)D_3$). Because of its serum half-life of weeks, this 25-hydroxylated form of vitamin D_3 is clinically measured as an indicator for the patients' vitamin D_3 level [1, 2]. The active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) or calcitriol, is synthesized in the kidney by the 1α-hydroxylase

CYP27B1. To a limited extent, vitamin D_3 can also be taken up with diet as well as vitamin D_2 (ergo-calciferol), which is largely found in food.

Calcitriol can perform its genomic actions via vitamin D receptor (VDR) binding. Hence this undergoes a conformational change and forms a complex with the retinoid X receptor (RXR) which interacts with the vitamin D response element (VDRE) to regulate the expression of numerous genes. Because of this ability of transcriptional modulation, vitamin D has influence on various cellular processes, for example, the mitochondrial function by maintaining the mitochondrial respiratory chain activity [3]. Dysfunctional mitochondria will normally be removed by autophagy, a process that is also promoted by vitamin D [4]. Damaged mitochondria could induce inflammation. Vitamin D is able to downregulate the expression of pro-inflammatory cytokines such as the tumor necrosis factor-α (TNF-α) or interleukin-6 (IL-6), thereby reducing inflammation [5]. Additionally to these cellular processes, vitamin D antagonizes oxidative stress by enhancing the expression of antioxidants and thereby reduces levels of reactive oxygen species (ROS) [6]. Furthermore the expression of the voltage sensitive L-type Ca^{2+} channels is suppressed by vitamin D to regulate intraneural calcium [7, 8]. Vitamin D also plays a role in DNA-related cellular processes, for example, epigenetic changes. These are influenced on the one hand by oxidative stress which is regulated by vitamin D, as described before, and on the other hand by histone methylation which is also modulated by vitamin D due to its influence on the transcription of key DNA demethylases [9, 10].

Based on the fact that up to 90% of the elderly population is suffering from a vitamin D hypovitaminosis because of a homebound lifestyle and the reduced ability of their skin to generate vitamin D_3, the idea arose to analyze the impact of a vitamin D deficit on the abovementioned cellular processes which are all involved in ageing [11]. Moreover, ageing is a risk factor for neurodegenerative disorders. Michael J. Berridge published a very detailed article 2 years ago reviewing the influence of vitamin D deficiency on ageing and age-related diseases. He figured out that hypovitaminosis D promotes those ageing-related processes, for example, due to a decline of mitochondrial respiration or the electron transport chain. This dysfunction leads to an increase in oxidative stress and inflammation, main drivers of ageing. Furthermore, he describes elevated Ca^{2+} levels in neurons during ageing which are accompanied by a decline in cognition. This observation can be restored by vitamin D due to its ability to reduce the levels of Ca^{2+}. Additionally, telomere shortening, a DNA-related process that is involved in ageing, is reported to be decreased by vitamin D [12]. Those upregulated ageing processes under vitamin D-deficient conditions in the elderly population could further lead to age-related disorders like dementia or declines in cognition.

A relationship between vitamin D and age-related cognitive disorders was further strengthened by the findings that vitamin D and its metabolites are able to cross the blood-brain barrier. Early research reported the presence of vitamin D metabolism intermediates and products in human cerebrospinal fluid [13]. Furthermore, due to the presence of the metabolizing hydroxylases in the brain, the active form of this hormone-like secosteroid can be synthesized in the human brain. The additive existence of the VDR in neuronal and glial cells suggests that vitamin D might influence functioning of the central nervous system (CNS) [14]. As mentioned earlier, the VDR-mediated nuclear functions of $1,25(OH)_2D_3$ influence cellular processes, for example, immune modulation and cell growth or differentiation. Those biological systems have also an impact on maintaining the function of the brain. Annweiler et al. reported increased risks of cognitive disorders for patients with $25(OH)D_3$ serum concentrations lower than 10 ng/ml [15]. Vitamin D influences the structure of the brain, like changes in volume and vasculature as

well as its metabolism [16]. Furthermore, in vivo studies using offspring of vitamin D-deficient rats could show that vitamin D plays an important role in the developing brain [17]. Eyles et al. give a detailed overview in their review over all the different animal studies that have been made in this context. One exemplary finding is that rats with a vitamin D deficiency during their development have impairments in their adult behavior [18].

In a retrospective study from 2007, Przybelski and colleagues reported that serum $25(OH)D_3$ can be positively associated with increased cognitive function [19]. Consistent finding results from a prospective cohort study in older adults that showed a vitamin D hypovitaminosis (levels <30 ng/mL) in 68% of the participants and moreover that these ones have lower baseline cognitive function and increased decline over the 4 years of follow-up [20]. In the same year, another prospective study including 1185 women also pointed out an association of higher plasma $25(OH)D_3$ levels and better cognitive performance [21]. A meta-analysis showed that participants with insufficient vitamin D levels have a 2.4-fold increased risk of cognitive impairments than those with sufficient levels [22]. In line with this, further systematic reviews and meta-analysis support an association between hypovitaminosis D and declined cognitive functions [23, 24]. Additionally, some longitudinal studies suggest a link between low serum $25(OH)D_3$ levels and cognitive performance. Toffanello et al. reported decreasing scores at Mini-Mental State Examination (MMSE) in participants having serum $25(OH)D_3$ levels below 75 nmol/L in their 4.4-year follow-up study including 1927 elderly subjects [25]. One year later, Miller et al. published a vitamin D-associated accelerated decline in cognitive performance in their study of a multiethnic cohort of older adults [26]. In line with these data, a more recent clinical study in the elderly US population (3325 participants) reported that low serum levels of vitamin D_3 are linked to an enhanced risk of cognitive impairment [27].

Due to the suggested role of vitamin D in brain function, several studies examining the effect of dietary supplemented vitamin D exist. Animal studies and epidemiological studies generate biological evidences for a relationship between vitamin D levels and brain health. Latimer et al. could show that a 6-month supplementation of vitamin D improves cognitive function in a rat model of aging [28]. One study from Annweiler and colleagues in the year 2010, including 5596 women with a mean age of 80.5 years, concluded that higher weekly dietary vitamin D intake is associated with better cognitive performance in older adults [29]. In line with this, a current cross-sectional and longitudinal study describes a significant association between serum vitamin D levels below 30 nmol/L and reduced general cognitive performance [30].

Unlike those animal and epidemiological findings, clinical studies using randomized controls examining the role of vitamin D in individuals without any form of dementia show heterogeneous data [31–34]. In general, it should not be disregarded that differences in clinical intervention studies might be because included participants who already had sufficient vitamin D levels at baseline could mask the benefit of supplementation on cognitive function. To prevent this, it would be beneficial including exclusively participants affected by a hypovitaminosis D at the baseline measurement. These inconclusive data also support the idea of individually adjusted supplementation protocols for each patient. Furthermore, methodological differences between the studies, for example, duration, form, and dose of vitamin D supplementation, have to be considered. A current study from Pettersen et al. [35] considered these abovementioned methodological pitfalls by administering 4000 IU/day of vitamin D_3 for 18 weeks to healthy adults and by baseline as well as endpoint measurements of serum $25(OH)D_3$ levels. Furthermore, they were able to distinguish between the two cognitive domains, verbal and visual memory, with

several cognitive tests. High doses of vitamin D enhanced visual memory, while low doses (400 IU/day) improved verbal memory. As summarized by the authors, there seems to be a small positive effect of vitamin D_3 supplementation on executive functioning, despite the outlined limitations (modest sample size with wide range of ages, no true placebo group) [35]. Recently, Aspell et al. give a well elaborated overview over a possible supporting role of vitamin D in cognitive function in age [36]. In the following chapter, we will present the results of current available studies examining the impact of vitamin D supplementation on brain-related disorders for each neurodegenerative disease itself. Up to this date, also the nutrition research field examines the influence of dietary components on brain health. In a recent review from Moore et al., the authors describe a potential protective role for vitamin D among others [37].

2. Alzheimer's disease

Alzheimer's disease (AD) can be described as a multifactorial, metabolic disease as this disease is characterized by impairments in multiple cellular processes. For example, AD pathology includes alterations in the sequential proteolytic processing of the amyloid precursor protein (APP) which results in the formation of neurotoxic Aβ plaques, in the phosphorylation of the microtubule-associated protein Tau, in lipid and energy metabolism, and in inflammation. Among others, AD pathogenesis is influenced by lipophilic vitamins [38].

Epidemiological studies indicate a relationship between vitamin D deficiency and AD. A systematic review and meta-analysis concluded that patients affected by AD have lower levels of serum 25(OH)D_3 than healthy controls [39]. Furthermore the level of vitamin D is shown to be significantly reduced in the cerebrospinal fluid of AD patients [40]. Another meta-analysis from Shen and colleagues shows that vitamin D hypovitaminosis (25(OH)D_3 level < 50 nmol/L) is associated with a 21% increased risk of AD [41]. Different results were obtained by a study, measuring serum level of 25(OH)D_3 via radioimmunoassay in patients with MCI or diagnosed dementia compared to healthy controls. Analysis of the cognitive performance showed significant differences between these groups. Vitamin D levels of 65.2 ± 17.9 nmol/L for controls, 61.4 ± 18.8 nmol/L for MCI, and 65.0 ± 20.3 nmol/L for AD were reported in this study; however the differences in vitamin D concentrations between these groups were not statistically significant. Interestingly, the authors show that approximately 12% of the MCI and AD patients used some kind of vitamin D supplementation, whereas only 5% of healthy controls did so. Furthermore, 80% of the MCI patients, 50% of the AD patients, and 62% of the control individuals regularly used nutritional supplements, which could have influenced the results as discussed by the authors. In conclusion, the authors comment that their "findings cannot exclude the possibility that targeted vitamin supplementation can act as a modifying measure, although it is less likely that vitamin intake can prevent dementia onset" [42].

Several longitudinal studies provide homogeneous results indicating that a deficiency in serum 25(OH)D_3 is linked to a greater risk and incidence of dementia and AD [43–47]. A current prospective study analyzed 25(OH)D_3 level, cognitive decline, and incidence of dementia in 916 patients for 12 years and reported a more pronounced cognitive decline and a threefold increased risk of AD in individuals with hypovitaminosis D [48].

In line, a study from 2018 analyzing the serum vitamin D level in AD patients described decreased levels in various stages of AD. Additionally, the authors indicate 25(OH)D_3 as significant predictor for severe AD and argue for vitamin D

supplementation in AD patients [49]. Moreover, Wang et al. recently reported a delayed onset of psychotic symptoms when vitamin D was used in AD patients and suggest variations in vitamin D-influenced genes as biomarkers for those individuals who can have a benefit from supplementation [50].

These studies consistently indicate a link between insufficient dietary intake of vitamin D and cognitive diseases like AD. However, these studies did not address the question whether vitamin D deficiency is a cause and thus risk factor for AD or a consequence of this neurodegenerative disease mediated by accompanying dietary and behavioral changes. Some more evidence of a causal relationship between hypovitaminosis D and AD was achieved by Mendelian randomization (MR) studies that use genetic associations on inherited alleles unaffected by confounding factors or disease progression. It was reported that two polymorphisms in the *VDR* gene are associated with AD risk in patients younger than 76 years [51]. A subsequent meta-analysis including six AD studies also revealed this relationship [52], as well as an earlier study using genotyping of 213 participants [53]. Wang et al. also provide this genetic evidence and could further generate functional data indicating a link between the VDR and the genetic AD risk [54]. Furthermore, the study of Mokry and colleagues supports genetically decreased vitamin D levels as causal risk of AD, by analyzing the effect of single-nucleotide polymorphisms (SNPs) involved in vitamin D metabolism on 25(OH)D_3 levels and the risk of AD in more than 56,000 participants [55]. A recently published MR study from Larsson and colleagues including 17,008 AD cases and 37,154 controls reported an inverse association between 7 SNPs related to elevated vitamin D_3 levels with AD, in which 2 of them were significant [56]. A study creating a vitamin D synthesis risk score analyzing more than 1000 Swedish men in a follow-up of 18 years is controversially discussed as this study revealed no link between baseline vitamin D status and the long-term risk of dementia [57].

Concerning clinical studies, randomized placebo-controlled trials analyzing the effect of vitamin D supplementation on AD risk and progression are still missing. However, recently nonrandomized studies showed positive findings about that relationship. A current study reported improvements in the cognitive status of MCI patients (n = 16) in an 18-month follow-up after 6 months of vitamin D supplementation. Additionally, vitamin D supplementation protected lymphocytes from oxidative stress [58].

In contrast to the missing clinical trials, several animal and cell culture studies underline a causal relationship between vitamin D and AD. One study described an increased neurogenesis and enhanced cognition after feeding a transgenic mouse model of AD (5xFAD mouse model) with a daily dose of 500 IU/kg vitamin D for 5 months [59]. Additionally, animal and cell culture studies allow analyzing combination therapies, for example, a supplementation of both vitamin D and resveratrol. This resulted in an improvement of cognitive function and reduced levels of $A\beta_{42}$ in the hippocampus along with decreased tau phosphorylation in the parietal cortex of a mouse model with AD-related memory impairment [60]. In line, a novel study from 2019 reported an improvement of the AD-related pathology in 5xFAD mice after intravenous injection of vitamin D-binding protein which was loaded on a biocompatible polymer (PLGA) [61]. Especially cell culture and animal-based studies are indispensable for clarifying the molecular mechanisms of vitamin D action in neurodegenerative diseases. They revealed that vitamin D exerts its protective effects via VDR-related, genomic, as well as non-genomic actions directed to processes like Aβ metabolism, neurogenesis, immune modulation, and neuronal calcium homeostasis. A study from Landel et al. examined the transcriptome of 5xFAD mice after 5 months of vitamin D_3 supplementation and reported a large number of differentially expressed genes. The authors suggest an

interaction of vitamin D with estrogen and insulin signaling to regulate the identified pathways [62]. Consistently, we could show that a deficit of vitamin D causes a dysregulation of numerous genes that are involved in multiple cellular processes like neurogenesis, inflammation, mitochondrial function, oxidative stress, signal transduction, and APP homeostasis in brains of hypovitaminosis D mice [63]. In respect to the impaired APP homeostasis, several studies using primary cortical neurons or human neuroblastoma cell lines were able to show beneficial effects of vitamin D and its analogues on anabolism and catabolism of the neurotoxic Aβ peptide [64, 65]. In line with these findings, a vitamin D_3-enriched diet leads to an increased Aβ clearance in mouse models of AD [66–68]. Consistent with the results of animal studies, Hooshmand et al. could demonstrate an association of increased plasma $25(OH)D_3$ levels with higher concentrations of CSF $A\beta_{1\text{-}42}$ in 75 patients, reflecting a decreased $A\beta_{1\text{-}42}$ aggregation in human brain parenchyma [69]. In contrast, a recent cross-sectional study failed to find significant associations between plasma vitamin D levels at baseline and Aβ load in different brain regions. But the authors themselves argue that those findings could be explained by an improper timing of measurements or rather no analysis over time or by a hypovitaminosis D-related cognitive decline independent of APP homeostasis [70]. The potential neuroprotective role of vitamin D_3 is based on findings that it regulates the transcription of the neurotrophin nerve growth factor, glial-derived nerve factor, and neurotrophin 3 which are important for neuronal survival [71–73]. Furthermore, the expression of the neuroprotective cytokine IL-34 was shown to be increased in dose- and time-dependent manner by calcitriol in neuroblastoma cells [74]. In respect to a relationship between vitamin D and neuronal calcium levels, a review describes a reduced autophagy due to impairments in calcium signaling as a consequence of hypovitaminosis D [6].

3. Parkinson's disease

As one of the most common neurodegenerative diseases, Parkinson's disease (PD) is characterized by the loss of dopamine-producing neurons in the substantia nigra pars compacta and typical Lewy bodies, aggregates of α-synuclein, provoking oxidative stress and further cell death, leading to impairments in cognition and behavior and to dysautonomia [75].

A number of epidemiological studies were able to indicate an association of vitamin D and PD. Several evidences exist that hypovitaminosis D is more frequent in PD patients [76, 77], and a cohort study with a 29-year follow-up reported a decreased risk of PD in individuals with higher vitamin D serum levels [78]. These findings are supported by the outcomes of recent studies describing significantly reduced levels of serum $25(OH)D_3$, daily vitamin D intake, and sunlight exposure in PD patients [79]. Besides significantly decreased serum $25(OH)D_3$ levels, also an association of vitamin D at baseline and disease motor severity after 36 months was observed in a recently published prospective observational study [80]. A clinical study found an inverse relationship between serum vitamin D concentrations and disease severity as well as an influence on balance function in PD patients [81]. A current study reported that vitamin D status of PD patients has no influence on nocturnal changes in blood pressure, a marker of cardiac autonomic dysfunction as non-motor symptom in PD [82]. However, the tight link between serum vitamin D concentrations and risk as well as severity of PD is also underlined by a recent systematic review and meta-analysis of Luo et al. [83].

PD is not only caused by environmental factors, for example, the vitamin D status, but also by genetic components. The first monogenetic mutation, which was

found to be associated with early onset familial PD, is located in the *SNCA* gene that encodes the α-synuclein protein [84]. A recent study was able to show an altered expression of *Snca* in brains of vitamin D-deficient mice, underlining a causal relationship between hypovitaminosis and PD [63]. Consistent with several previous publications, this is also supported by two recent studies analyzing SNPs in *VDR* and the vitamin D-binding protein. Besides the functional VDR polymorphism, FokI was reported to be associated with cognitive decline in PD and ApaI with the risk of PD, while vitamin D-binding protein gene was suggested as a risk factor for PD [85, 86]. In line with this, a double-blind, placebo-controlled intervention study from Suzuki and colleagues, including 114 PD patients, was able to reveal that supplementation of 1200 IU vitamin D_3/day prevents disease progression in a VDR FokI genotype-dependent manner [87]. Moreover, high-dose supplementation of vitamin D (10,000 IU/day) resulted in significant improved balance measured via sensory organization test in PD patients with an age of 52–66 but not in older individuals [88].

To elucidate the underlying molecular mechanisms by which vitamin D exerts its potential beneficial role, animal and cell culture experiments were performed. Vitamin D_3 seems to have a positive influence on synthesis and storage of dopamine in CNS by protecting against dopaminergic toxins such as 6-hydroxydopamine or hydrogen peroxide in rats [89]. This neuroprotective effect could be due to its ability to elevate the expression of the glial cell line-derived neurotrophic factor (GDNF) that influences the dopaminergic nigrostriatal system [90] and due to its antioxidative properties described before. Furthermore vitamin D_3 administration was shown to prevent zinc-induced oxidative stress in substantia nigra of rat brain [91]. Oxidative stress and elevated intracellular-free calcium promote the aggregation of α-synuclein synergistically, and a recent study could show that the vitamin D_3 analogue calcipotriol is able to induce the expression of calbindin-D28k, thereby inhibiting the calcium-mediated aggregation of α-synuclein in human neuroblastoma cells [92].

4. Multiple sclerosis

Multiple sclerosis (MS) is a multifactorial, chronic disease of the CNS characterized by demyelination, inflammation, and neurodegeneration.

Epidemiological studies indicate that genetic and environmental factors interact and influence the risk of MS, for example, several SNPs or environmental exposures like an infection with Epstein-Barr virus (EBV), vitamin D status, sunlight exposure, or smoking. There is a high prevalence for MS in areas with low sun/ultraviolet sun exposure [93, 94], and this could be explained by vitamin D [95]. A study from Lucas and colleagues revealed that vitamin D and sun exposure are independent risk factors of CNS demyelination [96]. For an overview over environmental factors and MS, we suggest an article from Ebers GC [97]. Genetic predispositions which are associated with MS are found in genes of the immune system. The strongest correlation was found for genes of the major histocompatibility complex (MHC), especially the HLA genotype HLA-DRB1 [98]. Findings from Ramagopalan and colleagues suggest a direct functional link between known environmental risk factors, for example, vitamin D, and established genetic predispositions. The authors described the localization of a vitamin D response element on the promotor region of *HLA-DRB1* and underlined its functional role by the finding that treatment with calcitriol results in an increased expression [99]. Later on this research group could show that mutations in the *CYP27B1* gene, involved in vitamin D_3 metabolism, are causative associated with the risk of MS [100]. Moreover, genetic variations

in the *CYP24A1* gene, involved in catabolism of vitamin D, were found to play a pathogenic role in MS [101]. For detailed information about the results of several genome-wide association studies analyzing the genetic risk of developing MS, we recommend a recent publication from Baranzini and Oksenberg [102].

Clinical studies indicate that vitamin D influences MS development and disease activity including the risk of relapse, gray matter volume loss, and clinical course of MS. A prospective study from Munger et al. included participants from the US military personnel and reported a significantly decreased risk of MS with elevated levels of $25(OH)D_3$ [103]. Furthermore, a cross-sectional study described a link of serum $25(OH)D_3$ levels with both relapse rate and disability in MS patients [104]. In line with this, an association of reduced serum $25(OH)D_3$ levels with a higher risk of relapse in MS was reported in a prospective longitudinal study including 73 individuals with relapsing-remitting MS [105]. Consistent findings are obtained from a retrospective study describing an association of a 10 ng/ml increase in $25(OH)D_3$ level with a 34% reduced relapse rate in pediatric-onset MS [106] and from a prospective study reporting a $25(OH)D_3$ elevation of 10 nmol/L to be associated with a 12% decreased risk of relapse in a cohort including 145 participants [107]. A 5-year longitudinal study from Mowry and colleagues using magnetic resonance imaging (MRI) as marker of disease activity provided evidences for a lower risk of developing new T2 lesions and gadolinium-enhancing lesions as well as reduced subsequent disability attended by an 10 ng/ml increase in $25(OH)D_3$ level [108].

As mentioned before in the context of AD, these clinical studies are not able to rule out the possibility of reverse causality. In this context MR studies are of great interest analyzing this potential causal relationship of vitamin D and MS risk. Mokry et al. identified four $25(OH)D_3$ level-associated SNPs in a large genome-wide association study for vitamin D, called SUNLIGHT, and performed an MR study to examine the influence of genetically reduced vitamin D_3 levels on the odds of MS in a large genetic association study for MS. This leads to the finding that genetically reduced vitamin D_3 levels are strongly linked to an elevated MS risk [109]. This thesis is furthermore supported by another novel MR study that described causal effects of decreased vitamin D_3 levels on pediatric-onset MS [110]. A very recent study from Graves et al. provides evidence for a causal link between $25(OH)D_3$ and MS relapses in children. The authors indicate that a vitamin D genetic risk score (vitDGRS) can support the identification of patients at greater relapse risk [111].

The protective role of vitamin D could be exerted on the molecular level mainly due to its several potential immunomodulatory effects. These are summarized in a review from Smolders and colleagues. The authors pointed out that vitamin D causes a shift to an anti-inflammatory immune response and increased regulatory T cells as well as reduced pro-inflammatory helper cells like Th1 and Th17 selectively [112]. In line with this, a study from Munger et al. reported an overlap of genes associated with vitamin D_3 and those associated with processes of immune regulation. Furthermore they suggest the sphingosine-1-phosphate receptor-dependent migration of lymphocytes from secondary lymphoid tissue as potential vitamin D_3-mediated mechanism [113]. Additional evidences for the MS-underlying molecular mechanisms arise from a recent study using the L-type calcium channel antagonist nimodipine, showing decreased neurodegeneration in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. The authors reported calcium channel-independent, microglia-specific effects: induction of apoptosis, reduced levels of NO and ROS, as well as positive effects on remyelination [114].

Based on the results of all these studies demonstrating a causal link between vitamin D hypovitaminosis and the risk of MS, the role of vitamin D_3 supplementation in MS therapy was investigated. A study from Munger et al. reported an approximately 41% reduced relative risk of MS due to the supplementation of

400 IU vitamin D/day in two large cohorts of women [115]. Several following studies also describe beneficial effects of vitamin D_3 supplementation on gadolinium-enhancing lesions, relapses, and T-cell proliferation without unrequested calcemic side effects [116–118]. Inconsistent with these results, another study revealed no positive effect of high-dose vitamin D_3 supplementation (20,000 IU/week) on course and activity of the disease, for example, the relapse risk. A possible explanation for this finding could be individuals with high vitamin D_3 levels in the placebo group. Furthermore the authors could not preclude that the used vitamin D dose was too low or that the sample size was too small [119]. In this context it should be mentioned that the individual vitamin D metabolism, which could be influenced by genetically mutations in the enzymes required for the anabolism and catabolism of vitamin D_3 as discussed before, has to be analyzed since this would influence the response to identical supplemented doses of vitamin D in different individuals [120]. Subsequent studies were able to show a reduction in relapse rate after supplementation of vitamin D_3 [121] and an improved cognitive performance [122]. Oral supplementation of 20,000 IU vitamin D_3/week in a 96-week randomized double-blind placebo-controlled study in 68 MS patients results in reduced levels of anti-EBV nuclear antigen 1 (EBNA1) protein and fragment antibody [123]. A previous study could show a remarkable overlap of EBNA2 with VDR binding sites and thereby demonstrates a genetic argument for an interaction between genetic and environmental risk factors of MS [124]. A current study reports that long-term supplementation for several months with high doses of cholecalciferol results in a significantly promoted aggravation of clinical and histological EAE, but simultaneously they find a direct, anti-inflammatory, beneficial effect of vitamin D on lymphocytes of human and murine origin [125].

5. Prion diseases

Prion diseases are a group of neurodegenerative disorders whose pathology is caused by the conversion of the cellular prion protein (PrPC) into a misfolded form of the protein, called prion or "scrapie prion protein" (PrPSc), a proteinaceous, insoluble, infectious particle which is resistant to proteases and seems to act as template for exponential transformation and further accumulation of prion proteins. One characteristic of prion diseases is their appearance by sporadic (spontaneously conversion, Jakob-Creutzfeldt disease), genetic (familial mutations in the prion protein gene, PRNP), or acquired (accidentally transmission or infection) mechanisms [126].

A study on transgenic mice was able to identify a naturally occurring polymorphism in the human PRNP which is very rare but seems to completely prevent prion disease [127]. Up to this date, no clinical trial on human prion disease was successful, and most of the analyzed chemical compounds failed as potential therapeutics because of their toxicity. A study from Suenaga and colleagues intended to identify compounds that interfere with the direct PrPC-PrPSc interaction by screening hydrophobic vitamins. The authors reported for the first time that vitamin D_2 was able to interact with a truncated form of human recombinant PrPC leading to a reduced oligomerization in vitro. The absence of such an effect mediated by vitamin D_3 could be due to structural differences between these two vitamin D forms. This study suggests vitamin D_2 as suitable therapeutic candidate to target PrPC in the brain of patients with prion disease because of its direct inhibition of PrPC oligomerization, its blood–brain barrier permeability, and its safety compared to other synthetic compounds [128]. But apart from that, more studies, especially clinical trials, are essential to elucidate the relationship of vitamin D and prion diseases.

6. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons in the brain and spinal cord resulting in paralysis [129]. It is characterized, similar to the abovementioned vitamin D_3-associated diseases, by oxidative stress, inflammation, mitochondrial dysfunction, and neurodegeneration [130].

Clinical studies result in inhomogeneous findings about a possible role of vitamin D on ALS. A retrospective study including 57 ALS patients reported neither significant differences in 25(OH)D_3/D_2 blood levels in comparison to 57 healthy individuals nor an improvement of the recorded clinical, ALS-related variables after oral supplementation of 100,000 IU of vitamin D_3/week for 4 weeks and thereafter 25,000 IU every 15 days compared to untreated participants. But as discussed by the authors, potential limitations of this study are its retrospective character and the sample size [131]. Two earlier studies also described an absent relationship between serum 25(OH)D_3 levels and prognosis in ALS [132, 133]. A very recent study examined the outcome of supplementation of 50,000, 75,000, and 100,000 IU vitamin D_3/month on motor dysfunction and clinical progression of ALS. After 6 months, they reported increased levels of serum 25(OH)D3 from approximately 14 ng/mL at starting point to approximately 40 ng/mL after supplementation of 75,000 and 100,000 IU vitamin D_3 monthly, but there were no statistically significant differences in the tested clinical ALS characteristics. As the authors mention, the sample size of 10–12 participants per group as well as the short duration of the follow-up study has to be taken into consideration [134].

In contrast to these findings, a study from Karam et al. reported vitamin D level less than 30 ng/mL for 81% of their patients with ALS and additionally improvements in the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score after supplementation of 2000 IU vitamin D/day for 9 months in 20 participants [135]. In line, a subsequent study described a neuroprotective role for the biologically active form of vitamin D_3 as well as a four-time accelerated decline and reduced expectation of life due to hypovitaminosis D [136]. These variable outcomes strengthen the need of further clinical studies with an adequate sample size to reach statistical power and follow-up period analyzing the effect of vitamin D_3 on ALS. A first evidence of a genetic link between vitamin D_3 and ALS arises from a study from Török and colleagues describing that a SNP in the *VDR* gene is associated with ALS [137]. Further suggestions of a potential role of the VDR in ALS derived from the finding that VDR-knockout mice have muscular and motor disruptions but no reduced cognitive performance [138].

In contrast to the ambiguous clinical studies, animal trials provide evidences for a positive effect of supplemented vitamin D_3 on ALS. Studies from Gianforcaro and colleagues reported beneficial functions of high-dose dietary vitamin D_3 supplementation on the paw grip endurance and motor performance of transgenic G93A ALS mouse model while decreased performance of functional outcome in vitamin D_3 deficient mice [139, 140].

In reference to the above itemized similarities in ALS characteristics with other neurodegenerative diseases, it is not remarkable that the molecular mechanisms of which vitamin D_3 is suggested to have a beneficial influence on ALS are overlapping. Vitamin D_3 could decrease the elevated levels of TNF-α or IL-6 found in ALS patients [141] or influence calcium metabolism by regulating the expression of calcium-binding proteins, known to be impaired in ALS [142]. More detailed information can be found in the publications from Gianforcaro et al. and Long et al. [143, 144].

7. Huntington's disease

Up to this date, only a few studies are available examining a possible relationship between vitamin D_3 and Huntington's disease (HD), a neurodegenerative disorder characterized by impairments in cognition, motor behavior, and psychiatrics. HD is caused on molecular level by an expansion of an autosomal dominantly inherited CAG trinucleotide repeat in the huntingtin (*HTT*) gene which is located on chromosome four in humans. This leads to the expression of a mutant huntingtin protein containing an abnormal long polyglutamine repeat [145]. The number of repeats is associated with the risk of developing HD [146]. A recent clinical review describes the consequences of mutant huntingtin on cellular level, including interferences in transcription and protein homeostasis as well as mitochondrial dysfunction and direct toxicity of the altered protein itself. This leads to a disruption in neuronal function and further cell death and neurodegeneration [147]. To our knowledge, there are no current epidemiological or clinical studies suggesting a relationship between vitamin D status and HD, just an explorative study from Chel and colleagues. They reported a high prevalence of deficient or insufficient serum vitamin D level (<50 nmol/L) in 28 individuals with manifest HD [148]. In line with this, a recent study on HD transgenic mice showed no effect on motor performance but a significantly prolonged lifespan after subcutaneous supplementation of 12,000 IU vitamin D_3 per kilogram weight [149]. Further evidences for an influence of vitamin D_3 on HD arise from a recent publication of Seuter and colleagues analyzing the epigenome-wide effects of vitamin D in THP-1 human monocytes. They identified 165 physiologically important target genes after supplementation of 1,25-dihydroxyvitamin D_3 being one of them the *HTT* gene [150].

8. Neuropsychiatric diseases

Beside neurodegenerative disorders also neuropsychiatric diseases affect the nervous system, and therefore we would like to give a brief summary of recent studies analyzing a possible influence of vitamin D on autism spectrum disorders (ASD), depression, and schizophrenia. Epidemiological studies demonstrated an elevated prevalence for ASD in children born at higher latitudes [151] and in offspring of highly pigmented women [152] as well as lower vitamin D levels of children with autism [153]. Furthermore maternal, gestational hypovitaminosis D is associated with a higher ASD risk [154, 155]. Recent findings of strong associations of ASD with polymorphisms in the VDR or other genes involved in vitamin D_3 metabolism [156, 157] hypothesize vitamin D_3 as environmental and genetic factor influencing ASD [158]. On the molecular level, cellular processes like oxidative stress or neuroinflammation were shown to play a role in ASD [159], and they could present a potential contact point for vitamin D_3. In line with the abovementioned link between ASD and vitamin D, a vitamin D-deficient rat model revealed broad behavioral similarities between vitamin D-deficient models and ASD-associated behavior [160]. Treatment of an ASD rat model with high-dose vitamin D revealed significant protective effects [161]. In contrast to supplementation in animal trials, the first randomized controlled clinical study analyzing the daily supplementation of 300 IU vitamin D3/kg for 4 months on 109 children with ASD, resulting in significant improvement of autism symptoms, was retracted 1 month ago [162]. This lets us conclude that vitamin D_3 can be suggested as possible preventive treatment for the prevention of ASD, but more studies supplementing pregnant women and their children with adequate levels of vitamin D_3 have to be performed.

A potential link between vitamin D_3 and depression is subject of current research, and for more detailed information about the potential role of vitamin D on major depressive disorder, we recommend a review from Casseb et al. [163]. A meta-analysis from Parker and colleagues concluded that there are increasing evidences for an influence of vitamin D on depression [164], and in line with this, another review also postulated hypovitaminosis D as risk factor for late-life depression [165]. Consistently, a very recent meta-analysis reported a negative association of serum 25(OH)D_3 levels with the risk of depression [166].

A following cross-sectional study including 100 women in reproductive age also shows that the depression score inverse correlated with the vitamin D serum level [167]. Contradictorily, a supplementation with 1200 IU vitamin D for 12 months failed to have an influence on the prevention of depression in a very recent, randomized clinical trial including 155 participants having clinically relevant depressive symptoms [168]. Furthermore, a recent MR study from Libuda and colleagues indicates no causal relationship between both depressive symptoms and broad depression and vitamin D levels due to a missing association of six vitamin D-related SNPs with depression [169]. In summary, the current research investigating the role of vitamin D_3 in depression is much less clear than other neurological disorders.

In respect to the chronic mental illness schizophrenia, it could be shown that hypovitaminosis D is common in patients [170]. This fits to the environmental risk factors that have been described for schizophrenia, like season of birth [171] and latitude [172]. Also a link between neonatal vitamin D levels and the schizophrenia risk was reported [173]. A recent randomized, placebo-controlled study from Krivoy and colleagues examined psychosis severity, mood, cognition, and metabolic profile in 47 schizophrenia patients during an 8-week supplementation of 14,000 IU vitamin D/week. The authors described no significant effects on psychosis, mood, or metabolic status, but a trend to an improved cognitive function accompanied by significant elevated vitamin D levels in the supplemented group. A possible explanation for these findings, given by the authors, could be that a medical score that measures the symptom severity in schizophrenia patients decreased during the study in the placebo as well as in the treated group and could thereby veil the influence of supplemented vitamin D [174]. An actual study reported beneficial effects of supplementation of vitamin D_3 in combination with probiotics in schizophrenia patients [175]. Addressing the underlying molecular mechanism, vitamin D could perform its suggested beneficial actions via modulation of immune system and inflammation processes since it was reported that patients with chronic schizophrenia have significantly elevated levels of TNF-α and IL-6 [176]. Furthermore, it could be shown that the expression of genes involved in the metabolism of vitamin D_3 (*VDR*, *CYP27B1*, *CYP24A1)* is significantly elevated in peripheral blood of schizophrenic patients [177], indicating a potential causal relationship between vitamin D_3 and schizophrenia, which should be the aim of future research.

9. Conclusion

In summary, in the current literature, several lines of evidence suggest a tight link between vitamin D_3 and neurodegenerative diseases (see **Table 1**). Besides epidemiological studies, vitamin D deficiency influences several pathways associated with neurodegenerative diseases, which also indicates a causal link of hypovitaminosis D and AD, MS, and PD. As a consequence, vitamin D hypovitaminosis is broadly assumed to be a risk factor for these diseases. In addition, recent literature underlines a potential link between prion diseases, ALS, HD, and neuropsychiatric

	Alzheimer's disease	Parkinson's disease	Multiple sclerosis	Prion disease	Amyotrophic lateral sclerosis	Huntington's disease
Epidemiological and clinical studies	Vit. D levels ↓ [39, 40] Risk factor [41, 43–48] Causal link [51–56]	Vit. D levels ↓ [76–79] Severity of PD [80–81, 83] Genetic link [85–86]	Risk factor [96, 103] Genetic predisposition [99–101] Causal link [109–111]	/	Non-consistent findings [132, 133, 136] Evidence of genetic link [137]	Vit. D levels ↓ [148]
Animal and cell culture studies	Causal link [63] Transcription [62]	Causal relationship [63]	Regulatory T cells ↑ [112]	Vit. D2 as suitable therapeutic candidate [128]	Potential role of the VDR [138] Benefits after supplementation [139, 140]	Vit. D supplementation ↓ prolonged lifespan [149] Epigenomic action on *HTT* gene [150]
Molecular mechanisms	APP homeostasis [64–69] Neurotrophins [71–73] Inflammation [74] Ca^{2+} homeostasis [6]	Dopamine homeostasis [89] Antioxidative properties [91] Ca^{2+} homeostasis [92]	Immunomodulatory effects [113] Ca^{2+} homeostasis [114]	Reduced oligomerization of prions [128]	Inflammation [141] Ca^{2+} homeostasis [142]	Potential transcriptional regulation of *HTT* gene [150]
Intervention studies	Beneficial [58]	Beneficial in dependence of genotype [87]	Beneficial [115–118] Vit. D as genetic and environmental risk factor [124]	/	Variable outcomes [131, 134]	/

Vit. D, vitamin D; APP, amyloid precursor protein; Ca^{2+}, calcium; PD, Parkinson's disease; VDR, vitamin D receptor; ↓ reduced.

Table 1.
Summary of the current research findings about the influence of vitamin D on selected neurodegenerative diseases Alzheimer's disease, Parkinson's disease, multiple sclerosis, prion disease, amyotrophic lateral sclerosis, and Huntington's disease in respect to epidemiological and clinical, animal and cell culture studies, identified molecular mechanisms, and intervention studies.

diseases and reduced Vitamin D level; however, it has to be emphasized that many aspects of vitamin D remain obscure in these diseases. Therefore, further studies to clarify and judge a potential beneficial effect of vitamin D_3 are needed to unravel the exact role of vitamin D in brain metabolism and neurodegenerative diseases. Especially determination of the individual serum 25(OH)D_3 levels by appropriate methods like mass spectrometry and taking the individual patient's competence to metabolize or form active vitamin D_3 into consideration will help to avoid ambiguous results. The fact that several prospective studies investigate a mixture of several different nutritional components makes it hard to trace the observed effects back to vitamin D in particular at the moment.

Interestingly some European countries, like Finland, already supplement vitamin D in general in food to prevent hypovitaminosis in the elderly population, and an optimal intake of 2000–4000 IU vitamin D or 10–20 min sunlight exposure per day is already recommended by experts, for example, at the Joint International Symposium Vitamin D in Prevention and Therapy and Biologic Effects of Light (2019). Further studies will help to validate the beneficial effects of these recommendations.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 8

Vitamin D_3 Modulates NF-kB/p65, 17β-Estradiol, and Vitamin D Receptors Expression at Estrogen Deficiency

Alexandra Koshkina, Olga Volkova and Julia Fedotova

Abstract

The aim of the present study was to focus on the effects of Vitamin D_3 (VD_3) supplementation (5.0 mg/kg, s.c.) on the NF-kB/p65, 17β-estradiol (17β-E_2)/VD_3 receptors expression in the hippocampus in the long-term ovariectomized (OVX) rats treated with low dose of 17β-E_2 (0.5 μg/rat, s.c.) submitted for the chronic unpredictable mild stress (CUMS) for 28 days. Sucrose preference (SPT), forced swimming (FST), and open-field (OFT) tests were conducted to estimate the anhedonia-/depression-like states. NF-kB/p65, 17β-E_2/VD_3 receptors levels in the hippocampus were evaluated by ELISA and Western blot assays. The findings demonstrated that VD_3 at high dose (5.0 mg/kg, s.c.) in a combination with low dose of 17β-E_2 decreased anhedonia in the SPT and depression-like behavior in the FST of the long-term OVX rats submitted to CUMS. VD_3 (5.0 mg/kg) resulted in significant decreased levels of hippocampal NF-kB/p65 protein expression, as well as to the normalization of hippocampal 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS. In conclusion, VD_3 (5.0 mg/kg, s.c.) in a combination with low dose of 17β-E_2 had a synergic antianhedonic- and antidepressant-like effects in the adult female rats following long-term ovariectomy submitted to CUMS.

Keywords: vitamin D_3, long-term ovariectomy, chronic unpredictable mild stress, NF-kB/p65, 17β-estradiol receptor, vitamin D_3 receptors

1. Introduction

The menopausal transition is often associated with a multiplicity of manifestations, the most standard being neuropsychiatric [1, 2]. The role of ovarian hormones in affect-related disorders is of great interest for women transitioning through menopause [2, 3]. Mood disorders during menopause could partly be explained due to a loss of estrogen is known to have neuroprotective effects on brain [3]. Numerous experimental and clinical studies have documented that estrogen deficiency during menopause increases the susceptibility to mood disturbances, including anxiety [4–6]. There has been a discussion that menopausal hormonal therapy (MHT) may improve the symptoms of affective-related disorders or decrease the risk of developing these, yet some uncertainty still exist around this topic because as research has

also found that MHT does not entirely stop the development of affective-related symptoms [7].

Females going through menopause are at higher risk of developing Vitamin D (VD) deficiency due to a VD poor diet, restricted outdoor activity resulting in less sun exposure as well as a decreased capacity to produce enough calcitriol as a result of an age related decline in hydroxylation by the kidneys [8]. Our previous experimental work has confirmed that hormonal profile in ovariectomized (OVX) female rodents is also characterized by VD deficiency or insufficiency [9, 10]. Traditional methods of affective-related disorders therapy, which also include antidepressants/anxiolytics, are unfortunately of limited effectiveness [11]. Nutrient imbalance, especially VD_3 deficiency, is considered as one of the critical causes, enabling the pathophysiological mechanisms for development of psychiatric disorders [12]. In the pathophysiological mechanisms of mood disorders, many trigger factors play a role, and it is argued that one of them could be a deficiency in VD_3 [12].

VD_3 deficiency has been proven to impact on the pathogenesis of various diseases, for example, autoimmune diseases, cardiovascular diseases, infections, osteoporosis, obesity, diabetes, and certain types of cancers [13–15]. A correlation between very low VD_3 levels and numerous neuropsychiatric diseases and a correlation between an impact of VD_3 levels and normal brain functioning have also been found in recent studies [14–16]. VD receptors (VDRs) have been found present in the central nervous system [17], in the brain structures involved in processes of mood regulation (cingulate cortex, hippocampus, thalamus, and hypothalamus) [18]. In this line, it can be assumed that VD_3 likely has humoral or neurohumoral activities in these brain structures. VD_3 involves in the neurogenesis, neuroplasticity, neuroprotection, and neuroimmunomodulation [19–21]. This fact creates a neurobiological basis to propose the involvement of VD in the mechanisms of neuropsychiatric disorders [22–26].

The neuroinflammation in the central nervous system is supposed to be one of the main trigger factors for the development of affective-related disorders [27, 28]. Taking this assumption into account, mood disturbances established in menopausal women might result from complex alterations in estradiol and VD_3 levels, as well as neuroinflammation.

Nowadays, nuclear factor-kappa B (NF-κB) is postulated as the proinflammatory transcription factor that controls proinflammatory cytokines expression and is involved in the mechanisms of many inflammatory and neuroinflammatory diseases [29, 30]. NF-κB is triggered by stress and might mediate cellular responses to stressful life events, thereby critically involved in development of affective-related disorders [31–33]. The enhancement of NF-κB might induce the elevated production of proinflammatory cytokines and diminished neurohormonal stress feedback [34]. Furthermore, NF-κB pathway is involved in antidepressant action of different psychotropic drugs that used for treatment of mood disorders [35]. Clinical studies using patients with mood disorders have shown that NF-κB levels are increased in the serum of such patients [35–37]. Using genetic and environmental model of depression, it was shown that the antidepressant effect of such pharmacological treatments was dependent on NF-κB-p65 acetylation [36, 37].

The hippocampus is one of the key structures of the brain, which plays a role in affective-related disorders [38]. Both estrogen and VD_3 have been associated with the successful functioning of the hippocampus [1, 21, 25]. Basic and clinical studies have suggested that alterations in NF-kB/p65 signaling and in 17β-E_2/VD_3 receptors expression in the hippocampus, as well changes of serum estradiol/VD contents are very often registered at affective-related disorders [1, 23, 39]. Animal studies have documented that the impaired behavioral profile in OVX rats is correlated with increased NF-kB/p65 levels in the brain [40, 41].

Recently, we found that VD_3 (5.0 mg/kg, s.c.) reduced anhedonia and depression-like behavior of long-term adult ovariectomized (OVX) rats exposed to the chronic unpredictable mild stress (CUMS) in the sucrose preference (SPT) and forced swimming (FST), respectively [42]. However, the therapeutic effects of VD_3 in a combination with low dose of 17β-E_2 in female rats with long-lasting decline of estrogens exposed to CUMS remain unknown. Furthermore, it is still unclear whether the antidepressant-like action of VD_3 plus 17β-E_2 application implicates NF-kB/p65 signaling pathway and modifications of 17β-E_2/VD_3 receptors expression in the hippocampus of long-term OVX adult rats with CUMS.

The current investigation was performed to clarify the antidepressant-like effect of a combination with VD_3 plus low dose of 17β-E_2 on a rat model of CUMS in the female rats with long-lasting decline of estrogens. Similar to previously published work [43], we used long-lasting estrogen deficiency caused by a post-ovariectomy period of 3 months. This animal model is widely utilized in preclinical behavioral research producing a menopausal-like state in women [44]. Such behavioral tests as sucrose preference (SPT), forced swimming (FST), and open-field (OFT) were carried out to examine the depression-like behavior. NF-kB/p65, 17β-E_2/VD_3 receptors levels in the hippocampus and serum estradiol and VD concentrations were determined to assess the possible mechanisms of the VD_3 effects on the depression-like behavior in long-term OVX rats given with low dose of 17β-E_2 subjected to CUMS.

2. Materials and methods

2.1 Animals

A total of 49 Wistar rats of 3 months age, female sex (weighing 200–220 g) were purchased in this work. Animals were divided into experimental groups with access to rat standard food and water ad libitum. The female rats were placed under a 12 light-dark scheme (light was given between 07:00 and 19:00 h) and room temperature (23 ± 2°C). All behavioral procedures and CUMS model were performed in compliance with the National research council's guide for the care and use of laboratory animals and approved by the Ethical committee for experimental studies of I.P. Pavlov Institute of Physiology (statement No.: 1095/1/25.06.2012). Stress model of depression was conducted with minimal pain for all groups of rats.

2.2 Ovariectomy

Three months before CUMS procedure, sham operation and long-term total ovariectomy with general anesthesia (ketamine 70 mg/kg and xylazine 10 mg/kg, i.p.) were performed. Long-term period (3 months) elimination of female gonadal hormones was chosen as experimental model of menopause in women [44, 45]. The removal of ovaries was carried out accordingly to our method as prescribed earlier [43]. After surgery or sham-operation (SHAM), the ovariectomized (OVX) females were placed in home cage with free access to food and water. During 12 weeks, sham-operated and OVX females had a recovery. Following 3 months of surgery, experimental rats were randomly distributed to the groups for the chronic stress procedure, except for SHAM non-stressed control rats.

2.3 CUMS model

Chronic unpredictable mild stress (CUMS) paradigm is a valid and significant animal model of depression induced by stress procedure. This behavioral model

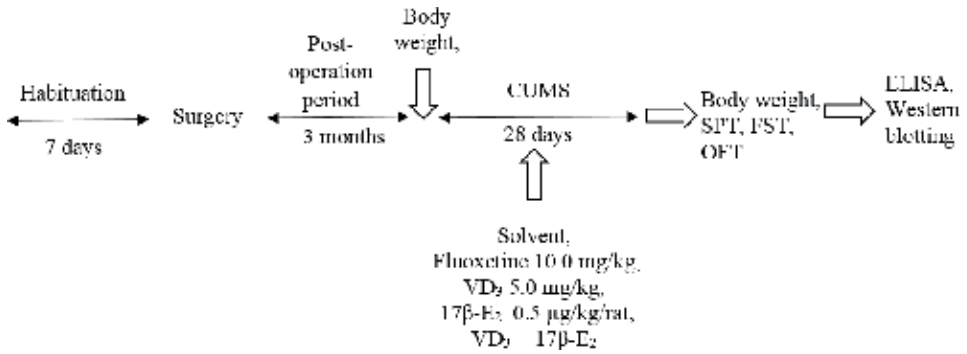

Figure 1.
Timeline of chronic treatment. Female Wistar rats were divided into 6 groups – non-CUMS SHAM rats treated with solvent (control), SHAM rats submitted to CUMS treated with solvent, long-term OVX rats exposed to CUMS given with solvent, fluoxetine as positive control (10.0 mg/kg/day), 17β-E_2 (0.5 μg/rat/day, s.c.) or VD_3 (5.0 mg/kg/day, s.c.) in a combination with low dose of 17β-E_2.

of depression state is strongly verified by both preclinical and clinical studies [46]. CUMS was made as described previously [47, 48]. The procedure included the exposure to different and unpredictable stress factors that are randomly changed during experimental days [49]. These manipulations are 24 h food deprivation, 24 h water deprivation, wet bedding overnight, titled cage overnight, unpredictable shocks (15 mA, one shocks/20s, 10 s duration, 20 min), 5 min swimming at cold water (4°C), tail hanging, 1 min, clip tail for 1 min, reversal of light/dark cycle [47, 48]. All stress triggers were performed individually and continuously. To prevent habituation and to ensure the unpredictability of the stressors, all stress manipulations randomly made accordingly to experimental scheme, repeated throughout the 4 weeks of CUMS protocol. The control SHAM females were placed in a separate room without any contact with the stressed groups of animals. These rats were maintained as undisturbed animals that are subjected only routine cage cleaning for 4 weeks. The total scheme of whole experiment is indicated on **Figure 1**.

2.4 Drugs

17β-E_2, fluoxetine hydrochloride and VD_3 as cholecalciferol were provided from Sigma Chemical Co. (St. Louis, MO, USA). The solution of female estrogen was prepared using sterile sesame oil. Vitamin D_3 was dissolved in 95% ethanol and then aliquoted and remained at −80°C. The solution of cholecalciferol for the injection into the experimental groups was diluted in sterile water, resulting in a solvent of VD_3 containing 2% ethanol. Fluoxetine hydrochloride was dissolved in sterile physiological saline. All drugs were injected subcutaneously (0.1 ml/rat) for the 4 weeks during the CUMS procedure – 30 min before the daily stressor action – and throughout the period of the behavioral tests. All behavioral measurements were made 60 min after the last drug administration.

2.5 Groups of animals

All animals were randomly assigned to the six experimental groups (n = 7 in each): non-CUMS SHAM rats treated with solvent (control), SHAM rats exposed to CUMS treated with solvent, long-term OVX rats exposed to CUMS given with solvent, fluoxetine as positive control (10.0 mg/kg/day), 17β-E_2 (0.5 μg/rat/day, s.c.) or VD_3 (5.0 mg/kg/day, s.c.) in a combination with low dose of 17β-E_2. In our preliminary studies, there were no significant differences between SHAM/OVX rats treated with physiological

saline as solvent for fluoxetine and SHAM/OVX females treated with sterile water with 2% ethanol as solvent for VD_3 or SHAM/OVX females treated with sesame oil as solvent for 17β-E_2 in behavioral trials (data are not shown). Since, we did not found any differences between these experimental groups, the sesame oil as solvent for SHAM/OVX females was used in the present work. The dose of VD_3 and dose of 17β-E_2 were based on our previous work on the behavioral effects of VD_3 on depression-like behavior of long-term OVX female rats submitted to CUMS [42, 43]. The dose of fluoxetine was utilized according to earlier studies demonstrating that the administration of fluoxetine decreases depressive-like behavior in rodents [42]. All drugs were injected subcutaneously (0.1 ml/rat) for the 4 weeks during the CUMS procedure – 30 min before the daily stressor action – and throughout the period of the behavioral tests. All behavioral measurements were made 60 min after the last drug administration.

2.6 Sucrose preference test

We performed SPT accordingly to our previous study [50, 51]. Before and after the initiation of the 4 weeks CUMS procedures, the experimental rats were subjected to the sucrose preference test (SPT) [42, 51]. This test is set up as follows: following a training trial, the rats are subjected to a 24 h deprivation of food and water. On the next day, the rats have 1 hour access to one bottle with 200 ml of water and a similar amount of sucrose solution. The experimenter measures the percentage of the consumed sucrose solution and water volumes as a measure of sucrose preference by calculating the value of the sucrose preference among all (sucrose plus water in mL) liquid consumption:

$$\%\text{sucrose preference} = \frac{\text{sucrose consumption}}{\text{sucrose consumption + water consumption}} \times 100 \qquad (1)$$

2.7 Forced swimming test

For testing of modifications of depression-like behavior, all groups of rats were submitted to the standard forced swimming test (FST) as described in earlier works (FST) [42, 43]. The three cylinders (60 cm tall and diameter 20 cm) were filled with 23–25°C water up to a 30-cm depth. On the first day, rats were pre-tested during 15 min in cylinders. Then, rats were dried with papers and placed at their home cages till the next day. On the second day (testing trial), OVX females with CUMS were examined into the apparatus for 5 min. The following parameters were registered: (1) immobility time (floating in the water with only movements necessary to keep the head above water); (2) swimming time (active swimming movements around glass cylinder); (3) climbing time (active movements with forepaws directed toward the walls). For recording of these values, a video camera was installed above the apparatus.

2.8 Open field test

The measurements of the behavioral activity in the OFT were carried out in a similar way to the method which has been published in a previous study [43]. The rats were set in the center square of the OFT and tested for 5 min. Motor activity and rearing and grooming behavior were recorded for 300 s in the OFT apparatus using a video camera, and equipment was cleaned in-between sessions.

2.9 Biochemical measurements

All rats underwent a narcosis after behavioral trials, and approximately 5 ml samples of blood were drawn from the animals to be centrifuged at 4000 *g* for

15 min at 4°C [43]. While doing so, the hippocampi of rats in the experimental group were dissected to be homogenized in cold lysis extraction buffer (0.2% sodium deoxycholate, 0.5% Triton X-100, 1% NP-40, 50 mM Tris-HCl pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 1 mM N-ethyl-maleimide, and 2.5 mM phenanthroline) [43]. After that, the hippocampal samples with the cold lysis buffer were sonicated for 15 s. Then, the hippocampi were centrifuged at 12,000 *g* for 15 min at 4°C. The Bradford method was used for the normalization of hippocampal supernatants to the total protein [52]. The serum samples and hippocampal protein normalized supernatants were stored at −80°C until the ELISA assays. The serum samples were used for the measurement of the 25-hydroxyvitamin D_3 (25-OH-VD_3) and estradiol levels using a commercially available rat ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, P.R. China) according to the manufacturer's instructions. The sensitivity and detection range of the 25-OH-VD_3 rat ELISA kits were 5.0 μg/l and 20–100 μg/l, respectively. The sensitivity and detection range of the estradiol rat ELISA kits were 4.0 pg/ml and 40–1500 pg/ml, respectively.

Hippocampal homogenates were used for the detection of the NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels by rat ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, P.R. China) according to the manufacturer's instructions. Briefly, 100 μl of hippocampal sample or standard was added to each well and incubated for 120 min at 37.0°C. Then, 100 μl of anti-NF-kB/p65/p65, anti-17β-E_2 receptor, and anti-VD_3 receptor antibodies were added to each different well and incubated for 60 min at 37.0°C. After 3 times of washing, 100 μl of HRP-avidin working solution was added to each well and incubated for 60 min at 37.0°C. Again, after 5 times of washing, 90 μl of tetramethylbenzidine solution was given to each different well and incubated for 15–30 min at 37.0°C. Then, 50 μl of stop solution was added to each well to terminate the color reaction. The NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels were measured using a MC Thermo Fisher Scientific reader (Thermo Fisher Scientific Inc., Finland) with an absorbance of 450 nm. The standard curve was used for the calculation of the relationship between the optical density and the NF-kB/p65/p65, 17β-E_2/VD_3 receptors levels. The BDNF content is presented as pg/mg of tissue. The sensitivity and detection range of the NF-kB/p65 rat ELISA kit were 5.0 μg/ml and 6.0–600 μg/ml, respectively. The sensitivity and detection range of the 17β-E_2 receptor rat ELISA kit was 0.39 pg/ml and 1.56–100 pg/ml, respectively. The sensitivity and detection range of the VD_3 receptor rat ELISA kit was 5.8 pg/ml and 23.5–1500 pg/ml, respectively. The assay exhibited no significant cross reactivity with other ligands. All samples were duplicated for the assay.

2.10 Western blots

Hippocampal tissues were homogenized in cold lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich, USA) for 1 h and centrifuged at 12,000 *g* at 4°C for 20 min [42]. The protein content was evaluated by a Bio-Rad protein detector (Bio-Rad, USA), and 100 μg of total protein from each sample was denatured with buffer (6.205 mM Tris-HCl, 10% glycerol, 2% SDS, 0.01% bromophenol blue, and 50 mM 2ME) at 95°C for 5 min. The denatured proteins were separated on an SDS page (10% sodium dodecyl sulfate polyacrylamide gel) and forwarded to a nitrocellulose membrane (Amersham Biotech, USA). After that, the membranes were probed with anti-NF-kB/p65/p65, anti-17β-E_2 receptor, anti-VD_3 receptor (1:1000, Santa Cruz), and β-actin (1:1000; Sigma-Aldrich, USA) monoclonal antibodies for 2 h and secondary antirabbit antibodies (1:5000; Santa Cruz, USA) conjugated to horseradish peroxidase for 1 h. Bands were detected by 5-bromo-4-chloro-3-indolyl phosphate with a nitro blue tetrazolium kit (Abcam, China) as a chemiluminescent substrate. Signals were measured by an image analysis system (UVIdoc, Houston, TX, USA).

2.11 Statistical analysis

All experimental data are expressed as the mean ± standard deviation of the mean. The treatment effects were determined with a one-way ANOVA followed by an LSD *post hoc* test using the Statistics Package for SPSS, version 16.0 (SPSS Inc., USA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 VD_3 alters the body weight in the long-term OVX rats treated with 17β-E_2 exposed to CUMS

The body weights of long-term OVX rats subjected to CUMS and treated with 17β-E_2 in a combination with all investigated doses of VD_3 are presented in **Figure 2**.

There was no difference in the initial body weight in all the experimental groups. Following 4 weeks, the body weight of SHAM rats with CUMS was significantly decreased compared to the control, non-CUMS SHAM group (**Figure 2**, $F(1,34) = 72.66$, $P < 0.001$). The body weight of long-term OVX rats with CUMS was significantly decreased compared to the non-CUMS/CUMS SHAM groups (**Figure 2**, $P < 0.001$). Administration of 17β-E_2 did not statistically enhance body weight of long-term OVX rats with CUMS compared to the non-CUMS control, CUMS OVX/SHAM groups (**Figure 2**, $P > 0.001$). However, there was a tendency to increase the body weight of long-term OVX

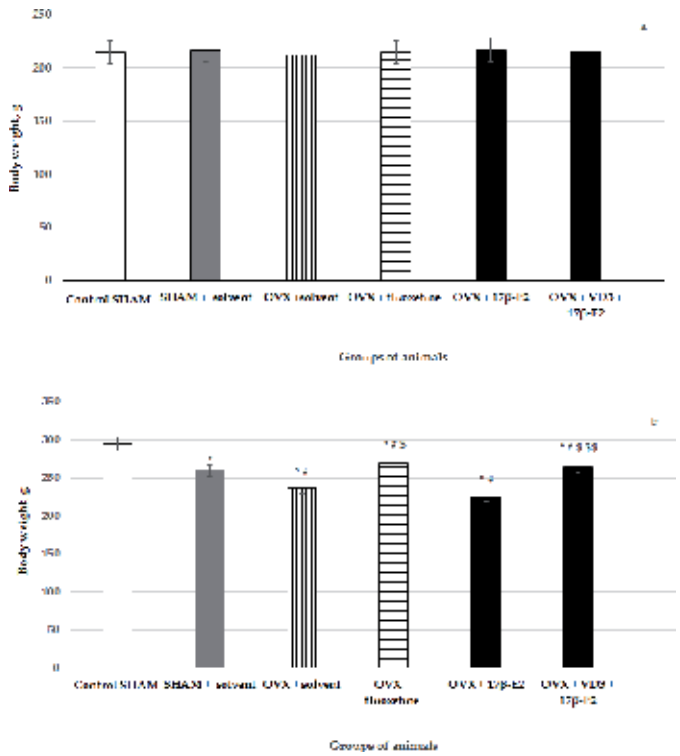

Figure 2.
*VD_3 corrects the body weight in the long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) Prior to CUMS and (b) After CUMS. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

rats with CUMS compared to the OVX rats plus CUMS given with solvent. Supplementation with VD_3 (5.0 mg/kg) plus 17β-E_2 significantly prevented the reduction of the body weight of long-term OVX rats with CUMS ($P < 0.001$) compared to the OVX plus solvent or 17β-E_2/SHAM rats exposed to CUMS (**Figure 2**, $P < 0.001$). This effect of co-administration of VD_3 (5.0 mg/kg) plus 17β-E_2 was similar to the effect of the reference drug fluoxetine (10.0 mg/kg) in long-term OVX rats with CUMS.

3.2 VD_3 increases sucrose preference in the long-term OVX rats treated with 17β-E_2 exposed to CUMS

Before the CUMS protocol, there was no significant difference among the experimental groups in the SPT (**Figure 3**). Following 28 days of the CUMS trials, the SHAM rats exhibited a decrease in sucrose preference when compared to the control non-CUMS SHAM group ($P < 0.05$). The sucrose preference in long-term OVX rats was significantly reduced compared to the non-CUMS/CUMS SHAM rats (**Figure 3**, $F(1,34) = 56.14$, $P < 0.05$). Low dose of 17β-E_2 increased sucrose preference in long-term OVX rats with CUMS compared to the OVX group with CUMS plus solvent (**Figure 3**, $P > 0.05$). Treatment with VD_3 at dose of 5.0 mg/kg plus 17β-E_2, as well as fluoxetine, markedly increased sucrose

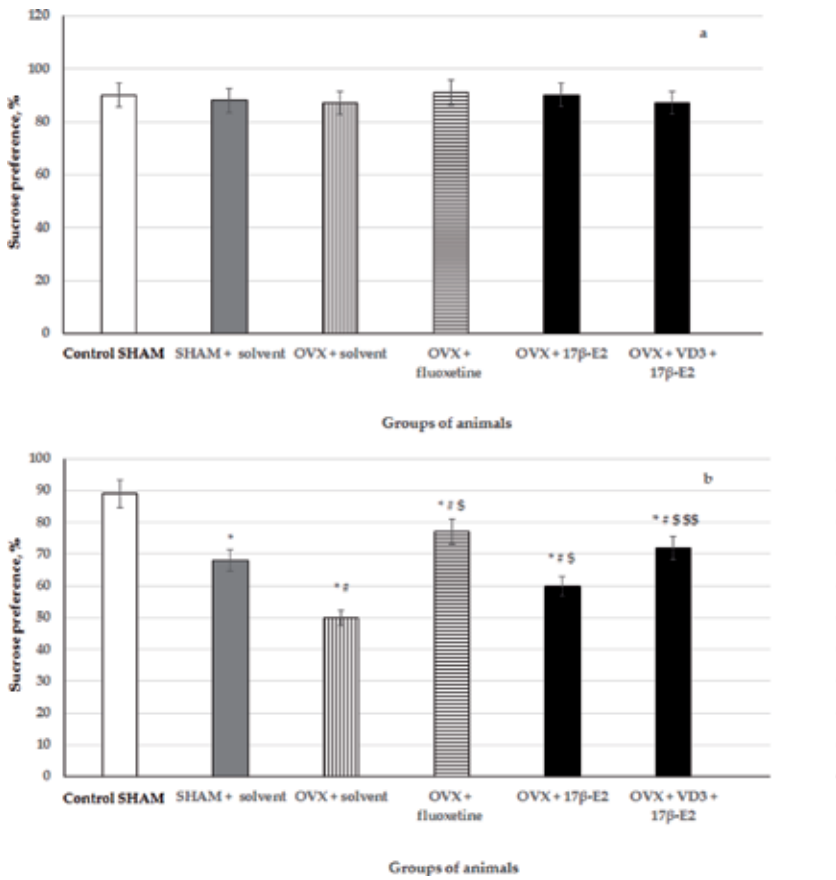

Figure 3.
*VD_3 increases sucrose preference in the long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) Prior to CUMS and (b) After CUMS. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

consumption in the long-term OVX rats exposed to CUMS when compared to the OVX plus solvent or 17β-E_2/SHAM rats submitted to the CUMS (**Figure 3**, $P < 0.05$).

3.3 VD_3 decreases depression-like behavior in the forced swimming test of long-term OVX rats treated with 17β-E_2 exposed to CUMS

CUMS produced a significant increase of the immobility time and decrease of swimming time in the long-term OVX compared to the non-CUMS/CUMS SHAM rats (**Figure 4**, $F(1,34) = 52.84$, $F(1,76) = 68.89$, $F(1,76) = 26.12$, respectively,

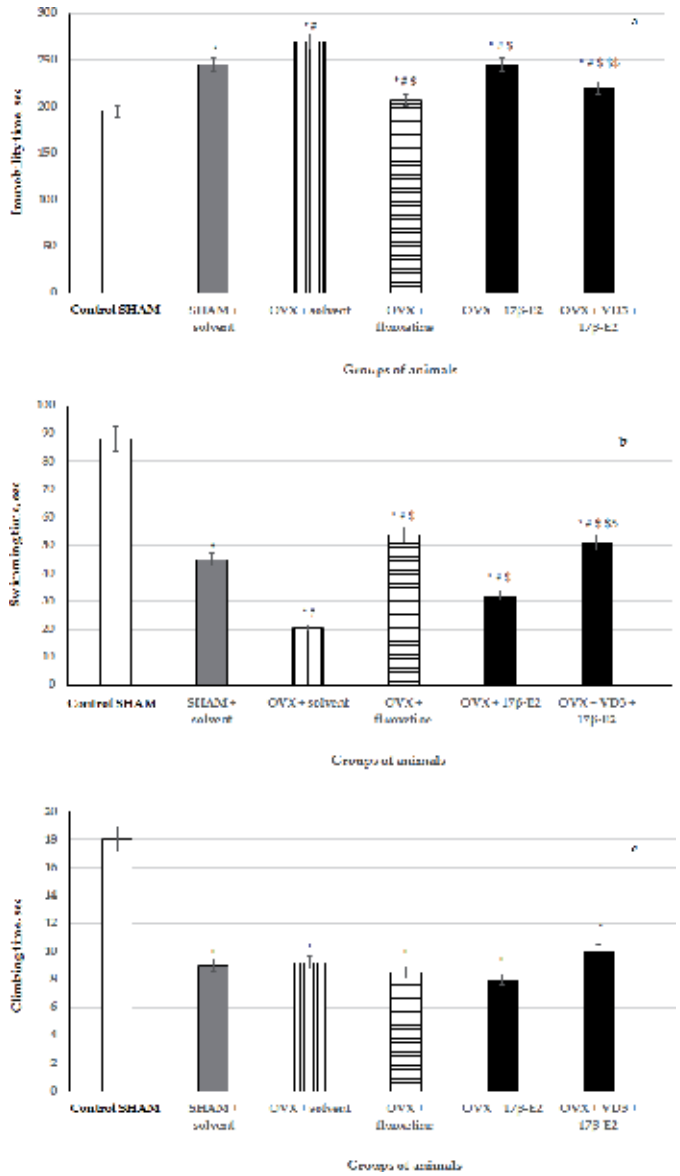

Figure 4.
*VD_3 decreased depression-like behavior in the forced swimming test of long-term OVX rats treated with 17β-E_2 submitted to CUMS: (a) immobility time, sec, (b) swimming time, and (c) climbing time, sec. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

P < 0.05). VD_3 (5.0 mg/kg), as well as fluoxetine treatment, significantly reduced the immobility time and increased the swimming time in the long-term OVX treated with 17β-E_2 compared to the OVX plus solvent or 17β-E_2/SHAM with CUMS groups (**Figure 4**, P < 0.05).

3.4 VD_3 changes the behavior in the open field test of long-term OVX rats treated with 17β-E_2 exposed to CUMS

Following 28 days of CUMS protocol, there were no statistically significant differences for grooming activities between all the experimental groups of animals in the OFT (**Figure 5**, F(1,34) = 0.82, P > 0.05). The long-term OVX rats with CUMS

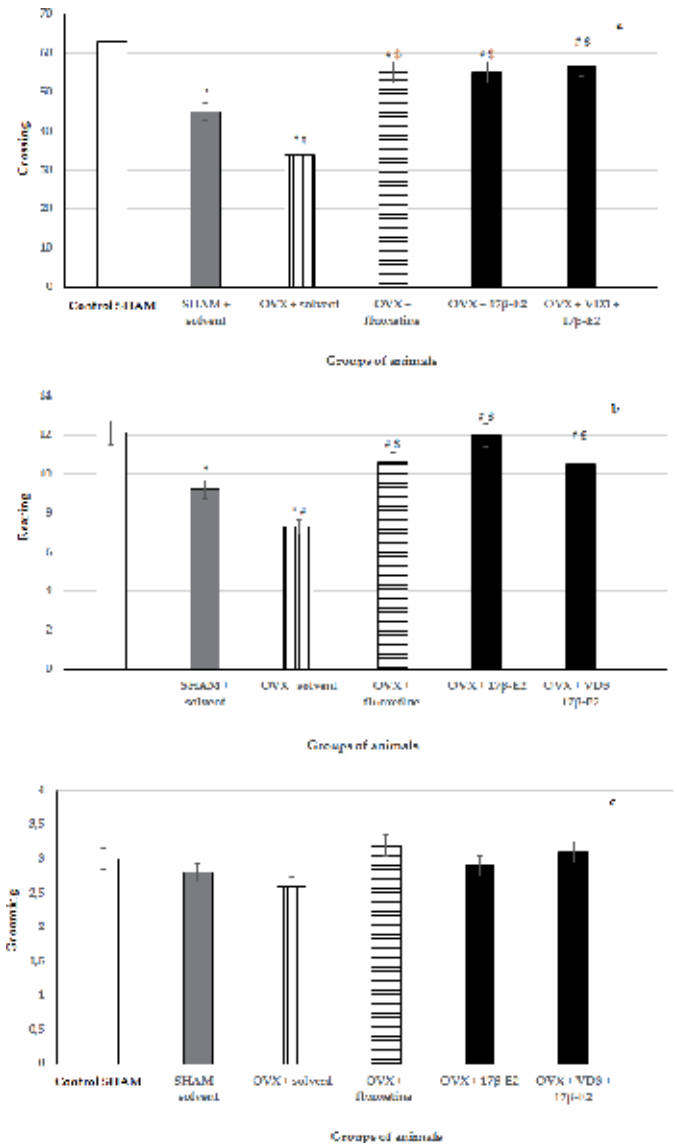

Figure 5.
*VD_3 alters the behavior in the open field test of long-term OVX rats treated with 17β-E_2 submitted to CUMS. (a) Crossing, (b) rearing, and (c) grooming. * – P < 0.05 versus the control group, # – P < 0.05 versus to the SHAM group with CUMS, $ – P < 0.05 versus to the OVX group with CUMS, and $$ – P < 0.05 versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

showed a decreased number of rearings and crossings when they were compared to the non-CUMS/CUMS SHAM groups (**Figure 5**, $F(1,34) = 14.14$, $P < 0.05$). Administration of fluoxetine, 17β-E_2, as well as treatment with VD_3 significantly elevated the number of rearings and crossings in the long-term OVX rats with CUMS compared to the OVX/SHAM rats with CUMS plus solvent (**Figure 5**).

3.5 VD_3 alters serum estradiol and VD_3 levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS

The ELISA assay revealed decreased estradiol and VD_3 concentrations in the long-term OVX rats with CUMS compared to the non-CUMS/CUMS SHAM groups (**Figure 6**, $F(1,34) = 78.56$, $F(1,34) = 56.12$, $F(1,34) = 22.21$, respectively, $P < 0.05$). Low dose of 17β-E_2 induced increase of estradiol levels in the serum blood to some extent in the long-term OVX rats with CUMS, however, it was not statistically significant ($P > 0.05$). The co-administration of VD_3 with 17β-E_2 significantly increased estradiol and VD_3 concentrations in the long-term OVX rats with CUMS compared to the OVX plus solvent or 17β-E_2/SHAM with CUMS rats (**Figure 6**, $P < 0.05$). Fluoxetine did not change the serum

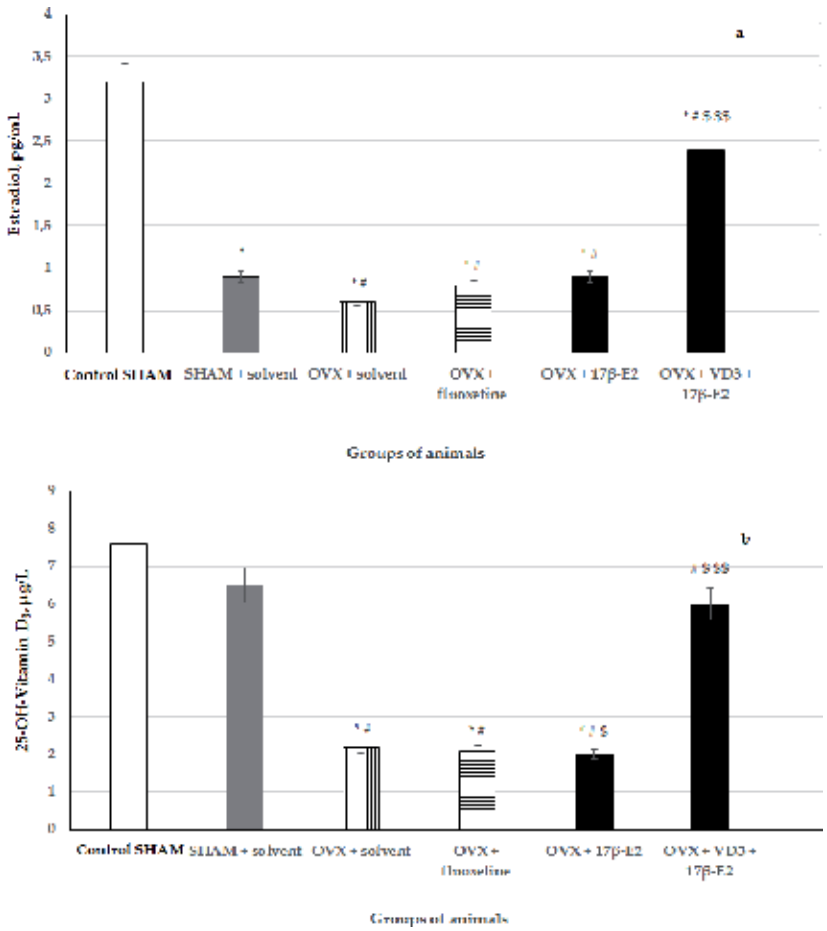

Figure 6.
*VD_3 alters serum estradiol and VD_3 levels in long-term OVX rats treated with 17β-E_2 submitted to CUMS. (a) estradiol, pg/ml and (b) 25-OH-VD3, μg/ml. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

estradiol and VD_3 levels in the long-term OVX rats exposed to CUMS (**Figure 6**, $P > 0.05$).

3.6 VD_3 modulates hippocampal NF-kB/p65/p65 and 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 exposed to CUMS

CUMS significantly increased NF-kB/p65/p65 levels and decreased 17β-E_2/VD_3 receptors concentrations in the hippocampus of SHAM rats compared to the non-CUMS control (**Figure 7**, $P < 0.05$). CUMS produced a increase of hippocampal

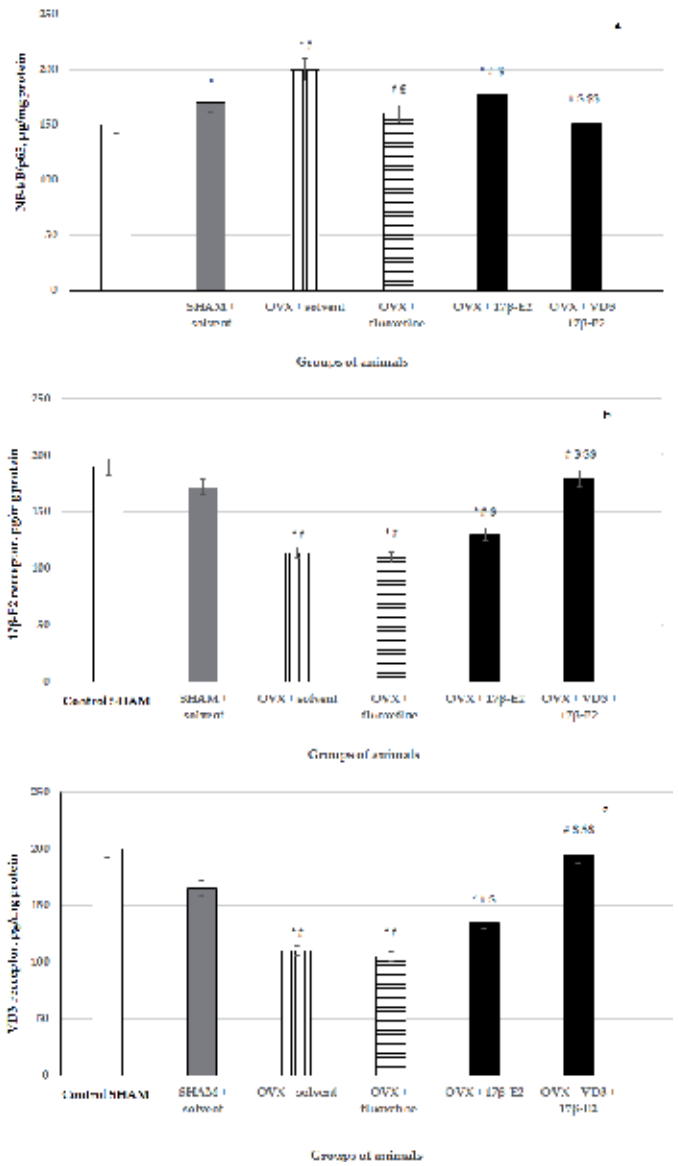

Figure 7.
*VD_3 modulates hippocampal NF-kB/p65 and 17β-E_2/VD_3 receptors levels in long-term OVX rats treated with 17β-E_2 submitted to CUMS tested by ELISA. (a) NF-kB/p65/p65, μg/ml, (b) 17β-E_2 receptor, pg/ml, and (c) VD_3 receptor, pg/ml. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

NF-kB/p65/p65 and decrease of 17β-E_2/VD_3 receptors levels in the long-term OVX rats compared to the non-CUMS/CUMS SHAM rats (**Figure 7**, $F(1,34) = 28.44$, $P < 0.05$).

Fluoxetine (10.0 mg/kg) decreased NF-kB/p65/p65 levels in the hippocampus of long-term OVX rats treated to CUMS compared to the OVX plus solvent/SHAM rats with CUMS (**Figure 7**, $P < 0.05$). Moreover, VD_3 plus 17β-E_2 reversed 17β-E_2/VD_3 receptors levels and reduced NF-kB/p65 levels in the hippocampus of the long-term OVX rats compared to OVX plus solvent or 17β-E_2/SHAM rats with CUMS (**Figure 7**, $P < 0.05$). Fluoxetine failed to modify 17β-E_2/VD_3 receptors levels in the long-term OVX rats exposed to CUMS (**Figure 7**, $P > 0.05$).

Western blotting analysis revealed that NF-kB/p65 protein levels in the hippocampus of SHAM rats submitted to CUMS were higher compared to non-CUMS control

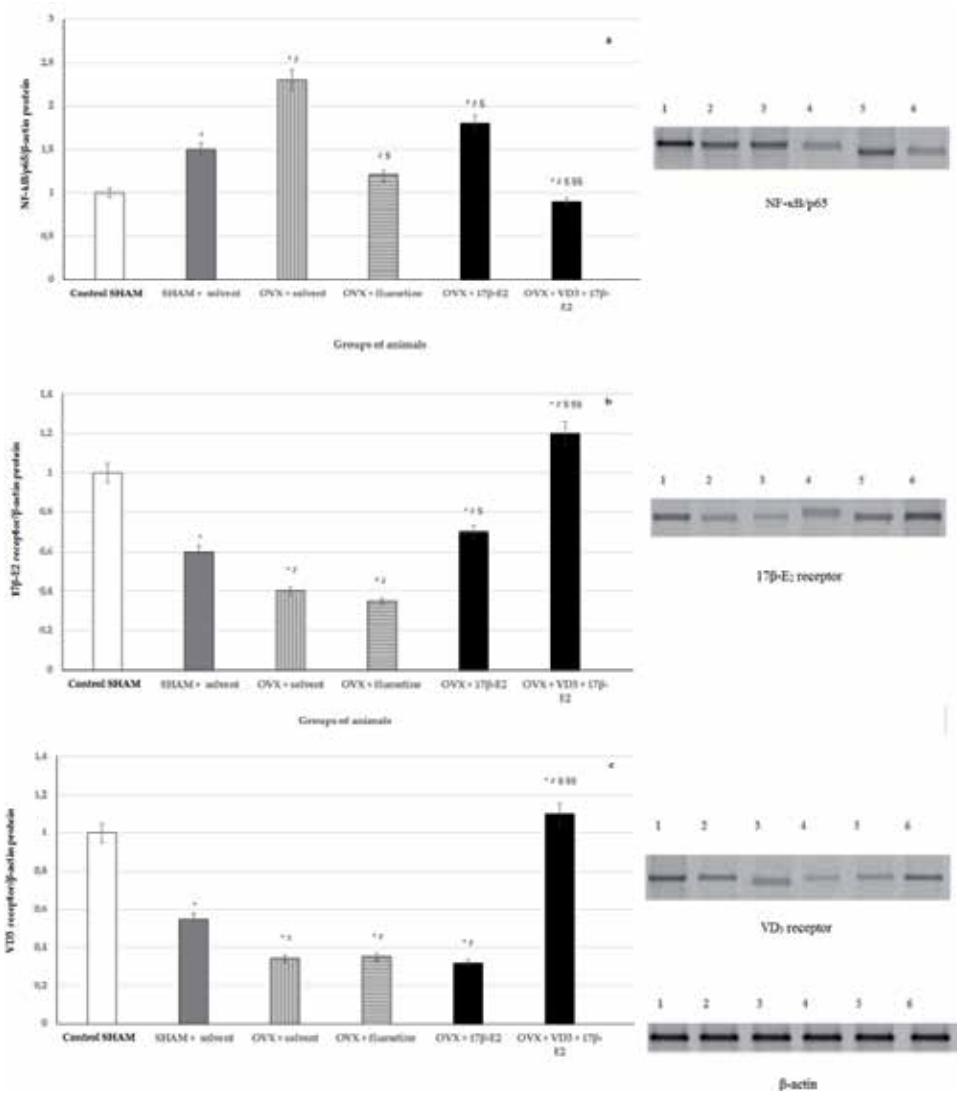

Figure 8.
*VD_3 modulates hippocampal NF-kB/p65 and 17β-E_2/VD_3 receptors expressions in long-term OVX rats with 17β-E_2 submitted to CUMS detected with western blotting treated. 1 – control SHAM, 2 – SHAM + CUMS + solvent, 3 – OVX + CUMS + solvent, 4 – OVX rats + CUMS + fluoxetine, 5 – OVX rats + CUMS + 17β-E_2, and 6 – OVX rats + CUMS + VD_3 + 17β-E_2. * – $P < 0.05$ versus the control group, # – $P < 0.05$ versus to the SHAM group with CUMS, $ – $P < 0.05$ versus to the OVX group with CUMS, and $$ – $P < 0.05$ versus to the OVX group with CUMS treated with 17β-E_2. The data are presented as mean ± SD; n = 7 in each group.*

females (**Figure 8**, $P < 0.05$). NF-kB/p65 levels were increased in the hippocampus of long-term OVX rats with CUMS compared to the non-CUMS/CUMS SHAM rats (**Figure 8**, $F(1,34) = 34.45$, $F(1,34) = 16.38$, respectively, $P < 0.05$). Fluoxetine (10.0 mg/kg) resulted in significant reduced levels of hippocampal NF-kB/p65 protein expression in long-term OVX with CUMS compared to the OVX plus solvent/SHAM rats with CUMS (**Figure 8**, $P < 0.05$). Co-treatment with VD_3 and 17β-E_2 decreased NF-kB/p65 protein levels and increased 17β-E_2/VD_3 receptors protein expression in the hippocampus of the long-term OVX rats compared to OVX plus solvent or 17β-E_2/SHAM rats with CUMS (**Figure 8**, $P < 0.05$). Fluoxetine did not alter 17β-E_2/VD_3 protein expression in the long-term OVX rats exposed to CUMS (**Figure 8**, $P > 0.05$).

4. Discussion

The present preclinical study analyzed the antidepressant-like effects of VD_3 (5.0 mg/kg, s.c.) in long-term adult OVX female rats given with low dose of 17β-E_2 subjected to the CUMS. A CUMS paradigm is a well-known experimental paradigm that has documented to consider as standard pathophysiological impairments in mood state linked to a clinical depressive disorders in humans [47–49]. In the present work, the implications of NF-kB/p65 signaling pathway, as well as the 17β-E_2/VD_3 receptors, in the mechanisms of VD_3 activity in depression were tested regarding to the affective-related condition of long-term adult OVX rats treated with low dose of 17β-E_2 exposed to CUMS.

The results of this study showed that in the adult long-term OVX rats undergoing CUMS, there were marked anhedonia-/depression-like behaviors, as assessed by SPT and LDT, respectively.

Moreover, long-term OVX rats exposed to CUMS exhibited decreased locomotor and rearing activities in the OFT. The ELISA assay clearly demonstrated lower estradiol and VD_3 concentrations in adult long-term OVX rats subjected to CUMS. In addition, the increased NF-kB/p65 concentration/protein expression and decreased 17β-E_2/VD_3 receptors levels were found in the hippocampus of long-term OVX rats exposed to CUMS.

Administration of 17β-E_2 failed to completely restore behavioral and biochemical parameters in the long-term OVX rats exposed to CUMS. Fluoxetine decreased anhedonia-like and depression-like states and decreased NF-kB/p65 levels in the hippocampus of the long-term OVX female rats exposed to CUMS. Data of literature have demonstrated that fluoxetine corrected depression-like profile of OVX rats in stress depression model [53]. The results of the study indicate that CUMS provokes marked behavioral, neurochemical, neurohormonal, and neuroinflammation alterations in adult OVX rats with long-lasting estrogens decline. Our data are in agreement with our recent data and other findings, which indicated that long-term estrogen deprivation in female rodents subjected to a CUMS procedure results in a profound affective-like profile [54].

The most important findings of the present study is linked to the antidepressant-like effects of VD_3 in the long-term adult OVX rats treated with low dose of 17β-E_2 under conditions of CUMS. VD_3 given with a dose of 5.0 mg/kg reversed anhedonia-like and depression-like states in the SPT/LDT paradigms in the long-term OVX rats treated with 17β-E_2 subjected to CUMS, which was similar to the effects of the fluoxetine treatment. Moreover, the VD_3 application reversed the behavioral impairments observed in the OFT in the long-term OVX rats supplemented with 17β-E_2 subjected to CUMS. Biochemical assays found that VD_3 increased the serum VD_3 and estradiol levels, as well decreased the hippocampal NF-kB/p65 content in the long-term OVX rats treated with 17β-E_2 exposed to CUMS. Additionally, VD_3

increased 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. Western blot analysis revealed that VD_3 reduced NF-kB/p65 and increased 17β-E_2/VD_3 protein expression in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. These data suggest that VD_3 attenuates the CUMS-produced behavioral impairments and normalized the serum VD_3 and estradiol levels, as well NF-kB/p65 and 17β-E_2/VD_3 production in the hippocampus of long-term OVX rats.

Inflammation is now recognized to be the one of the key components of affective-related development, with the NF-κB involved in both the early and late stages of the inflammatory processing [33, 41, 55]. NF-κB is the main transcriptional factor which controls the expression of various genes implicated in multiple cell functions and is triggered by different types of extracellular stimuli stimulated neuroinflammation [33, 34]. Deterioration of NF-κB signaling in the brain negatively influence on neuroplasticity and neuromorphology, as well as cognitive functions. However, NF-κB overstimulation is deleterious, and this detrimental effect can be reversed suppressing NF-κB signaling. That is why, NF-κB signaling is fundamental for normal brain function [36–39, 55]. The inhibition of neuroinflammation may be critical for the antidepressant action of VD_3 that was noted in our study. The increased pro-inflammatory cytokines have been found repeatedly in both animals and depressed patients [37–39]. Clinical studies indicate that inhibition of neuroinflammation by nonsteroidal antiinflammatory drugs can attenuate depression-like behaviors in depressed rodents and humans [36–39]. The inhibitory effect of VD_3 on CUMS-induced increase in pro-inflammatory cytokines in the hippocampus of the long-term OVX rats is strongly in accordance with the neuroinflammation hypothesis of depression [39]. A better comprehension of NFκB-dependent mechanisms in antidepressant action of VD_3 needs further studies.

Based on our findings, it can be assumed that VD_3 in the present study is implicated in the modulation of NF-κB signaling in long-term OVX rats with CUMS. On the other hand, VD_3 normalized 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats treated with 17β-E_2 subjected to CUMS. Such complex effects of VD_3 on neuroinflammation and 17β-E_2/VD_3 receptors might promote a greater effect of combination of VD_3 plus 17β-E_2 than application only 17β-E_2. This is the first study to show the action of VD_3 in the behavioral and neuroinflammation and biochemical consequences of a CUMS in adult long-term OVX rats treated with low dose of 17β-E_2. The inhibition of NF-kB/p65 activity by VD_3 treatment is a promising fact of study for treatment of neuroinflammatory diseases that are associated with low levels of VD_3. These results suggest an anti-inflammatory role for VD_3, which may be one of the fundamental components of its activity.

Thus, the possible mechanism of VD_3 action might be explained by the stimulation of 17β-E_2/VD_3 receptors identified in the different brain structures involved in mood control [13–15]. The possible mechanisms of such action of VD_3 in the long-term OVX rats can be connected with cross-talk protein-protein interactions. Moreover, VD alters the neuroinflammation response via NF-kB/p65 signaling at the affective-related state, thereby improving depression state [39, 55]. Low VD levels appear in the majority of postmenopausal women [52, 56, 57]. Therefore, VD supplementation may be very useful for treatment of mood disorders in postmenopausal women with a low level of VD and supplemented with MHT. However, the exact role of VD supplementation in the prevention and treatment of mood disorders associated with menopausal consequences has not been completely identified.

In conclusion, the present study supports evidence for repeated administration of VD_3 in a chronic unpredictable stress model having an anti-anhedonia-like and antidepressant-like effects in long-term OVX adult rats treated with low dose of 17β-E_2. Moreover, the biochemical and western blotting assays suggest the

implications of NF-kB/p65 and 17β-E_2/VD_3 production modulation in the antidepressant-like activity of VD_3. Further studies should however explore the precise mechanism of VD_3 action, due to the necessity of an improvement of therapies focusing on mood-repair in females with long-lasting estrogen deficiency.

5. Conclusions

This study demonstrated that VD_3-induced antianhedonic- and antidepressant-like effects in the adult female rats following long-lasting estrogens decline treated with low dose of 17β-E_2 submitted to CUMS. Treatment with VD_3 modulates NF-kB/p65 concentration/protein expression and 17β-E_2/VD_3 receptors levels in the hippocampus of long-term OVX rats exposed to CUMS treated with low dose of 17β-E_2. Our study yields new knowledge into the mechanisms by which VD_3 affects to alleviate anhedonia- and depressive-like behaviors in female rodents with long-term estrogen deficiency in stress model of depression.

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Conflicts of interest

The authors declare no conflict of interest.

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Section 3

Vitamin D Deficiency and Metabolic Diseases

Chapter 9

Vitamin D and Obesity

Sabrina Ait Gacem and Moyad Jamal Shahwan

Abstract

Obesity is a very common issue worldwide, and it is one of the risk factors for mortality. Several studies were done to identify the causes of this issue and to investigate factors that can affect this condition. Vitamin D is claimed to have an impact not only for maintaining bone health but also for having an association between its deficiency and obesity as some studies found that the concentrations of this vitamin are low in obese individuals. The suggested mechanisms and a discussion of the latest findings as well as the possibility of integrating supplementation in the treatment of obesity are covered in this book chapter. It was concluded that vitamin D deficiency is prevalent in many parts of the world and the supplements are an affordable option, but further studies are required to address different confounding factors that will result in clear data interpretation and will contribute to the future planning of health policies and guidelines used by healthcare professionals.

Keywords: vitamin D, deficiency, supplements, obesity, health, BMI

1. Introduction

Obesity is one of the most commonly observed health issues and can be defined as the condition in which there is an abnormal or excess fat accumulation in the body that can affect the health of an individual [1].

A measure of the obesity and other body weight status is the body mass index (BMI) which is a tool that uses the weight and the height of an individual. The weight is divided by the square of the height. If the BMI is 30 or more, the individual will be considered obese. BMI provides a measure of obesity and overweight that is general for female and male adults from different age groups, but for children the age has to be taken into consideration. Due to its inconsistent representation and correspondence for the level of fat in various individuals, it should be used as a rough guide [1].

According to the World Health Organization (WHO), obesity is known to be high especially in developed countries (high-income countries), but recently it was observed to increase even in middle- and low-income countries as well which is a serious issue because obesity is one of the main risk factors for several serious chronic diseases that are noncommunicable diseases (NCDs) like respiratory diseases, cardiovascular diseases, diabetes, and cancers causing an estimated 38 million deaths annually according to the WHO statistics [1].

According to the Global Health Observatory (GHO), data from the WHO statistics of the prevalence of obesity among adults aged 18+ (1975–2016) represented 27.8% in the United Kingdom (UK) and 31.7% in the United Arab Emirates (UAE) among adults [1]. According to the National Health Survey, adult obesity in the UAE stands at 27.8% in 2019 [2]. Obesity is caused by several reasons, and one of them is

the imbalance between calories expended and calories consumed due to changes in physical activity and dietary patterns [3].

The prevalence of obesity was found to be due to several factors especially change in lifestyle habits besides the genetic contribution and other factors, and this indicates that certain healthcare awareness activities and certain interventions can help and contribute to overcome this issue [4].

Vitamin D is a very important vitamin and plays a role in the maintenance of bone tissues, the balance of phosphorus and calcium, as well as other cell functions [5].

During sunlight exposure, the skin is penetrated by ultraviolet B photons by which pre-vitamin D3 is isomerized into vitamin D3 by the body heat [6].

Although many controversies still exist suggesting that vitamin D is not connected to any health condition and it is just a marker of general good health, there is also a good evidence of the relation between vitamin D level and malignancies as well as mental health, bone health, and other health issues [7].

Vitamin D has a vital role in maintaining good health, and its benefits or importance comes from its role in absorbing calcium and phosphorus from the gastrointestinal track, which is why it can be used to treat and prevent bone and muscle aches as well as chronic fatigue, teeth and dental problems, and osteoporosis [8].

Vitamin D deficiency contributes to many chronic metabolic and endocrine diseases [9, 10]. Vitamin D deficiency causes rickets and growth retardation and an enhanced risk of adult fractures [11, 12]. Obesity was found to be significantly associated with low calcium serum levels especially abdominal obesity [13, 14].

It is also associated with other conditions such as enhanced risk of common cancers, infectious conditions, cardiovascular diseases, and other diseases [15, 16].

In a recent study of 2018, it was observed that vitamin D supplements only increase bone density in adults with 25-hydroxyvitamin D $\leq$ 30 nmol/L, and the study suggested the implementation or use of supplements [17].

Vitamin D deficiency is connected as well to high myocardial diseases and cardiovascular risk; however, the way it works is yet to be investigated in further details [18]. A recent study held in 2018 showed a significant association between low plasma vitamin D levels and prevalence of hypertension. There was a statistically significant correlation between vitamin D deficiency and acute coronary syndrome [19].

On the other hand, a study found that the supplementation of calcium in addition to vitamin D did not diminish the danger of chronic heart disease or stroke and did not have an effect on the incident of hypertension [20, 21].

Several patients with low vitamin D level remain undetected, with bone chemistry values within the reference ranges, unless clinical suspicion is raised. Clinical suspicion based on history and awareness of risk factors should remain the gold standard for requesting vitamin D measurements [22].

2. Suggested mechanisms of action

The body obtains energy from the ingested food, and part of this energy is utilized to maintain the regular body activities while the other part can be stored in the adipose tissue that is able to sense the body energy state. When the body has excess energy compared to the demand, the adipose tissue starts to store the energy as triglyceride in the lipid droplets [23, 24].

The process of lipolysis in which the triglycerides get broken into free acids is activated by catecholamines by the cAMP signaling pathway which activates the protein kinase A. The hormone-sensitive lipase enzyme (HSL) that promotes

the translocation of lipid droplet which will lead to access to triglyceride stores is phosphorylated by protein kinase A [24, 25].

The activity of the proteins can be changed after being formed through a mechanism called phosphorylation. A phosphate group which is provided by ATP is added to a protein by enzymes referred to as kinases [24].

When the body requires energy, lipolysis occurs. The adipose triglyceride lipase (ATGL) enzyme starts hydrolyzing the triglycerides into diacylglyceride. The diacylglyceride is then broken down into monoglyceride by the enzyme hormone-sensitive lipase (HSL), and then the monoglyceride ester bond is cleaved to release glycerol [24, 26].

The lipid droplets in the fat-storing cells in adipose tissue are coated by a protein called perilipin which acts as a protective coat from the body's natural lipases like hormone-sensitive lipase (HSL) that breaks triglycerides into glycerol and free fatty acids for use in metabolism during lipolysis. Phosphorylation of perilipin is important for the mobilization of fats in adipose tissue which is important for the regulation of lipid storage [27].

Insulin suppresses the enzyme hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) as well because insulin enhances the amount of perilipin around the lipid droplets to prevent their access to triglycerides [24].

Vitamin D deficiency is known to occur due to several reasons and through several mechanisms, and one of the stated reasons is the low sun exposure due to decreased mobility due to obesity [14, 24].

Vitamin D is obtained from different sources taken up by the adipose tissue that was suggested to store this vitamin for conditions when the production is reduced. The levels of adipose tissue are inversely correlated with this vitamin level [28].

Several hypotheses were proposed to correlate obesity with vitamin D deficiency, and few of which are due to low physical activities and other reasons which led to limited sun exposure. There was evidence that vitamin D storage, action, and metabolism influences adiposity, and an observational study had shown that there is an increased risk of deficiency among obese individuals, but detailed explained causes are not clear [29].

The hormonal form of vitamin D is (1,25 dihydroxyvitamin D), and besides its known action in the regulation of calcium level, the vitamin D hormone has many other activities such as the regulation of adipocytes [24].

Another hypothesis suggested that the level of vitamin D stimulation enzyme 1-α-hydroxylase in the adipose cells may explain the greater local use of 25(OH)D.

Medical practitioners measure the level of this metabolite to identify a patient's vitamin D status [30, 31]. 25-Hydroxyvitamin D (25(OH)D) is also known as calcifediol, which is a prehormone produced in the liver by hydroxylation of vitamin D3 (cholecalciferol) by the enzyme cholecalciferol 25-hydroxylase. Vitamin D3 gets converted into calcifediol, and this process takes approximately 7 days; then calcifediol is converted in the kidneys (by the enzyme 25(OH)D-1α-hydroxylase) into calcitriol (1,25-(OH)2D3), a secosteroid hormone that is the active form of vitamin D [32].

Variations in serum 25(OH)D and vitamin D reserves can be directly linked to the amount of subcutaneous body fat according to this hypothesis [10, 33]. However, in a cohort study, it was reported that this theory was not enough to address the relationship between vitamin D deficiency and obesity [10, 33].

The active form of vitamin D (1,25-dihydroxyvitamin D) can affect the free fatty acid mobilization from the adipose tissue [34]. Animal studies found that high doses of vitamin D can lead to elevation in energy expenditure because of uncoupling of oxidative phosphorylation in adipose tissues [35].

It has also been suggested that the weather may contribute as obesity can result from an adaptive winter action and vitamin D obtained from the sun is limited during winter which can play a role in the tendency to elevate fat mass during cold weather [36].

Another theory for the correlation between obesity and vitamin D could be that vitamin D is stored in the adipose tissue, and, hence, perhaps the most likely explanation is that the bigger the storage capacity for this vitamin in obese people, the less the circulating [25(OH)D] concentrations in the blood [37].

Another suggested association was between genetic variants that imitate the impact of modifiable environmental exposure and the outcome of interest [38].

A genetic variant linked with lower 25(OH)D concentrations should be linked with BMI if lower vitamin intake is causally linked to obesity. The genetic associations are considered less subjected to confounding factors and socioeconomic and lifestyle factors as genotypes are invariant [39].

Different researches on vitamin D had been made based on the fact that several human cell types carry vitamin D receptor (VDR) which can contribute to several cell functions and regulation [40].

The role of vitamin D receptor (VDR) in the regulation of body energy in vivo was explained in previous studies, and it was observed that when these receptors were inhibited in some animal studies, several body tissues were affected, which made it difficult to interpret the data and make a clear correlation [24, 41]. Some studies suggested that 1.25(OH)D inhibits adipogenesis through actions modulated by vitamin D-dependent receptors, so the decrease in vitamin D can lead to excessive differentiation of pre-adipocytes to adipocytes [10, 42].

It was observed in some studies that obese participants who were subjected to a dose of ultraviolet (UV-B) radiation showed a small response compared to normal-weight participants [37, 40].

The detailed mechanism explaining how this vitamin might be kept in fat was not clearly mentioned, but although the previous mechanism is not detailed, it suggested that vitamin D is relatively tightly bound in tissue depots and not appropriately released to maintain the serum vitamin amount in the blood [40].

Several different levels of sun exposure are not a likely explanation for the link between adiposity and vitamin D deficiency as observed in some studies. Alternatively, it was suggested that extra fat holds the vitamin D metabolites and that the cholecalciferol is partially sequestered by the fat before being transported to the liver for the first hydroxylation [10, 37].

A study concluded that vitamin D serum levels were observed to be (53%) lower among obese participants. Some studies suggested that vitamin D deficiency can favor higher adiposity by promoting elevated parathyroid hormone levels and more calcium inflow into adipocytes which will increase lipogenesis through which acetyl-CoA is converted to triglyceride for storage in fat and packaged within lipid droplets [10, 43].

Identifying and understanding the mechanism beyond low vitamin D status in obesity has a great importance in deciding on appropriate vitamin D replacement doses for obese individuals [40].

However, further studies are required to clarify further the mechanism and the confounding factors that may interfere with the data interpretation such as physical activity, educational level, diet intake, secondary hyperparathyroidism, and other factors.

3. Vitamin D and obesity findings

Several studies were done to identify the causes of obesity and to investigate factors that can affect this condition. Vitamin D is claimed to have an impact not

only for maintaining bone health but also for having an association between its deficiency and obesity as some studies found that the concentrations of this vitamin are low in obese individuals.

Although many controversies still exist suggesting that vitamin D is not connected to any health condition and it is just a marker of general good health, there is also a good evidence of the relation between vitamin D level and several health conditions [7].

It was observed in many studies including a meta-analysis study that vitamin D concentrations were linked to the decrease in the risk of occurrence of metabolic syndrome, diabetes, and cardiovascular diseases [10, 26].

Several studies have shown a positive correlation such as some observational studies that showed a correlation between vitamin D deficiency and obesity with no clear evidence for the detailed causes [9, 10, 44, 45].

Results of a meta-analysis study showed that the deficiency of this vitamin was linked with obesity regardless of the age of participants, and it was concluded that there was no significant correlation with age and many studies were held to assess the increasing risk of developing metabolic syndrome and other disorders like hypertension, excess weight, and cancer with vitamin D deficiency [9, 10, 46].

A recent meta-analysis study revealed as well no significant correlation with latitude or the development status of the country, so it can be concluded that it was found that the correlation between vitamin D deficiency and obesity is not affected by latitude, country status, or age, but a positive association between body mass index (BMI) and vitamin D deficiency was observed and due to the study designs included, it was difficult to clarify the underlying causes [7, 47].

It was concluded from some studies that investigated the relation between vitamin D status and body mass index that on the basis of a bidirectional genetic approach that reduces confounding, a bigger BMI leads to lower 25(OH)D and decreasing BMI is expected to reduce the prevalence of vitamin D deficiency. When obese and nonobese participants were given 50,000 IU of vitamin D orally or exposed to simulated sunlight, the results showed that the obese participants were able to increase the vitamin blood levels by no more than 50% compared with other participants, and this was explained that it is because the body fat sequesters the fat-soluble vitamin which makes obese individuals at higher risk [48, 49].

Similar results were observed in another study that the observed serum vitamin D3 levels in obese individuals were less than that in normal-weight individuals and blood vitamin D2 concentrations after the intake of 50,000 IU of vitamin D2 are inversely correlated with BMI [37].

Similarly, another study concluded that obesity is linked with a lower bioavailability cutaneous synthesized vitamin D and dietary intake which was explained similarly to be due to the sequestration of vitamin D into the adipose tissue [50].

Similarly, other researches have shown that obese individuals tend to have lower blood concentrations of vitamin D3 and 25(OH)D3 than those with normal weights [51–53]. Several studies including epidemiological studies showed a high prevalence increased BMI and low vitamin D status [33, 54–59].

On the other hand, other researches revealed a weak correlation between vitamin D concentrations, and BMI a negative correlation between anthropometric variables and vitamin D level from different races and age groups [10, 47].

4. Integration of vitamin D in obesity treatment

Obesity is caused by many factors, but despite the genetic contribution, it was observed to occur mainly due to lifestyle habits, which indicates that it can be

modified through some interventions or health awareness campaigns. The WHO has identified physical inactivity and unhealthy diet as one of the risk factors for noncommunicable diseases (NCDs), and it urges all efforts to contribute in reducing them to prevent deaths from NCDs [45].

Identifying and understanding the mechanism beyond low vitamin D status in obesity has a great importance in deciding the needed and required vitamin D replacement doses for obese individuals [40].

A study discussed the possibility of integrating vitamin D supplementation with current strategies, and it was suggested that the induction of adipocyte death through apoptosis is a very promising strategy to manage obesity [60–62].

When the adipocytes reach a maximum size, elevation in adipose tissue mass includes as well an elevation in adipocyte number. So, weight loss can result from not only a decrease in adipocyte size but also adipocyte number and can result in the loss of adipose tissue mass. The removal of adipocytes by a process called apoptosis decreases body fat and can contribute to the long-lasting control of weight loss [60–62]. The effects of the hormonal form of vitamin D, 1,25(OH)2D3, on apoptotic cell death are mediated through several signaling pathways on cellular calcium Ca^{2+} [60–62].

High calcium and vitamin D3 intake is linked to the stimulation of the calcium-dependent apoptotic proteases in adipose tissue. The 1,25(OH)2D3-induced cellular calcium signal acts as an apoptotic initiator that directly recruits calcium-dependent apoptotic effectors that are able of causing apoptosis in adipose tissue. It was observed that 1,25(OH)2D3 induces a prolonged elevation in intracellular calcium concentration (the apoptotic Ca^{2+} signal) and is also associated with low lipid accumulation in mature adipocytes [63].

Some studies revealed that vitamin D deficiency was closely associated with enhanced risk of major adverse cardiovascular diseases (CVD) [64, 65]. Some trials revealed a tendency toward a decrease in CVD risk with vitamin D supplementation as well, but the correlation was not significant [66]. Observational studies have indicated that high 25-hydroxyvitamin D [25(OH)D] levels were associated with a favorable serum lipid profile [67]. However, a solid rationale for such association is hard to identify unless there is an effect of vitamin D supplementation on serum lipids in placebo-controlled randomized trials. Unfortunately, the intervention studies gave different results ranging from positive to negative effect [67].

However, some randomized controlled trials (RCTs) studying the effect of supplementation on weight loss in overweight or obese people showed inconsistent results [68].

5. Conclusions

As long as vitamin D deficiency is prevalent in many parts of the world and the supplements are an affordable option, the deficiency of vitamin D may be a common and easily treatable risk factor for several health issues including obesity, but further studies are required to address different confounding factors and variabilities especially prospective studies to study the causal relationship between the deficiency of this vitamin and obesity as well as focus on the safety as well as the required dose regimen. Strong well-structured studies with limited confounding factors that will result in clear data interpretation will contribute to the future planning of health policies and guidelines used by healthcare professionals.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 10

Vitamin D Deficiency and Diabetes Mellitus

Ihor Shymanskyi, Olha Lisakovska, Anna Mazanova and Mykola Veliky

Abstract

Vitamin D (VD) is a molecule that can be synthesized directly in the humans' body or enter the organism with food in the form of inactive precursors. To exert its biological action, VD undergoes two-stage hydroxylation (at the 25th and 1st position) catalyzed by cytochromes P450, the presence of which has already been shown in almost all tissues of the human body. The product of hydroxylation is hormone-active form of vitamin D–1,25$(OH)_2$D. 1,25$(OH)_2$D binds to specific vitamin D receptor (VDR) and regulates the expression of genes involved in bone remodeling (classical function) and genes that control immune response, hormone secretion, cell proliferation, and differentiation (nonclassical functions). VD deficiency is prevalent around the globe and may be one of the key factors for diabetes development. The direct association between vitamin D deficiency and type 1 (T1D) and type 2 (T2D) diabetes has been proven. Detection of VDR in pancreas and adipose tissue, skeletal muscles, and immune cells allowed implying the antidiabetic role of vitamin D by enhancing insulin synthesis and exocytosis, increasing the expression of the insulin receptor, and modulating immune cells' functions. This chapter summarizes data about relationship between VD insufficiency/deficiency and development of T1D and T2D, and their complications.

Keywords: cholecalciferol, vitamin D deficiency, vitamin D receptor, CYP27B1 (1α-hydroxylase), type 1 diabetes mellitus, type 2 diabetes mellitus, immune response

1. Introduction

Vitamin D (VD) is a unique bioregulatory molecule as it can be synthesized in the skin in addition to its dietary sources. VD in its metabolically active form, 1,25$(OH)_2$D (calcitriol), is a secosteroid hormone produced after hepatic (at carbon atom 25) and, not exclusively, kidney (at carbon atom 1) hydroxylations. The well-studied function of VD is associated with its ability to regulate metabolic processes in skeletal tissue by affecting mineralization, maintaining a balance between the formation and resorption of bone tissue, and thereby contributing to the prevention of osteoporosis and the occurrence of fractures. In addition to being involved in calcium-phosphate metabolism, the variety of physiological effects of VD also extends to extra-skeletal tissues, since the vast majority of their species possesses vitamin D receptor (VDR). Furthermore, most of these tissues also express the cytochrome P450 enzyme, CYP27B1, responsible for converting 25-hydroxyvitamin D (25OHD), the main circulating metabolite of VD, to hormonally active

form–1,25$(OH)_2$D. 1,25$(OH)_2$D through VDR controls the expression of both those genes that participate in mineral homeostasis and bone remodeling, and genes (about 500) that participate in various cellular pathways that affect physiological and cellular mechanisms, such as immunomodulation, hormone secretion, inhibition of cell proliferation, and induction of cell differentiation.

Recent epidemiological studies have indicated the association between VD deficiency and both type 1 (T1D) and type 2 (T2D) diabetes mellitus. Moreover, impaired glucose tolerance and diabetes have been shown to ameliorate in VD-deficient individuals after VD supplementation. Vitamin D deficiency, which may be a key factor for diabetes development, is prevalent around the globe, with an estimated one billion people being vitamin D deficient. The role of VD in diabetes became clearer after the discovery of VDR in the pancreas, adipose tissue, skeletal muscle cells, and immune cells, which indicates a regulatory effect of VD on glucose homeostasis. Vitamin D can directly enhance insulin synthesis and its release from pancreatic β-cells as well as increase the expression of the insulin receptor in peripheral tissues. It can also indirectly exert an antidiabetic effect by acting on cells of the immune system that secrete pro-inflammatory cytokines as mediators affecting weight gain, systemic inflammation (contributes to insulin resistance), and autoimmune-mediated destruction of pancreatic β-cells. These findings suggested that VD deficiency probably has a causal relationship with diabetes mellitus. Some studies have also reported that VD deficiency was not the cause, but the result of diabetes. Regardless of whether this deficiency is one of the causes of diabetes or its consequence, it is obvious that low levels of VD are closely associated with poor regulation of diabetes and its complications; however, the extent of this relationship and its clinical relevance are not well established.

The aim of the present chapter is to summarize the latest evidence linking VD insufficiency/deficiency with the development of T1D and T2D and their complications. We also analyzed different intervention studies with VD supplements to determine their influence on glucose metabolism and delineated the underlying mechanisms. Previous reviews on the role of VD in diabetes mellitus have been published in recent years. Here, priority was given to the most recent and convincing available evidence.

2. Role of vitamin D in immune regulation and inflammatory responses

The first data concerning the potential role for VD and its active metabolite 1,25$(OH)_2$D in modulating the immune response were obtained as a result of the treatment of tuberculosis and leprosy caused by mycobacteria [1]. However, the mechanisms underlying these observations have been clarified more recently with several important discoveries: (1) the upregulation of CYP27B1 and VDR expression in activated human inflammatory cells, thus providing their ability both to produce 1,25$(OH)_2$D in the site of inflammation and to respond to this hormonally active metabolite; and (2) the participation of 1,25$(OH)_2$D in modulating the multiple pathways of the innate and adaptive immune system. The influence of 1,25$(OH)_2$D on the different cell types of these immune system segments is outlined in **Figure 1**.

Innate immune response involves the activation of Toll-like receptors in monocytes/macrophages as well as in a number of cells such as placenta trophoblasts, keratinocytes, and epithelial, intestinal, lung, and corneal cells, representing first-barrier defenses. VD affects innate immunity through its stimulatory action on the synthesis of defensin β2 and cathelicidin antimicrobial peptide (CAMP) upon Toll-like receptors' activation. These low molecular weight host defense

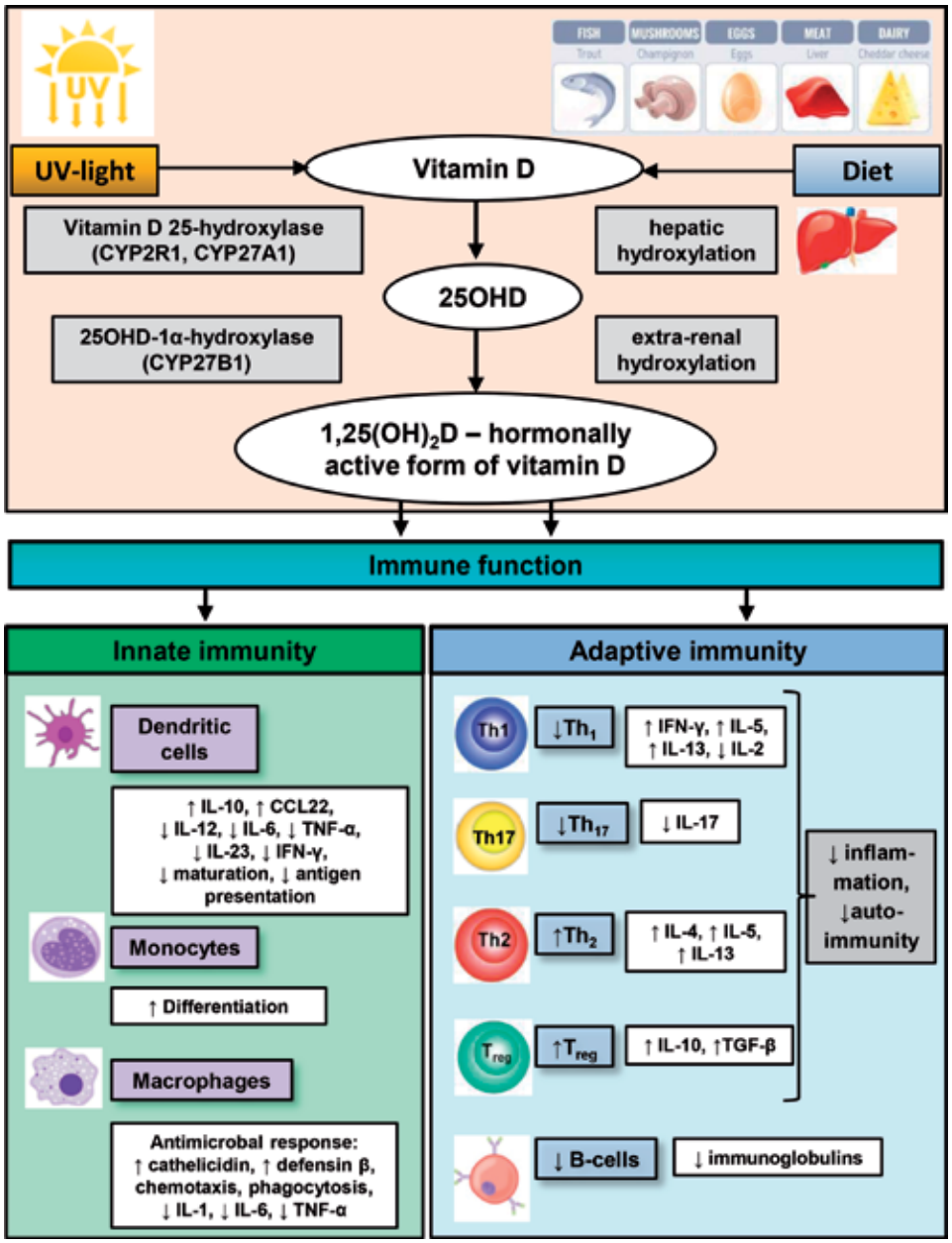

Figure 1.
Vitamin D in immune modulation. 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25OHD, 25-hydroxyvitamin D; IFN-γ, interferon-γ; ILs, interleukins; TGF-β, transforming growth factor β; Th1, type 1 T helper; Th2, type 2 T helper; Th17, type 17 T helper; TNF-α, tumor necrosis factor-α; Treg, regulatory T cells.

antimicrobial peptides demonstrate a broad spectrum of activity against bacteria, viruses, and fungi in the immune cells and are also synthesized in a variety of other cell types [1]. CAMP is known to be a direct transcriptional target of VD, which is induced by binding of $1,25(OH)_2$D-VDR/retinoid X receptor (RXR) complex to the VD response elements (VDRE) in the gene promoter [2]. VD can also modulate innate immune system by increasing chemotaxis, autophagy, and phagolysosomal fusion of phagocytic cells. Notably, VD's action on macrophages was established to be modulated by interleukins. In particular, VD increases the antimicrobial activity of macrophages formed after the IL-15 stimulus, while phagocytic macrophages do

not respond to vitamin D after the IL-10 stimulus, regardless of their high phagocytic activity [1, 3].

Vitamin D shows an inhibitory action on the adaptive immune system, the responses of which include the ability of T and B lymphocytes to produce cytokines and immunoglobulins, respectively, to specifically combat antigens presented to them by macrophages and dendritic cells (DCs). Experimental studies have yielded encouraging results on the immunomodulatory effect of calcitriol on T helper (Th) cells. In particular, $1,25(OH)_2D$ was shown to suppress the immune responses mediated by Th1 cells capable of producing such pro-inflammatory cytokines as IL-2, IL-6, interferon γ (IFN-γ), and tumor necrosis factor-α (TNF-α) [4]. The lack of IFN-γ prevents further antigen presentation to T lymphocytes and their recruitment, while lower IL-2 production impedes T lymphocyte proliferation and differentiation. It has been recently demonstrated that calcitriol also increases formation and activity of CD4+/CD25+ regulatory T cells (Treg) as seen by elevated FoxP3 and IL-10 expression [5]. Increased levels of IL-10 as well as other cytokines with anti-inflammatory properties, induced by calcitriol, block Th1 differentiation, thus shifting the balance from Th1 to Th2 cell phenotype [6]. Many of the effects of VD on Th1 cells, which were previously considered to be implicated in the pathogenesis of several autoimmune diseases, can now be attributable, at least in part, to the inhibitory action of $1,25(OH)_2D$ on the formation and activity of Th17 cells, producing IL-17 [5]. The overall impact of VD on Th cells is related to the suppression of antigen-presenting cells (APCs) of the innate immune system, including the most potent dendritic cells. This modulatory effect of $1,25(OH)_2D$ induces a "tolerogenic state" associated with the differentiation of Treg cells, autoreactive T cell apoptosis, reduced production of inflammatory cytokines, and increased levels of the anti-inflammatory cytokines.

Chromatin immunoprecipitation assay revealed VDR binding to a VDRE in the proximal area of IL-10 promoter in antibody-producing cells of the immune system, or B-cells [7]. $1,25(OH)_2D$ blocked the proliferation of activated B-cells and stimulated their apoptosis. It also inhibited maturation of activated B-cells into plasma cells and memory cells that is consistent with the inhibitory action of VD on the secretion of IgM and IgG [8]. Several observational trials showed an inverse relationship between serum IgE and 25OHD levels, while others indicated a positive correlation [9].

Due to the ability of VD to suppress the adaptive immune system, the role of VD deficiency and supplementation in inflammatory and autoimmune diseases acquires more comprehensive support. In a number of animal models, including autoimmune diabetes, inflammatory arthritis, experimental allergic encephalitis, and different mouse models of enterocolitis, calcitriol prevented the initiation and reduced the disease progression. However, despite strong experimental evidence, human studies are less convincing to prove a role for VD in the modulation of adaptive immune system of individuals affected by autoimmune diseases. In this respect, some trials have confirmed beneficial effect of VD on different inflammatory disease progression, inflammatory markers, and T cell subsets, whereas others have not shown any promising result [10, 11].

3. Vitamin D in maintaining pancreatic β-cell function and regulating insulin sensitivity

As demonstrated in the previous section, VD is one of the key players in the control of immune homeostasis, and here, we will examine in more detail the molecular mechanisms, showing how inadequate VD status and inflammation can

contribute to pancreatic β-cell dysfunction and the formation of insulin resistance (IR). Comprehensive results of experimental and clinical studies have shown that vitamin D is a potential regulator of pancreatic β-cell survival, Ca^{2+} levels, insulin secretion, and insulin signaling (**Figure 2**).

Vitamin D plays an immunomodulatory role in preventing pancreatic β-cell dysfunction and death, via VDR, which is expressed along with Cyp27B1 in APCs, activated T cells, and islet pancreatic β-cells [12]. These effects have been demonstrated in many studies of nonobese diabetic mice using $1,25(OH)_2D$ or analogs. Conversely, $1,25(OH)_2D$-deficient mice showed a tendency to develop more aggressive form of T1D, if the deficiency is present at an early age. The bioactive form of VD protects against the development of insulitis in the pancreas or reduces their

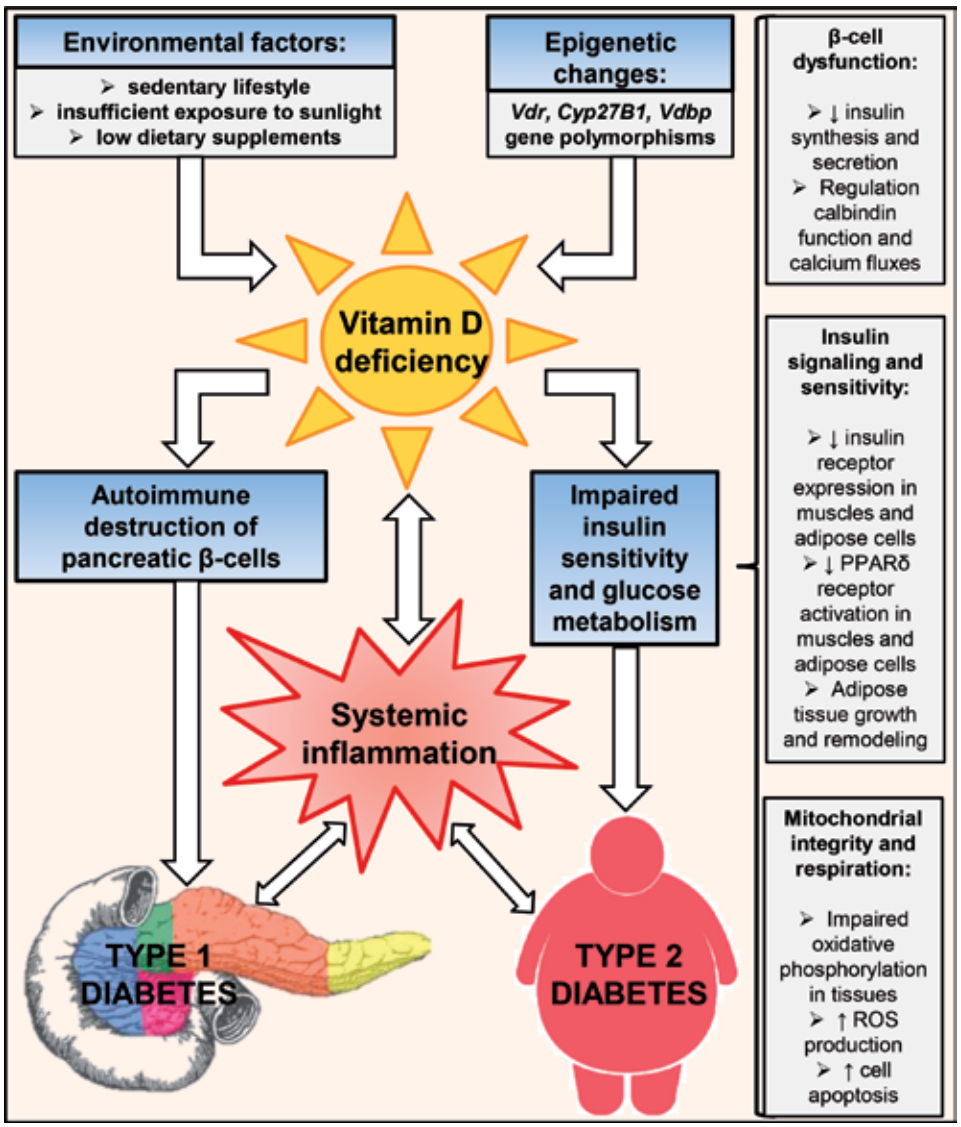

Figure 2.
Mechanisms of glucose homeostasis deregulation related to vitamin D deficiency status. Cyp27B1, 25-hydroxyvitamin D 1-alpha-hydroxylase gene; Vdr, vitamin D receptor gene; Vdbp, vitamin D-binding protein gene.

severity through a dual mechanism of action on both pancreatic β-cells and immune cells [13].

In pancreatic islets, $1,25(OH)_2D$ decreases, as was shown in *in vitro* and *in vivo* experiments, the expression of pro-inflammatory cytokines (e.g., IL-6), which are involved in the pathogenesis of T1D, making β-cells less chemoattractive and less prone to inflammation [14]. This leads to a decrease in T-cell recruitment and infiltration, an increase in regulatory cells, and a delay in the autoimmune process. In addition, $1,25(OH)_2D$ reduces the expression of MHC class I, leading to a decrease in the vulnerability of islet β-cells to the action of cytotoxic T lymphocytes [13, 14].

At the level of the immune system, $1,25(OH)_2D$ inhibits the differentiation and maturation of DCs and promotes their apoptosis, preventing them from becoming APCs, which is the first step in initiating an immune response. It has also been shown that $1,25(OH)_2D$ restores suppressor cells, reduces cytokine formation by Th1 cells responsible for β-cell death, and shifts the immune response toward Th2 cell activation, leading to more benign inflammatory response in pancreatic islets. $1,25(OH)_2D$ suppresses the formation of IL-6, a direct stimulator of Th17 cells involved in the pathogenesis of various autoimmune diseases, including T1D [15]. On the other hand, $1,25(OH)_2D$ exerts an antiapoptotic effect on cytokine-induced apoptosis of pancreatic β-cells. It induces and maintains high protein levels of the A20 (anti-inflammatory protein; inhibits NF-κB signaling), leading to a decrease in nitric monoxide (NO) levels. In fact, NO is able to directly induce β-cell dysfunction and death, or indirectly may affect β-cell function through the induction of Fas expression. Fas is a transmembrane cell surface receptor and a member of the TNF receptor superfamily. Activation of these receptors occurs under the influence of inflammatory cytokines secreted by mononuclear cells that infiltrate islet cells. Reduction of the NO level leads to inhibition of all the above mechanisms and allows realizing cytoprotective effect on islet β-cells. The ability of $1,25(OH)_2D$ to counteract the cytokine-induced expression of Fas in human pancreatic islets at both mRNA and protein levels, modulating the cell death signal cascades and preventing β-cell apoptosis, was established [16].

Several trials have reported that VD deficiency caused impairment of glucose-mediated secretion of insulin in rat pancreatic β-cells, which was restored after VD supplementation. However, the results of clinical studies are not unambiguous as VD adequacy was not always associated with the improvement of insulin secretion. This stimulatory effect of VD is important for the prevention of T2D and may have different explanations. The bioactive form of VD is able to induce insulin secretion through direct binding of VDR-RXR complex to VDRE previously identified in the promoter of insulin gene in pancreatic β-cells [17]. In accordance with this finding, mice with a lack of functional VDR showed impaired insulin secretion after stimulation with glucose [18]. It is noteworthy that VDRE can stimulate not only the transcription of the insulin gene but also many other genes involved in the organization of the cytoskeleton, cell growth, differentiation, and survival of pancreatic β-cells.

In addition to genomic effects, rapid nongenomic mechanism of VD action appears to be involved in depolarization-stimulated insulin exocytosis by regulating intracellular Ca^{2+}. This effect of calcitriol is realized through a membrane VDR-mediated increase in the synthesis of inositol trisphosphate and phospholipase C that promotes the release of Ca^{2+} from endoplasmic reticulum and diacylglycerol-mediated PKC activation. In turn, activated PKC phosphorylates the ATP-dependent K^+ channels and L-type voltage-dependent Ca^{2+} channels. Ultimately, these effects lead to depolarization of the cytoplasmic membrane and the opening of Ca^{2+} L-type and T-type channels that increase intracellular Ca^{2+} level and, accordingly, insulin secretion [19]. Activation of PKA signaling pathways by calcitriol, apparently, is also involved in the regulation of L-type voltage-dependent Ca^{2+} channels.

It has been suggested that increased intracellular Ca^{2+} induced by VD may enhance the expression of cAMP-responsive element-binding protein (CREB), responsible for maintaining efficient transcription of insulin gene and insulin exocytosis, as well as for the glucose sensing and pancreatic β-cell survival [20]. Increased expression of proteins involved in providing low resting Ca^{2+} level, such as calcium-binding proteins (parvalbumin, calbindin-D_{28k}, and calbindin-D_{9k}), plasma membrane Ca^{2+}-ATPase, and Na^{+}/Ca^{2+}-exchanger, can be another mechanism by which vitamin D affects insulin secretion [21]. Furthermore, preclinical studies have shown that VD improves β-cell function by reducing the excess activity of the renin-angiotensin-aldosterone system [22].

Optimal intracellular levels of Ca^{2+} are essential not only for the proper function of pancreatic β-cells but also for insulin-responsive tissues, including liver, adipose tissue, and skeletal muscles. Impaired regulation of extracellular and intracellular Ca^{2+} concentrations due to abnormal transduction of insulin signaling in target tissues may evoke dephosphorylation and decreased activity of glucose transporter-4 (GLUT-4), leading to a phenomenon known as peripheral insulin resistance. The results of several studies confirmed that VD deficiency is involved in the onset of IR. Moreover, an adequate VD level was shown to improve insulin resistance associated with T2D [23]. In addition to the effect of calcitriol on insulin sensitivity related to regulation of extracellular Ca^{2+} concentration and its influx into cells through cell membranes, the active metabolite of VD seems to be an inducer of insulin receptor expression, which in turn improves insulin sensitivity [23]. Another mechanism underlying the beneficial effects of calcitriol on insulin sensitivity is related to activation of the peroxisome proliferator-activated receptor delta (PPARδ) [22]. Activated PPARδ, as a transcription factor, reduces fatty acids-evoked IR in adipose tissue and skeletal muscles. A secondary elevation of parathyroid hormone (PTH) in response to VD deficiency can also increase the concentration of intracellular Ca^{2+} in insulin-sensitive tissues and exacerbate IR by decreasing the number of GLUT1 and GLUT4 in the cell membranes of adipose tissue, liver, and muscle, thereby reducing glucose uptake [24].

An important endocrine and metabolic organ, playing a crucial role in glucose homeostasis and energy balance, is adipose tissue. This tissue is also the major site of VD storage in an organism that can sequestrate a fat-soluble prehormone and significantly decrease its level in blood circulation. It was found that VD exerts an important effect on the expression of genes implicated in promoting adipogenesis and adipose tissue remodeling. VDR is expressed in adipocytes in early stages of adipogenesis and mediates inhibitory effect of calcitriol on adipocyte differentiation through Wnt/β-catenin and mitogen-activated protein kinase (MAPK) signaling pathways [25]. Suppressive action of $1,25(OH)_2D$ on transcription factors, such as PPARγ and CAAT/enhancer-binding protein α (C/EBPα), alters the expression of numerous genes involved in lipolysis, lipogenesis, secretion of adipokines, insulin sensitivity (via GLUT4 expression), and transfer of fatty acids across the membrane [26].

The association between obesity, IR, and VD deficiency is a subject of intense research. A characteristic feature of hypertrophic enlargement of adipose tissue is elevated release of pro-inflammatory cytokines (TNF-α, IL-6, IL-8, MCP1, and resistin) by adipose-resident macrophages and activated T lymphocytes, whereas secretion of adiponectin, an anti-inflammatory and insulin-sensitizing bioregulatory molecule, by adipocytes is reduced [27]. Thus, one of the harmful consequences of obesity is impaired secretion of adipokines and systemic inflammation, which coexists with IR and favors the development of T2D as a key contributing factor. VD is known to protect against IR associated with inflammation by modulating the function of immune cells and secretion of adipokines (adiponectin and

leptin). It has been reported in numerous trials using animal models and in several human observational studies that higher VD levels are accompanied by lower inflammatory markers including TNF-α, IL-6, and C-reactive protein in healthy persons, and in those with inflammation-associated diseases, such as arteriosclerosis, inflammatory polyarthritis, and diabetes [28]. As for adipokines, positive correlation was shown between VD and adiponectin, and inverse correlation between VD and leptin [29]. Finally, VD by targeting mitochondrial respiratory functions through multiple mechanisms also attenuates oxidative stress and exerts key beneficial effects on controlling inflammation, impaired energy metabolism, and cell apoptosis. However, this topic is beyond the scope of this chapter. Vitamin D functions associated with the regulation of β-cell function and insulin sensitivity are summarized in **Figure 2**.

4. Vitamin D deficiency and type 1 diabetes mellitus

Type 1 diabetes mellitus is an autoimmune disorder caused by the progressive T-cell-mediated destruction of insulin-producing β-cells in the pancreas. T1D is commonly diagnosed in childhood and young adults, who are ultimately at risk of the long-term complications of diabetes [30]. This autoimmune condition is characterized by a state of hypoinsulinaemia and insulin-like growth factor (IGF-1) deficiency. T1D is triggered by a combination of both genetic and environmental factors including viral infections, dietary antigens, disruption in the gut microbiota, and VD deficiency [31].

Data regarding the presence of VDR in immune cells (B- and T-lymphocytes) and their ability to produce hormonally active form of VD locally, which acts on immune cells in auto-/paracrine manner, give the evidence that VD is an important regulator of multiple pathways of innate and adaptive immunity. In addition to immune-modulating properties, VD seems to play a role in the regulation of insulin secretion from β-cells. Respectively, VD insufficiency/deficiency is frequently reported to be associated with immunological disorders such as T1D, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, inflammatory bowel disease, hepatitis, asthma, and respiratory infections [32]. The link between the state of VD deficiency and T1D is a latter-day considerable area of interest; however, the presence of the clear association and especially causal relationship between low VD status and the occurrence of T1D still remains disputable and controversial.

Most of the international epidemiological and clinical studies have provided evidence to this causal relationship primarily in children. It has been reported previously that low 25OHD concentrations are fairly prevalent in the UK children with T1D [33]. Moreover, it has been shown that genetic factors affecting the VD metabolic pathway during the pregnancy can be related to the development of T1D. Another study emphasized that the fetal environment, including maternal VD metabolism, may be one of those factors that can lead to the early onset of T1D in Finnish children [34]. The study of VD level and its associated factors in Korean youth with T1D showed that serum 25OHD and $1,25(OH)_2D$ levels were lower in T1D cases than in healthy controls [35]. Nevertheless, in other cross-sectional study of subjects in Seoul National University Children's Hospital, there was no significant difference in the frequency of VD deficiency or serum 25OHD level between healthy and pediatric T1D patients [36].

There is much less data available regarding adult patients with T1D. It has been observed that the serum concentration of VD is negatively associated with IR in adult diabetic patients recruited in Poland [37]. In Algerian population, the

link between VD deficiency and an increased risk of T1D was also found [38]. In contrast, there was no difference reported between Turkish adult T1D patients and healthy controls according to their vitamin D levels [39]. The exact reason for these conflicting results is unclear; thus, we can assume that the interplay of genetic, nutritional, and environmental factors seems to affect the circulating level of VD status marker (25OHD) in adult T1D patients.

The cellular and molecular mechanisms underlying the VD deficiency in patients with TID deserve further thorough and comprehensive study. More recently, in addition to measuring the level of 25OHD, increased attention has been paid to new experimental directions, in particular, the investigation of the state of the VD auto-/paracrine system, including the following key components: VDR, CYP27B1, and 24-hydroxylase (CYP24A1).

Different genetic factors including mutations are known to modify serum 25OHD concentration. Several single nucleotide polymorphisms (SNPs) in the metabolic pathway of VD contribute as the genetic component to VD status. There is a set of studies dedicated to the associations between T1D and mutations related to VD metabolism genes such as VD-binding protein (VDBP), VDR [34, 40, 41], and CYP24A1. Moreover, polymorphisms in CYP2R1 gene encoding the enzyme involved in 25-hydroxylation of VD were also shown to be associated with a higher risk of T1D. Thus, polymorphisms in VD metabolism genes may contribute to susceptibility to T1D in Korean children [35]. Another study linked T-cell proliferation with VDBP level and reported higher levels and frequencies of serum anti-DBP antibodies in patients with T1D vs. healthy controls. This study postulated that VDBP, which was shown to be expressed in cells of pancreatic islets, can act as an autoantigen in T1D [42]. Furthermore, it has been reported that lower maternal third trimester VDBP levels and cord blood VDBP levels have been associated with a higher risk of T1D in offspring [43, 44]. At the same time, there is a lack of studies related to the investigation of the role of CYP27B1 in immune cells, such as monocytes, macrophages, and T-cells, which could shed light on the involvement of impaired VD metabolism in the pathogenesis and/or prevention of T1D. Thus, as VD biosynthesis and its signaling are regulated by genes encoding the VDR and enzymes of VD activation/catabolism, their polymorphisms may significantly alter the bioavailability and specific effects of VD metabolites.

Taken together, these data point to a role of VD deficiency in increasing the risk of T1D progression that provides the basis for further prospective studies on developing guidelines for vitamin D intake to prevent VD deficiency in patients with T1D and to treat this disease.

Since VD is considered a potential diabetes risk modifier, more studies appear to evaluate the role of vitamin D as an adjunctive therapy in improving glycemic control. The recent international study revealed that the majority of participants in Finland, Germany, and Sweden (97–99%) and 50% in the US receiving VD supplements during infancy demonstrated a reduced risk of T1D [45]. In another clinical trial, patients with T1D and low 25OHD concentrations were treated with different doses of cholecalciferol once daily for 3 months depending on their VD status, and as a result, it has been established that cholecalciferol can potentially improve the glycemic control [33]. Recent studies have shown favorable changes in HbA1c, C-peptide, insulin dose, and insulin sensitivity in VD-supplemented patients; therefore, cholecalciferol is increasingly attracting attention as a potential additional therapy in patients with T1D. In a double-blinded randomized controlled trial, which included Indian children with T1D, oral VD supplementation was used for six months in addition to insulin therapy. It has been proven that VD treatment may serve as an adjuvant to insulin therapy for children with T1D due to its effect on augmenting residual β-cell function and improving insulin secretion [46]. Some

representative studies on mechanisms of VD action in T1D described a beneficial effect of its supplementation on regulatory T-cells, with an increase in their percentage [47], suppressive capacity [48], and reduced progression to undetectable C-peptide.

However, some other studies have not demonstrated a beneficial effect of VD supplementation in preventing/improving the course of T1D or its complications. The prospective Environmental Determinants of Diabetes in the Young (TEDDY) Study demonstrated no benefit of maternal VD supplementation during pregnancy on the risk of islet autoimmunity in the offspring [49]. According to the review [32], there was no beneficial impact of VD supplementation on β-cell function, HbA1c levels, or insulin requirement.

The reason for these conflicting results is unclear. Nevertheless, we can presume the presence of a plethora of factors that may affect the results. Differences in study design, seasonal differences, stages in the progression of diabetes, ethnic origin of the populations, age and gender of patients may contribute. Therefore, further randomized controlled trials with a larger sample of patients are needed to gain more insight into the relationship between VD and T1D and to investigate VD replacement in preventing T1D.

The life expectancy of T1D patients has increased substantially during the last decades due to the availability of exogenous insulin, though it is still shorter than that of healthy people and associated with the development of chronic complications. Traditionally, the diabetic complications have been classified as either microvascular (retinopathy, nephropathy, and neuropathy) or macrovascular (cardiovascular disease, cerebrovascular accidents, and peripheral vascular disease). Although intensive glycemic control significantly reduced the incidence of microvascular and macrovascular manifestations, the majority of patients with T1D are still developing these outcomes. Most clinical trials related to the influence of VD supplementation on diabetes-associated complications have been performed in patients with T2D. To date, a limited number of experimental and clinical trials are available regarding the effect of VD on complications associated with T1D.

Diabetic ketoacidosis, which is the most dangerous and life-threatening complication of mainly T1D that results from insulin deficiency or excess of adrenaline or cortisol, is found to be associated with low VD level. VD is known to protect against viral and bacterial infections, which were shown to be triggering factors for diabetic ketoacidosis [50]; as a result, VD supplementation can become an integral part of diabetic ketoacidosis prevention and management. Nephropathy is another well-characterized complication of T1D, resulting in proteinuria and urinary loss of micronutrients. It has been previously found that the dietary supplements may modulate VD balance, attenuate polyuria, proteinuria, and renal hypertrophy in experimental T1D [51]. In addition, it has been reported that VD may reduce diabetic nephropathy not only by improving blood glucose and insulin levels but also by modulating hexosamine pathways in kidneys [52]. More recently, it has been shown that $1,25(OH)_2D$ may improve diabetic cardiomyopathy in T1D rats by modulating autophagy through the β-catenin/TCF4/GSK-3β and mTOR pathway [53]. Several studies have also demonstrated an association between low VD levels and diabetic peripheral neuropathy. Since VD is a well-known neurosteroid, a possible beneficial effect of its supplementation on preventing diabetic peripheral neuropathy can be assumed; nevertheless, further studies are needed.

Type 1 diabetes mellitus is a secondary cause of osteoporosis, characterized by reduced bone mass and disturbed bone microarchitecture. Patients with T1D have increased fracture risk that may be determined by the low 25OHD levels. Diabetic retinopathy, advanced cortical cataracts, and diabetic neuropathy are the risk factors for increased number of falls and, as a result, fracture because of

visual impairment and alterations in balance [54]. Replacement of VD along with calcium has been found to improve the bone mineral density in children with T1D; therefore, an adequate calcium level and VD supplementation are important for the prevention of T1D-associated osteoporosis [55].

According to available experimental and clinical data, new recommendations for T1M patients have been developed including obligatory assessment of serum 25OHD level and prescription of personalized doses of vitamin D in order to avoid the development of T1M complications or at least detain its progression.

5. Vitamin D deficiency and type 2 diabetes mellitus

Type 2 diabetes mellitus, formerly known as adult-onset diabetes, is a complex chronic metabolic disorder that has become one of the most serious public health-care problems worldwide. According to the data of the World Health Organization, 2.2 million people died from diabetes in 2012 and 1.6 million people died in 2015, and diabetes is expected to be the 7th cause of death by 2030. The incidence of T2D is estimated to account for 90% of all diabetes cases. T2D is characterized by dysfunction of pancreatic β-cell, systemic inflammation, and hyperglycemia due to insufficient insulin production, insulin action, or both [56]. A high diabetes-associated concentration of glucose in the blood over an extended period can cause heart disease, diabetic retinopathy, renal failure, poor blood circulation in the limbs, and, as a consequence, amputations.

T2D mainly develops as a result of the summation of genetic, environmental, and other risk factors [57]. To date, an increased risk of developing T2D in monozygotic twins with a statistical reliability of about 96% has been convincingly shown. Moreover, the risk of developing this disease in children from diabetic parents is 40% higher than in the offspring of healthy parents. T2D is now regarded as an endocrine-metabolic disease of a polygenic nature. About 75 loci have already been identified, damage to the sequences of which can be directly associated with the risk of developing T2D. These genes encode proteins with very different functions, such as ion channels (KCNJ11; potassium inwardly rectifying channel, subfamily J, and member 11), various transcription factors (TCF7L2, transcription factor 7-like 2), receptors (IRS1, insulin receptor substrate 1; MTNR1B, melatonin-receptor gene; and PPARγ2), growth factors (IGF2BP2, insulin-like growth factor two binding protein 2), as well as CDKN2A (cyclin-dependent kinase inhibitor 2A), HHEX (hematopoietically expressed homeobox protein), and FTO (fat mass and obesity-associated protein) [58–60].

Despite the growing body of data on the relationship between the risk of developing T2D and certain genes, improper food behavior and the sedentary lifestyle are still considered the key reasons for the development of the disease. In a number of experimental and clinical studies, VD has been shown to exhibit various non-skeletal properties that significantly regulate glucose metabolism. Furthermore, human studies have clearly revealed an inverse association between vitamin D status and the prevalence of T2D. It was found in numerous observational studies that the concentration of 25OHD negatively correlates with deteriorated glucose homeostasis, IR, and impaired β-cell function [61]. Low blood serum 25OHD levels were associated with the negative changes in a number of metabolic parameters, indicative of IR, including BMI (body mass index), HOMA-IR (homeostatic model assessment for IR), TG (triglycerides), HDL (high-density lipoproteins), LDL (low-density lipoproteins), TC (total cholesterol), and HbA1c [62]. More recently, large-scale epidemiological studies have been carried out as for the dependence of the risk of T2D developing on the availability of VD. VD deficiency has been shown

to be widespread in both men and women in different age groups among Saudi citizens, and this was accompanied by hyperglycemia in 90 percent of patients [63]. T2D patients studied in India also showed VD deficiency of varying severity in 77% of subjects [64]. The association between a low serum of 25OHD concentration and an increased risk of developing T2D can be partially explained by an increase in fat mass. A study of serum 25OHD in patients with T2D from the urban area of Cairo with limited exposure to the sun and overdressing habit revealed a decrease of its level by 13% compared with the control. Depleted level of the prohormone has been found to be related to a higher risk of insulin resistance [65].

However, as it turned out, not all studies confirmed a decrease in VD contents in patients with T2D. Using the method of high-performance liquid chromatography in tandem with mass spectrometry, significantly higher levels of VD were demonstrated in patients with T1D and T2D in comparison with the control group [66].

Overall, most of the data presented here suggest a pivotal role of VD in the regulation of insulin secretion and confirm that the decreased insulin sensitivity at target organs may be attributable to VD inadequacy [67]. The relationship between VD deficiency and IR could be realized at the level of modulation of immune processes and inflammation, since VD deficiency is associated with an increase in inflammatory markers. In addition, genetic polymorphisms of VD-related genes such as VDR, CYP2R1, and CYP27B1 may predispose to impaired glycemic control and T2D [68].

As we mentioned earlier, VD mediates its biological activity through VDR, which belongs to the family of steroid hormone receptors. Many VDR gene SNPs have been identified to be related to T2D, in particular to insulin synthesis and release [69]. The most reported VDR SNPs associated with diabetes are Fok1, Bsm1, Taq1, and Apa1. Recently, the study of these polymorphisms is becoming increasingly popular because their detection can be a reliable diagnostic characteristic in determining the risk of T2D development. The association between VDR polymorphisms and abdominal obesity has shown that patients with Bsm1 and Apa1 polymorphisms have low vitamin D status, which is accompanied by an increase in the concentrations of TC, LDL, and TG [69]. The results of another study show that body weight and BMI were significantly associated with polymorphisms Bsm1 and Taq1, while Bsm1 strongly correlated with elevated HbA1c level. The frequency of the heterozygous genotype of the Bsm1 polymorphism was significantly greater in type 2 diabetics than in controls [70]. A study of this parameter in a group of patients with T2D from India revealed that it was Taq1 and Bsm1 polymorphisms that were closely associated with diabetes [71]. Thus, it can be concluded that the detection of different types of VDR gene polymorphisms is a reliable prognostic parameter for the risk of T2D. In addition, the type of polymorphism appears to be race-specific.

Despite a large amount of data on the association of CYP27B1 polymorphisms with the risk of T1D, the effect of different SNP variants of this gene in the development of T2D is unclear to this day [72, 73].

While the closest relationship between VDR polymorphism in T2D has long been established, the discovery of the effect of CYP2R1 gene polymorphisms on the risk of developing this disease was a real step forward. Among a large group of tested SNPs of CYP2R1 gene, only two of them showed reliable association with the incidence of T2D. However, none of the tested polymorphisms were independently associated with serum 25OHD levels [74].

The effect of VD supplementation on glucose metabolism in patients with T2D remains controversial. It was shown that replenishing VD deficiency with high doses of cholecalciferol helped to reduce non-HDL cholesterol and caused significant normalizing changes in metabolic parameters of glucose homeostasis

(fasting glucose and serum insulin) and a decrease in oxidative stress and DNA damage [75, 76]. VD supplementation also caused an increase in insulin secretion and insulin sensitivity in T2D patients [77]. Nevertheless, some research groups did not find any effect, or only a slight decrease in fasting plasma glucose and an improvement in IR were seen. Basically, patients with VD deficiency and impaired glucose tolerance at baseline demonstrated these latter effects [68].

In addition to the ability of VD to reduce the risk of T2D, an adequate level of this vitamin in the body is also important for correcting diabetes-related pathologies such as retinopathy, nephropathy, neuropathy, and secondary osteoporosis caused by diabetes. Moreover, patients with already diagnosed T2D also have a risk of developing VD deficiency, in particular, due to the progression of nephropathy caused by diabetes [78]. Serum 25OHD was shown to be significantly reduced in patients with diabetic nephropathy [79]. Thus, the relationship between VD deficiency and the incidence of type 2 diabetes and related complications is likely to be bilateral.

Continuing the discussion on diabetic complications, we may note that vitamin D deficiency correlates with the risk of diabetic retinopathy. However, the reliable establishment of this relationship is problematic due to a number of limiting factors: cross-sectional structure, small sample size, ethnic variation, and heterogeneity in criteria that do not contribute to the identification of VD deficiency. Nevertheless, at least two state-of-the-art meta-analyses of observational studies indicate that D hypovitaminosis among T2D patients is associated with a significantly increased risk of diabetic retinopathy [80, 81].

A meta-analysis of data on the relationship between VD deficiency and the development of diabetes-induced neuropathy also revealed a strong correlation. Recovery of insulin secretion, increasing insulin sensitivity of target tissues, and reducing the inflammatory response have been proposed as potential mechanisms for improving the clinical manifestations of diabetic neuropathy following VD supplementation [82]. Furthermore, several independent studies have established serum 25OHD levels to predict cardiovascular complications or to assess the possible protective role of VD intake in patients with T2D. Diabetic patients with 25OHD < 36 nmol/L manifested approximately 21% higher risk for developing macrovascular disease [83].

There is no doubt that achieving normal levels of 25OHD is an important factor in preventing the onset of T2D or for eliminating the complications associated with the disease. However, at the moment, the question of the VD dosage and duration of the complex therapy of patients with T2D remains unresolved. Based on interventional trials, it can be postulated that the VD dose needs to be more than 2000 IU per day to raise blood 25OHD levels above 80 nmol/L, which is considered to be sufficient to reduce the risk of T2D [84]. However, the results of a double-blinded, placebo-controlled, randomized trial with 4000 IU of VD did not improve HbA1c or OGTT (oral glucose tolerance test)-based indices of β-cell function or insulin secretion in patients with stable T2D. The ambiguity of the results may be due to the following factors: the baseline 25OHD concentration was too high (<27 ng/mL), whereas a significant reduction in HbA1c and fasting glucose after VD supplementation was reported only among patients with baseline 25OHD < 20 ng/mL; VD may have no detectable effect in persons with well-controlled diabetes (good glycemic control, HbA1c-6.6%); and/or metformin treatment, which may have masked a small effect of VD supplementation [85]. These data indicate the need for individual VD therapy for each patient with a risk of development or with an already established diagnosis of T2D. When choosing this type of therapy, it is necessary to take into account the initial level of 25OHD in the patient's blood, changes in this level during therapy, as well as the presence of other drugs in the treatment regimen that can affect the manifestation of the beneficial effects of VD.

6. Conclusion

A two-way relationship between VD status and T1D and T2D can be argued. At the moment, it seems undeniable that there is a causal association between the risk of diabetes mellitus development and numerous polymorphisms in genes responsible for metabolism (CYP2R1 and CYP27B1) and signaling (VDR) of VD. Despite the fact that diabetes mellitus is a multifactorial disease, and it is unlikely that VD deficiency is the main cause of this pathology, there is no doubt that it may be used in the complex treatment of diabetes mellitus and its complications. The importance of more comprehensive and randomized clinical trials to determine the therapeutic role of vitamin D in preventing the progression of glucose intolerance in groups at high risk of developing T2D should be noted.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25OHD	25-hydroxyvitamin D
APCs	antigen-presenting cells
BMI	body mass index
CYP	CYP2R1 vitamin D-25-hydroxylase
CYP24A1	25-hydroxyvitamin D-24-hydroxylase
CYP27B1	25-hydroxyvitamin D-1-alpha-hydroxylase
DCs	dendritic cells
IFN-γ	interferon-γ
IGs	immunoglobulins
ILs	interleukins
IR	insulin resistance
NF-κB	nuclear factor kappa B
PPARs	peroxisome proliferator-activated receptors
PTH	parathyroid hormone
RXR	retinoid X receptor
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
TGF-β	transforming growth factor β
Th1	type 1 T helper
Th17	Th17 type 17 T helper
Th2	type 2 T helper
TNF-α	tumor necrosis factor-α
Treg	regulatory T cells
VD	vitamin D
VDR	vitamin D receptor
VDRE	vitamin D response element

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Section 4

Vitamin D Deficiency and Chronic Diseases

Chapter 11

Vitamin D and Cardiovascular Disease: The Final Chapter?

Jeremy I. Purow and Seth I. Sokol

Abstract

Vitamin D deficiency is globally prevalent and has been associated with the pathogenesis and complications of cardiovascular disease (CVD) and its risk factors. Defining these relationships has been challenging, and the clinical applications of vitamin D screening and supplementation for CVD risk prevention and modification have only recently become clearer. Most of the available evidence includes large observational studies and smaller randomized trials that scarcely evaluate CV outcomes as primary endpoints. Additionally, these studies include methodological inconsistencies, making it difficult to ascertain the benefits of vitamin D supplementation. However, more recently, randomized trials have been conducted which utilize CVD outcomes as primary endpoints, while assessing the effects of high dose vitamin D supplementation on CV health. Despite observational evidence as well as a conventional consensus that vitamin D supplementation improves CV health, these studies suggest that vitamin D supplementation likely has no benefit in this regard, at least in the follow-up period and populations evaluated.

Keywords: vitamin D deficiency, cardiovascular disease, endothelial function, hypertension, heart failure, renal disease, prevention, vitamin D

1. Introduction

Cardiovascular disease (CVD) is the most common cause of death in the developed world and is forecasted to be the leading cause of death and morbidity in developing countries by 2020 [1]. According to the American Heart Association, 9.0% (24.3 million in 2016) of adults (≥20 years of age) in the United States live with CVD (including coronary heart disease, heart failure, and stroke). This number increases to 48.0% when including hypertension [2]. CVD is a multifactorial disease that includes a complex interplay between genetics, environmental factors, and risk factors.

Despite effective measures for control and modification of traditional risk factors, a significant amount of risk remains. Therefore, the identification of easily modifiable novel risk factors has been heavily investigated over the past few decades.

In addition to the well-known relationship between vitamin D and bone health, there has been considerable interest of the possible linkage between vitamin D and CV health due to the expression of the vitamin D receptor (VDR) on cardiomyocytes and vascular cells [3, 4]. Vitamin D plays an extensive role in the regulation of numerous pathways implicated in CVD pathogenesis. Interestingly, various studies note that CVD events are higher in the winter months, a time when vitamin D

levels are known to be at their lowest due to lack of sunlight [5, 6]. A similar trend is noted in certain populations with poor cutaneous vitamin D production, such as African Americans, who are more prone to developing hypertension and CV disease [6]. Lastly, low vitamin D levels (<20 ng/mL) have been independently linked to increased morbidity and mortality [7, 8]. Although convincing, this evidence does not demonstrate causality, but supports a hypothesis for further study.

Prior to 2017, randomized controlled trials had mostly relied on surrogate or secondary endpoints for CV risk reduction. Study methodologies have been heterogeneous, and results have often been conflicting. In the absence of results from these trials, regular supplementation has not been recommended for CV risk modulation. Despite the lack of recommendations, use of vitamin D supplements for this purpose had risen dramatically.

However, more recent trials have been conducted that have assessed CV risk reduction as a primary endpoint. These trials have given researchers and clinicians a better understanding of the effects of vitamin D supplementation and whether it should be indicated to reduce the risk of developing CVD [9, 10].

The following chapter will discuss the prevalence of vitamin D deficiency, describe vitamin D synthesis and metabolism, and provide an overview on the biologic plausibility and current state of the evidence linking vitamin D to CV health and disease.

2. Vitamin D deficiency

Vitamin D deficiency is found in 30–50% of the general population, and prevalence estimates suggest that more than 1 billion people worldwide are vitamin D insufficient or deficient [4, 11].

Vitamin D deficiency is indicated by serum levels of 25(OH)D < 20 ng/mL [4]. Serum levels >30 ng/mL are likely optimal for bone health, but some studies have shown benefits with lesser values. Parathyroid hormone (PTH) suppression appears to plateau at levels between 30 and 40 ng/mL [12]. There has been no agreement on optimum 25(OH)D levels required for purported health benefits beyond skeletal health. One study suggested that 25(OH)D levels below 11–14 ng/mL signify increased CVD risk [13]. Levels in the range of 21–29 ng/mL are considered by some as insufficient, a definition that would label the majority of the U.S. population vitamin D insufficient [4].

The Endocrine Society does not recommend screening for vitamin D deficiency in individuals who are not at risk for it [12]. Many of the risk factors for vitamin D deficiency have been identified. Some of these include inadequate cutaneous synthesis stemming from insufficient sun exposure or dark skin pigmentation and inadequate dietary intake. Other noted risk factors include aging, obesity, renal disease, liver disease, disorders that affect fat absorption (e.g. celiac disease, inflammatory bowel diseases, types of bariatric surgery), increased catabolism due to medications (e.g. steroids, anticonvulsants etc.), and other hereditable (e.g. rickets) and acquired disorders (e.g. hyperthyroidism) [4].

3. Vitamin D metabolism

Vitamin D's active form, 1a,25-dihydroxyvitamin D (1,25[OH]2D3) plays a critical role in influencing a myriad of metabolic pathways [14].

Natural sunlight exposure contributes to more than 90% of vitamin D (D3-cholecalciferol) production in humans [15]. UV-B irradiation absorbed by skin

keratinocytes triggers photolysis of 7-dehydrocholesterol (pro-vitamin D3) in the plasma membrane, which is then swiftly modified into vitamin D3 by heat [4, 16].

Dietary supply of vitamin D (D2-ergocalciferol) contributes to the remainder of the total amount of vitamin D in the body. Foods containing vitamin D include oily fish (salmon, sardines, and mackerel), cod liver oil, egg yolk, mushrooms, and fortified milk, orange juice, cereals, and cheese [11, 12].

D3 and D2 from the skin and diet, respectively, each undergo two sequential hydroxylation's: 25-hydroxylation in the liver and then 1,25-dihydroxylation in the kidney. In order to assess vitamin D status from oral intake and endogenous production, the primary metabolite of vitamin D, 25(OH)D, should be measured [11, 15, 17]. The hydroxylation of 25(OH)D to its biologically active form, 1,25(OH)2D3, is controlled by PTH [11, 18].

Most of the known biological effects of 1,25(OH)2D3 are mediated through the vitamin D3 receptor (VDR), part of the superfamily of nuclear hormone receptors, which mediates transcriptional gene regulation [19].

Over 200 genes are regulated by 1,25(OH)2D3. These include genes directly or indirectly responsible for renin and insulin production, anti-inflammatory cytokine release, proinflammatory cytokine suppression, and regulation of vascular smooth muscle cell (VSMC) and cardiomyocyte proliferation [11, 20].

1,25(OH)2D3 is also involved in non-genomic mediated intracellular signaling, demonstrating immunomodulatory, antiproliferative, and pro-differentiative activities in experimental settings [19].

4. Biologic plausibility

Characterizing vitamin D deficiency as a primary risk factor for CVD is challenging due to the multitude of complex interacting pathways involving vitamin D. The vitamin D receptor is nearly ubiquitous in human cells including vascular smooth muscle cells (VSMC), endothelial cells, cardiac myocytes, juxtaglomerular, and most immune cells, all implicated in the pathogenesis and progression of CVD [11, 18].

Immune cells such as activated CD4+ and CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells are capable of converting 25OHD3 into 1,25OHD3, its active form. Moreover, 1,25 hydroxylase, the rate-limiting enzyme in this pathway, is present in activated macrophages [21–23]. Lastly, VSMC and endothelial cells also express 1,25 hydroxylase, suggesting that these cells have an autocrine mechanism allowing them to modulate the effects of vitamin D on the vasculature [24, 25].

Vitamin D has various direct and indirect effects on CV function. 1,25(OH)2D3 directly modulates VSMC and expression of vascular endothelial growth factor via the VDR and CYP27B1 expression in VSMC's and endothelial cells. 1,25(OH)2D3 has an inhibitory effect on hypertrophy and proliferation of VSMC in vitro and in cultured cardiac myocytes. It also plays an important role in inflammation and thrombosis [21, 24]. In a swine model of atherosclerosis, vitamin D deficiency accelerated plaque progression by enhancing inflammation in epicardial adipose tissue [26]. Inverse associations between vitamin D deficiency and thrombogenicity, vascular inflammation, and vascular calcification have been demonstrated [27–29].

Indirectly, the expression of renin in vivo is strongly regulated by vitamin D, and an inverse relationship between vitamin D levels and renin expression has been demonstrated experimentally [30–32]. 1,25(OH)2D3 binds to the renin promoter region and inhibits renin transcription [30]. VDR knockout mice were shown to have increased levels of renin and angiotensin II and, therefore, a higher prevalence

of hypertension [32]. Thus, vitamin D is implicated in blood pressure regulation and myocardial thickening.

Another indirect effect of vitamin D on CVD is the regulation of matrix metalloproteinase 2 and 9 production. Increased metalloproteinases have been associated with cardiac fibrosis, hypertrophy and heart failure in mice [33, 34].

Vitamin D deficiency may also indirectly harm the CV system by inducing hyperparathyroidism, which may act upon parathyroid hormone (PTH) receptors within the blood vessel wall and the myocardium [35]. Multiple studies have been able to demonstrate an association between elevated PTH levels and hypertension, cardiac dysfunction and vascular disease [35, 36].

Lastly, hyperlipidemia has also been associated with vitamin D deficiency. This is likely a result of decreased transcriptional activity of the VDR, leading to the increase of hepatic cholesterol production [37].

5. The clinical evidence

Vitamin D deficiency is prevalent in CVD patients [11]. Most studies showing an association between inadequate 25OHD3 and poor outcomes in CV health are observational, hindering the establishment of a causal relationship. Furthermore, significant differences across studies hamper the ability to make valid and consistent conclusions. These differences include varying definitions of vitamin D deficiency and lack of seasonal adjustment and properly defined CV outcomes. Further difficulties include the use of single baseline measurements of vitamin D (which may be an inaccurate assessment of overall vitamin D status), a poor understanding of the role of high PTH on CVD, and the use of other disease modulating drugs such as calcium and statins in active and placebo groups, which may affect study results.

However, newer studies implement clearly defined primary endpoints, differing frequencies of vitamin D supplementation, and a diverse cohort of participants. These studies allow for a clearer understanding of the effects of vitamin D supplementation on CVD health.

6. Observational data

Several large-scale observational studies have been completed over the past decades. The NHANES III national cohort registry found a significant inverse relationship between 25(OH)D levels and all-cause mortality, but a non-significant association between 25(OH)D levels and CVD mortality [8].

In the Intermountain Heart Collaborative Study Group, significantly higher rates of diabetes, hypertension, hyperlipidemia, and peripheral vascular disease were found in those with serum 25(OH)D levels below 30 ng/mL. Additionally, low serum 25(OH)D levels were also linked to coronary artery disease, myocardial infarction (MI), heart failure, stroke and incident death [38].

In the Health Professionals Follow-up Study, men deficient in 25(OH)D (≤15 ng/mL) had a higher risk of MI than men with sufficient levels (≥30 ng/mL) [39].

In contrast, other prospective studies have had discordant results. In the MIDSPAN family study, with a median follow up of 14.4 years, plasma levels of 25OHD less than 15 ng/mL were associated with all-cause mortality, but not the risk of CV diseases [40].

Similiarly, in the MrOS Sleep Study, no relationship between circulating 25(OH)D levels and risk of CVD events was found [41].

Additionally, in a study involving 746 patients undergoing coronary angiography, no correlation was found between vitamin D levels (<20 ng/mL vs. >20 ng/mL) and the degree and severity of coronary artery disease [42].

7. Randomized controlled trials

Prior to 2017, most randomized interventional studies have assessed surrogate endpoints rather than hard CV outcomes. In the available studies, there has been considerable variation in defining baseline vitamin D status, assessing adequate vitamin D dosage, and ascertaining study outcomes.

In a double-blind, placebo controlled, randomized trial where elderly participants in the United Kingdom received vitamin D3 supplements of 100,000 IU every 4 months for 5 years, no benefits on CVD outcomes were shown [43].

Additionally, in a systematic review of 18 randomized trials studying the efficacy of supplementation with vitamin D (with or without calcium) on various cardiometabolic outcomes, only four reported on incident CVD. There were no significant reductions in CVD risk found in these trials [44].

Postmenopausal women in the Women's Health Initiative (WHI) receiving calcium carbonate (1000 mg/day) and vitamin D (400 IU/day) had no reduction in their risk of coronary events or stroke during the 7 year follow-up period. Treatment with calcium and vitamin D also had no effect on blood pressure reduction, hypertension development and coronary artery calcification. In the overall participant cohort, supplementation did not reduce the overall incidence of heart failure, yet some benefits were noted in participants considered to be at low risk for heart failure. Lastly, in a follow-up 4.9 years after the culmination of the study, no positive effects on CVD were found [45–49].

More recent studies on vitamin D supplementation have made use of well-defined CV outcomes as primary endpoints. In 2017, the results of the Vitamin D Assessment study (VIDA), a randomized, double blinded, placebo-controlled study on the effects of high-dose, monthly vitamin D supplementation in the general population aged 50–84, were published. 5,110 patients were randomized to receive either an initial oral vitamin D dose of 200,000 IU followed by 100,000 IU monthly or placebo. Primary outcomes included incident CVD and death. Secondary outcomes included MI, angina, heart failure, hypertension, arrhythmias, chronic ischemic heart disease, arteriosclerosis, stroke, and venous thrombosis. Additionally, pre-specified subgroup analysis for the primary endpoint was done for patients with vitamin D deficiency (<20 ng/mL) and established CVD. Results of the study showed that high doses of monthly vitamin D supplementation provided no benefit to CV health over placebo. However, the study is limited by a low event rate and decreased power, especially for subgroup analysis in those deficient at baseline. Supplementation was monthly, leaving the question of whether daily dosing may be better. Finally, the funding of the clinical trial only allowed for a median follow-up time of 3.3 years and, therefore, the long-term effects of high dose, monthly vitamin D supplementation remain in question [10].

Another recent placebo-controlled trial, known as the VITAL study, assessed the effects of more frequent vitamin D supplementation (2,000 IU of vitamin D and 1 g of omega-3 per day) on CVD and cancer. The primary endpoint in the CV arm was major CV events, which included MI, stroke, and cardiac related death. 25,871 subjects participated in the study and the median follow-up time was 5.3 years. The trial found that daily vitamin D supplementation had no benefit on CV health. The strengths of the study included daily vitamin D supplementation, longer follow-up times, and participant diversity [9].

A recent updated meta-analysis, including VITAL and VIDA, with a total of 83,291 patients, indicated that vitamin D supplementation had no benefit on CV health. Only 4 of the 21 trials used CVD as a primary endpoint. It has also been shown that vitamin D supplementation provided no benefit toward the secondary endpoints of MI, stroke, CVD mortality, and all-cause mortality [50]. This meta-analysis, though, lacks complete and specific patient level data and, therefore, subgroup analysis is difficult.

8. Hypertension

Low vitamin D status has been heavily linked to an increased prevalence of hypertension. A prospective examination of 1,211 non-hypertensive men, over a 15 year follow-up period, demonstrated an inverse association between vitamin D levels and hypertension development [51].

In addition to the demonstrated link between low vitamin D levels and hypertension development, the Framingham Offspring Study suggested that low vitamin D levels may also increase the risk associated with already existing hypertension, which may substantially augment the risk of future CV events [52].

Contrary to observational evidence, randomized controlled trials of vitamin D repletion have not shown significant changes in blood pressure in vitamin D deficient individuals with prehypertension or hypertension [53, 54].

In a randomized, double blind trial involving 283 black subjects given either placebo, 1,000, 2,000, or 4,000 IU/day of vitamin D for 3 months, significant modest reductions in systolic blood pressure were seen. Systolic blood pressure decreased by 0.2 mmHg for every 1 ng/mL increase in vitamin D, but no effect on diastolic pressure was demonstrated [55].

In VITdish, another small (N = 159) randomized, double-blind, placebo-controlled trial, there was no effect of vitamin D supplementation on blood pressure or other markers of vascular health in older adults with systolic hypertension [56].

A 6 month study of vitamin D3 supplementation including patients with resistant hypertension showed similar results. Effects on left ventricular hypertrophy were also negligible, although the short follow-up may have limited this assessment [57].

In the VIDA study, with monthly high dose vitamin D supplementation, there were no significant differences between the vitamin D and placebo groups regarding incident hypertension [10].

Lastly and likewise, in the VITAL study using high daily doses of vitamin D, hypertension incidence was not significantly different in the vitamin D and placebo groups [9].

9. Endothelial dysfunction

Vitamin D deficiency has been associated with endothelial dysfunction. A study involving 23 asymptomatic subjects demonstrated impaired brachial artery flow-mediated dilatation (FMD) in subjects with significant vitamin D deficiency. Improvement was seen with vitamin D repletion [58].

In contrast, there was no improvement in endothelial-dependent vasodilation with active treatment (ergocalciferol 50,000 IU/week) versus placebo in an 8 week trial in non-hypertensive, overweight, vitamin D deficient individuals [59].

Similarly, a prospective placebo-controlled pilot study evaluated the effects of vitamin D repletion on endothelial function and inflammation in subjects with both vitamin D deficiency and coronary artery disease. The study was conducted over a 12-week period in 90 subjects. No significant differences between the groups were found in reactive hyperemia index (using RH-PAT), blood pressure, and levels of hs-CRP, IL-6, IL-12, interferon gamma (INF-gamma), and CXCL-10 [60].

Additionally, in the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) (N = 852), vitamin D levels were positively related to endothelial-independent vasodilation in women only. There were no significant relationships between vitamin D levels and indices of endothelium-dependent vasodilatation in both men and women [61].

Lastly, a recent systematic review including 31 trials and individual-level meta-analysis (2,571 patients) assessed the relationship between vitamin D and various markers of vascular function. The analysis found that most vascular markers studied were not significantly effected by vitamin D supplementation [62].

10. Heart failure

The impact of vitamin D supplementation on patients with heart failure has not been the focus of large randomized trials.

The RECORD trial (Randomized Evaluation of Calcium Or vitamin D) was a trial designed for the secondary prevention of fractures in 5292 participants aged ≥70 years (conducted between 1999 and 2002). Subjects received oral vitamin D3 (800 IU/d) plus calcium (1,000 mg calcium carbonate/d), vitamin D3 alone, calcium alone, or a placebo. An analysis of unpublished data from the trial, suggested that vitamin D supplementation may decrease heart failure events in the elderly. The trial had pre-specified CV endpoints of time to first cardiac failure, time to first MI, time to first stroke, and time to first composite outcome of cardiac failure, MI, or stroke. The trial, though, was not designed as a CV outcomes trial, and outcomes were not subject to an adjudication committee nor verified against medical records. Furthermore, significance for heart failure event reduction was reached only when off-trial data were used [63].

Several small placebo-controlled studies have been conducted to analyze the effects of vitamin D supplementation on differing endpoints in patients with heart failure. Results have been conflicting.

In a small randomized, double-blind, placebo-controlled trial (N = 105) in older adults with vitamin D deficiency (25-vitamin D < 20 ng/mL) and systolic heart failure, subjects were given 100,000 IU of oral vitamin D2 or placebo at baseline and 10 weeks. Functional outcomes, quality of life and biomarkers (B-type natriuretic peptide (BNP) and tumor necrosis factor (TNF alpha)) were measured at baseline, 10 and 20 weeks. BNP was significantly reduced in the active treatment group versus placebo, but TNF alpha was not. Despite reduced BNP levels, physical function, as measured by the 6-minute walk test and the timed get up and go test, did not improve. There was also no change in the Functional Limitations Profile measure in the active versus placebo group. A small, but significant worsening in quality of life with active treatment was noted, despite a non-significant increase in activity level, suggesting a chance finding [64].

Schleithoff et al. examined cytokine profiles with vitamin D3 supplementation in younger patients with heart failure. A dose of 2,000 IU per day reduced the pro-inflammatory marker TNF-alpha levels and increased the anti-inflammatory cytokine interleukin-10 levels, but no significant effects on BNP levels were seen [20].

A recent randomized, controlled trial assessed the effects of 50,000 IU of weekly vitamin D or placebo for 6 months on various measures of physical performance (primary endpoint: peak VO2; secondary endpoints: 6-Minute Walk test, timed get up and go test, isokinetic muscle strength) in patients with heart failure. The study failed to demonstrate any benefits, despite considerable increases in serum 25(OH)D levels in the active treatment group [65].

In VINDICATE, a double-blind, randomized, placebo-controlled trial, subjects with systolic dysfunction (ejection fraction (EF) ≤ 45%) and vitamin D deficiency (<20 ng/mL) were randomized to 4,000 IU of vitamin D3 or placebo. At 12 months, there was no significant difference in 6-minute walking distance (primary endpoint), but there was significant improvement in left ventricular (LV) systolic function and a reduction in LV end diastolic and end systolic diameter with active supplementation [66].

In the EVITA trial, 400 patients with heart failure were randomized to receive 4,000 IU of vitamin D or placebo daily for 3 years. There was no benefit of supplementation on the primary endpoint of all-cause mortality. Additionally, secondary endpoint analysis suggested that vitamin D supplementation was associated with an increased frequency of implantation of mechanical circulatory support [67].

11. Renal disease

Observational studies show that there are potential benefits to vitamin D supplementation in patients with chronic kidney disease (CKD). In a meta-analysis of some of these studies, it was determined that receiving treatment with any vitamin D derivative reduces all-cause mortality as well as CV mortality [68]. In another, more recent, meta-analysis of observational studies, it has been shown that vitamin D treatment may pose benefits toward reducing all-cause mortality and CV related mortality [69].

To further assess some of the available randomized, clinical trials for renal patients, a meta-analysis was done to test the efficacy of vitamin D supplementation in these patients. The 13 trials used for the meta-analysis showed no significant benefits for serious adverse CV events, all-cause mortality, or CV related mortality in CKD patients who supplemented with vitamin D. Unfortunately, there is a lack of patient level data relating to patients with CKD, making it difficult to draw any conclusions regarding the effects of vitamin D supplementation on CV health in this population [70].

In many of the previous studies in patients with CKD, the dosage of vitamin D has been limited in order to protect patients from developing hypercalcemia. Additionally, a lack of standardization in vitamin D formulations has made it especially difficult to compare results in different trials. Larger randomized trials with well-defined primary outcomes need to be conducted in order to further define vitamin D's effect on CVD in renal patients. In 2025, the SIMPLIFIED trial is expected to conclude and expectantly give insight into the effects of vitamin D supplementation on hard endpoints of all-cause mortality and CV related mortality in CKD patients [71, 72].

12. Conclusions

Epidemiologic, observational and laboratory evidence have implicated vitamin D deficiency in the pathogenesis and complications of CVD. This lent extensive biologic plausibility to the theory that vitamin D levels would be an effective target

for CVD prevention. However, like many other trials of vitamin supplementation, large randomized placebo-controlled trial data from the general population have refuted this theory. Therefore, vitamin D supplementation for the purpose of CVD prevention is not recommended for the general public.

13. Future directions

Based on the findings in the most recent clinical trials, it appears that the last chapter may have been written regarding the role of vitamin D for CVD prevention in the general population. However, many questions still remain and will likely fuel ongoing investigation and debate. Was the follow-up in these recent trials long enough? Do large trials randomizing by baseline vitamin D status need to be conducted since the mean vitamin D levels in VITAL and VIDA were both above the deficient threshold of 20 ng/mL? [9, 10]. Are there benefits to vitamin D repletion for heart failure outcomes? What is the true benefit of vitamin D repletion on CVD outcomes in the chronic kidney disease and dialysis population? These questions and others regarding both CVD and other chronic disease states will likely keep the book open on vitamin D for the foreseeable future.

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Chapter 12

Vitamin D and Autoimmune Diseases

Ifigenia Kostoglou-Athanassiou, Lambros Athanassiou and Panagiotis Athanassiou

Abstract

Vitamin D has many and profound effects on the immune system. Vitamin D deficiency is known to be related to the development of autoimmune diseases. In particular, vitamin D deficiency is related to the development and the severity of rheumatoid arthritis (RA). RA develops in patients with vitamin D deficiency, and the activity of the disease is related to vitamin D deficiency. Vitamin D deficiency is also related to the development of systemic lupus erythematosus (SLE). SLE develops in patients with vitamin D deficiency, and the activity of the disease is also greater in patients with vitamin D deficiency. Vitamin D deficiency is also related to the development and the severity of multiple sclerosis. Vitamin D should be administered to patients with multiple sclerosis, and this seems to mitigate the symptoms of the disease and to prevent disease progression. Vitamin D deficiency is also observed in patients with inflammatory bowel disease and may be related to disease severity. Low vitamin D levels have also been observed in patients with autoimmune Hashimoto's thyroiditis. Low vitamin D levels have been observed in patients with systemic sclerosis, especially in the diffuse form of the disease. Optimal vitamin D levels appear to be required for normal immune function and for the prevention and treatment of autoimmune diseases.

Keywords: vitamin D, autoimmunity, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, autoimmune Hashimoto's thyroiditis

1. Introduction

Vitamin D is a secosteroid hormone, which is known to be related to the regulation of the musculoskeletal system. It affects calcium and phosphate metabolism and is related to bone health. Recently, the extraskeletal effects of vitamin D are under intense research and have attracted the interest of the scientific community [1–6]. In particular, the relationship of vitamin D with the immune system is in the focus of scientific evaluation [7–9]. In the chapter herein, the effects of vitamin D on the immune system will be discussed, and the relationship of vitamin D deficiency with the development of autoimmune diseases will be reviewed.

2. Vitamin D and the immune system

The classic function of vitamin D is to enhance intestinal absorption of calcium by regulating several calcium transport proteins in the small intestine [4]. However, various cells express the vitamin D receptor (VDR) and the vitamin D activating enzyme 1-α-hydroxylase. Various cells of the immune system also express the VDR and harbor 1-α-hydroxylase [10, 11]. Thus, cells of the immune system respond to vitamin D and also activate vitamin D in a paracrine or autocrine fashion. The extra-renal 1-α-hydroxylase is not upregulated by PTH, and thus, production of $1,25(OH)_2D_3$ is dependent on concentrations of the substrate $25(OH)D_3$, and it may be regulated by inflammatory signals, such as lipopolysaccharide and cytokines [12, 13]. Cells of the immune system, which express the VDR and harbor 1-α-hydroxylase, are macrophages, T cells, dendritic cells, monocytes, and B cells (**Figure 1**) [9]. Vitamin D is involved in the regulation of the innate immunity as it enhances the defense system of the organism against microbes and other pathogenic organisms, and it modulates the adaptive immune system through direct effects on T-cell activation and on the phenotype and function of antigen-presenting cells, particularly dendritic cells.

2.1 Vitamin D and the innate immune system

The innate immune system is a first line of defense against infection. Vitamin D is a regulator of the innate immune system [1, 14]. The first data on the effect of vitamin D on the innate immune system have been generated on the treatment of diseases caused by mycobacteria, such as tuberculosis and leprosy [15–18]. Vitamin D has been used as a treatment of infections for more than 150 years. In 1849, Williams reported favorable results with the use of cod-liver-oil, an excellent source of vitamin D, in the treatment of patients with tuberculosis [19]. Fifty years later, Niels Finsen received the third Nobel Prize in Medicine for his description of using UV light, an effective method to increase vitamin D status, to treat lupus vulgaris, a cutaneous form of tuberculosis [20, 21]. Alfred Windaus contributed to the discovery of the chemical structure of vitamin D_2 and vitamin D_3 found in cod-liver-oil and received the Nobel prize [22–24]. Thereafter, several groups used vitamin D_2 and D_3 as a treatment for tuberculosis [22, 25]. Rook et al. [26] demonstrated in the 1980s that $1,25(OH)_2D_3$ inhibited the proliferation of *Mycobacterium tuberculosis* in culture. Vitamin D enhances the production of defensin β2 and cathelicidin in response to infection by macrophages, monocytes, and keratinocytes [12]. Humans have only

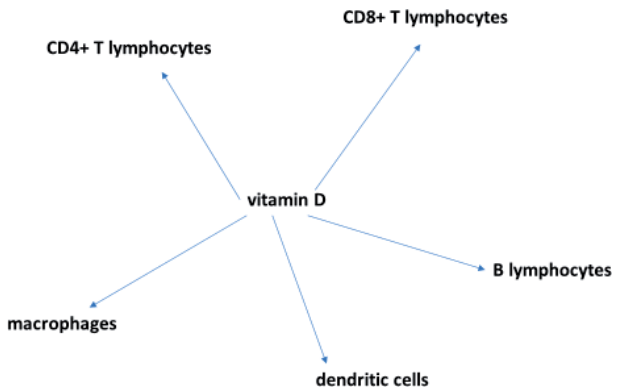

Figure 1.
Cells of the immune system regulated in part by vitamin D.

one cathelicidin, which is cleaved to form LL-37 [27]. Cells of the immune system including neutrophils and macrophages and cells lining epithelial surfaces that are constantly exposed to potential pathogens such as the skin, the respiratory, and the gastrointestinal tract produce cathelicidin [28–30]. Cathelicidin has broad antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as certain viruses and fungi [31]. The killing mechanism of cathelicidin involves bacterial lysis by destabilizing cell membrane [32]. Treatment with $1,25(OH)_2D_3$ upregulates cathelicidin mRNA in several cell lines. Thus, it appears that $1,25(OH)_2D_3$ upregulates antimicrobial peptide production, primarily cathelicidin, on a variety of different cells [33]. Studies indicate that $25(OH)D_3$, the major circulating form of vitamin D to determine vitamin D status, is important for local production of $1,25(OH)_2D_3$ to upregulate cathelicidin production in the skin and macrophages. Exposing human monocytes to pathogens increases the expression of both $1,25(OH)_2D_3$ and VDR, thus increasing both the local production of $1,25(OH)_2D_3$ and the ability of the cell to respond to it [12]. Since keratinocytes also possess 25-α-hydroxylase, UV light may directly stimulate cathelicidin production by providing the substrate $25(OH)D_3$ directly from vitamin D_3 produced within the skin [34, 35]. Macrophages also respond to vitamin D increasing their antimicrobial activity, however, heterogeneously [36, 37]. Macrophages formed after interleukin-15 stimulus respond to vitamin D increasing their antimicrobial activity, whereas macrophages formed after stimulation by interleukin-10 respond to vitamin D stimulus weakly.

Data regarding other infections also exist. Thus, children with low vitamin D status may be more prone to urinary tract infections due to low production of cathelicidin and defensin β2 [38, 39]. Also, adults with asthma may be less prone to infection after treatment with vitamin D due to increased production of cathelidicin and modulation of inflammatory cytokines [40, 41]. Low levels of vitamin D may be related to chronic obstructive pulmonary disease severity [42]. Vitamin D may increase resistance to HIV infection. Low levels of vitamin D have been associated with disease progression and mortality [43]. The ability of the immune cells to hydroxylate $25(OH)D_3$ locally suggests that in patients with infections, it may be better to administer $25(OH)D_3$ rather than hydroxylated metabolites to allow for local production and the feedback system to function.

2.2 Vitamin D and autoimmunity

The natural history of autoimmunity remains largely unknown. However, the theory is that both genetic susceptibility and environmental factors play a role in the development of clinical autoimmune disease. Vitamin D has known immunomodulatory effects on a wide range of immune cells, including T and dendritic cells [44, 45]. Each of these immune cells expresses VDR and produces the enzymes 1-α-hydroxylase and 24-hydroxylase and is therefore capable of locally producing active $1,25(OH)_2D_3$ [46–49]. Activation of CD4+ T cells results in a significant increase in VDR expression enabling regulation of many genes responsive to $1,25(OH)_2D_3$ [50]. $1,25(OH)_2D_3$ suppresses T-cell receptor induced T cell proliferation and changes their cytokine expression. The overall shift is away from T helper Th1 phenotype toward a more tolerogenic Th2 response [51–53]. Vitamin D appears to directly inhibit Th1 cells and may additionally modulate a skewing toward a Th2 response [54]. Th17 cells are a subset of CD4+ T cells involved in organ-specific autoimmunity playing a role in maintaining inflammation, which can lead to tissue damage. $1,25(OH)_2D_3$ suppresses autoimmunity and tissue destruction by inhibiting the Th17 response at several levels [55, 56]. Altogether, the evidence suggests an important role for vitamin D in influencing T-cell responses and in tempering inflammation and tissue damage.

Vitamin D appears to have a direct effect on B cells and inhibits immunoglobulin production [57]. Additionally, differentiation of B cells is interrupted when exposed to $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ also has effects on dendritic cells. Dendritic cells have important functions in maintaining both protective immunity and self-tolerance [58, 59]. Physiologic levels of $1,25(OH)_2D_3$ inhibit maturation of dendritic cells and maintain an immature and tolerogenic phenotype with inhibition of activation markers such as MHC class II, CD40, and others and upregulation of inhibitory molecules [60, 61]. Thus, it appears that the maturational state of dendritic cells can be modulated by $1,25(OH)_2D_3$, making it possible that the vitamin D status of an individual is likely to have important immunologic consequences.

3. Vitamin D and autoimmune diseases

There are several animal models of autoimmunity, in which disease could either be prevented or ameliorated with the administration of either $1,25(OH)_2D_3$ or one of its analogues. These animal models are models of autoimmune encephalomyelitis, collagen-induced arthritis, type 1 diabetes mellitus, inflammatory bowel disease, autoimmune uveitis, and lupus [44, 56, 62–76]. These studies show that treatment with active vitamin D is effective in modulating immune function and ameliorating autoimmune disease. Vitamin D deficiency is a risk factor for the development of some autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), diabetes mellitus type 1, multiples sclerosis, inflammatory bowel disease, and Hashimoto's thyroiditis [49, 69, 74, 77–85] (**Figure 2**). Additionally, vitamin D deficiency has been observed in patients with systemic sclerosis [86].

3.1 Vitamin D deficiency and rheumatoid arthritis

A meta-analysis showed that low vitamin D intake is associated with the development of RA [87]. Thereafter, several studies performed in various areas all over the world showed that vitamin D deficiency is observed in patients with RA and that vitamin D deficiency is associated with disease activity [78, 82, 83, 88–97]. A meta-analysis of the good quality studies performed regarding the association between vitamin D deficiency and RA showed that vitamin D deficiency is observed in RA patients significantly more than in a control group and that vitamin D levels are inversely correlated with disease activity, meaning that low vitamin D levels are associated with high-disease activity [98]. Moreover, an

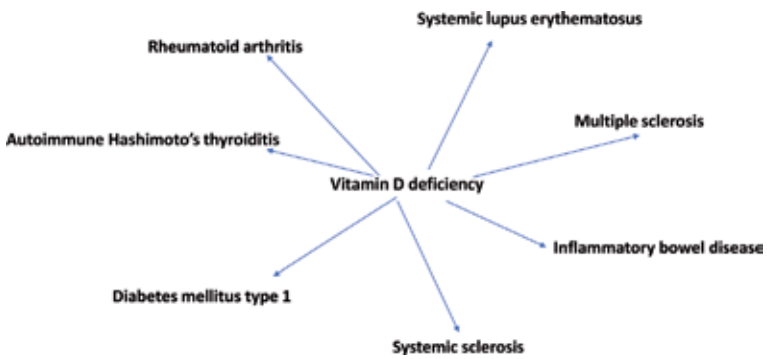

Figure 2.
Autoimmune diseases related to vitamin D deficiency.

association has been shown between VDR polymorphism and RA. Specifically, the FokI F allele of the VDR may be a risk factor for the development of RA [99]. Further studies are needed to unravel the exact association between vitamin D deficiency and RA and to determine the best method of vitamin D supplementation and whether it may be used for the prevention of RA or for the best management of the disease [77, 100]. In addition, it has been proposed that vitamin D may contribute to the management of pain in RA and may be used along with TNF-α inhibitors in RA treatment [77, 101].

3.2 Vitamin D deficiency and systemic lupus erythematosus

In SLE, the inflammatory milieu drives the development of T cells into proinflammatory pathways, defective function of Tregs, and survival and activation of B cells, which produce autoantibodies [78, 81]. Patients with systemic lupus erythematosus have lower $25(OH)D_3$ levels compared to controls, suggesting that vitamin D deficiency may be a risk factor for SLE [81, 84, 102–107]. The majority of studies have also found higher SLE disease activity associated with lower levels of $25(OH)D_3$ [84, 103]. As patients with SLE have often photosensitivity and are advised to avoid direct sun exposure, detecting vitamin D deficiency and replacing $25(OH)D_3$ with oral supplementation is critical and may impact disease activity [108].

3.3 Vitamin D deficiency and type 1 diabetes mellitus

Type 1 diabetes mellitus is one of the most prevalent chronic diseases with onset in childhood and is the result of immune-mediated destruction of pancreatic insulin producing β cells. There appears to be a geographic variation in incidence following a gradient in latitude, which is the inverse of the global distribution of ultraviolet B irradiation, critical for the production of vitamin D within the skin [109]. Studies have shown higher incidence of vitamin D deficiency in patients with type 1 diabetes [110–113]. One environmental factor thought to be protective against the development of type 1 diabetes mellitus is early supplementation with vitamin D [114]. A number of large case control studies showed that the risk of type 1 diabetes mellitus was significantly reduced in infants who were supplemented with vitamin D compared to those who were not supplemented [115–117]. Additionally, a lower incidence of type 1 diabetes was observed in infants born to mothers who were administered cod liver oil during pregnancy [118]. A birth cohort study in Finland, now more than 50 years ago, evaluated the effects of vitamin D supplementation on rickets and the development of type 1 diabetes mellitus [85]. All women due to give birth in 1966 were enrolled. There was an 80% reduction in the risk for type 1 diabetes mellitus in children having received >2000 IU vitamin D/day compared to those receiving less or not receiving supplementation with vitamin D. Evidence from both human and animal studies shows that vitamin D may be protective as far as the development of type 1 diabetes mellitus is concerned [68, 71, 76]. Thus, the administration of vitamin D may prevent diabetes mellitus type 1; however, once the destruction of pancreatic beta cells has taken place, it will not act therapeutically to reverse diabetes mellitus type 1.

3.4 Vitamin D deficiency and multiple sclerosis

Multiple sclerosis is characterized by inflammation, demyelination, axonal or neuronal loss, and astrocytic gliosis in the central nervous system, which can result in disability. Epidemiological studies have suggested that vitamin D insufficiency

may contribute to the risk of multiple sclerosis [62, 63, 75, 119, 120]. Moreover, several genetic studies in multiple sclerosis patients have shown that diverse abnormalities in vitamin D metabolism are related to the risk of the disease. It appears that vitamin D deficiency may interact with genetic and environmental protective and risk factors, such as the allele HLA BRB1*1501, infections, obesity, smoking, and sexual hormones and may modulate the risk of the disease [63, 74, 80]. Thus, vitamin D deficiency may be a risk modulating factor for the development of multiple sclerosis. Vitamin D acts as an immunomodulatory factor affecting T and B lymphocytes, and it may exert neuroprotector and neurotrophic actions within the central nervous system. Several studies have shown that vitamin D supplementation exerts multiple beneficial immunomodulatory effects in multiple sclerosis [121–124]. On the contrary, a Cochrane review states that there appears to be no benefit from vitamin D supplementation in patients with multiple sclerosis; however, the level of evidence is very low [125]. Nevertheless, it should be noted that robust statistical models used in association studies have already predicted a favorable vitamin D effect reducing relapses by 50–70% [121]. There is little doubt that vitamin D exerts a beneficial action on multiple sclerosis, the inflammatory component in particular, less so the degenerative. Until more information becomes available, vitamin D supplementation of multiple sclerosis patients, using a moderate physiological dose essentially correcting their vitamin insufficiency, is recommended.

3.5 Vitamin D and inflammatory bowel disease

Vitamin D deficiency has been observed in patients with inflammatory bowel disease, Crohn's disease, and ulcerative colitis [126]. It was found to be related to disease activity in Crohn's disease and ulcerative colitis. Vitamin D supports the integrity of the intestinal barrier and is related to microbiota homeostasis in this cohort of patients [127, 128]. Thus, vitamin D may contribute to the prevention of inflammatory bowel disease by supporting the integrity of the intestinal barrier, contributing to bacterial homeostasis and ameliorating disease progression via anti-inflammatory action. Vitamin D deficiency in inflammatory bowel disease is aggravated by decreased absorption of the vitamin via the gastrointestinal tract [128].

3.6 Vitamin D deficiency and autoimmune Hashimoto's thyroiditis

Studies have observed an association between autoimmune Hashimoto's thyroiditis and low vitamin D levels [79, 129]. These studies have not observed low vitamin D levels in patients with Graves' disease. A meta-analysis of 26 observational studies confirmed an association between vitamin D deficiency and autoimmune Hashimoto's thyroiditis [130]. The aforementioned meta-analysis found that although there was heterogeneity between the results of the various studies performed all over the globe, studies had similar results in populations from different countries and also in populations in different age ranges, in particular pediatric and adult populations.

3.7 Vitamin D deficiency and systemic sclerosis

Systemic sclerosis is a chronic, inflammatory, fibrotic disorder thought to be related to autoimmune etiology. Vitamin D deficiency has been observed in patients with systemic sclerosis [86, 131], especially in patients with the diffuse type of the disease [131].

4. Optimal levels of 25(OH)D_3

The molecule used to assess vitamin D sufficiency in a population is 25(OH)D_3 [9]. It appears that vitamin D has physiologic effects beyond those related to bone physiology and mineral homeostasis. It may be that the alarming prevalence of vitamin D deficiency observed all over the globe may be contributing to the development of autoimmune diseases. Based on bone-related biomarkers such as intact parathyroid hormone, calcium absorption, and bone mineral density, maintaining a 25(OH)D_3 level of at least 32 ng/ml appears sufficient.

5. Conclusions

It appears that vitamin D is a potent immunomodulator. It has multiple and diverse effects on the immune system. In particular, it potentiates the innate immune response enhancing the production of cathelicidin from human macrophages, monocytes, and keratinocytes, thus enhancing and potentiating the immune response against external pathogens. It affects the adaptive immune response shifting the phenotype of the adaptive immune response toward a more tolerogenic phenotype. Vitamin D deficiency is related to various autoimmune disorders. Vitamin D deficiency appears to be related to the development of RA and correlates with disease severity. Vitamin D deficiency is observed in patients with SLE. It was found to be related to disease severity and activity in some but not all studies. Vitamin D deficiency is observed in patients with multiple sclerosis, and vitamin D administration may ameliorate disease severity. Vitamin D deficiency is also observed in patients with inflammatory bowel disease, Crohn's disease, and ulcerative colitis, and it is related to disease activity. Vitamin D contributes to the integrity of the intestinal barrier and bacterial homeostasis. In addition, vitamin D absorption is decreased making supplementation important. Vitamin D deficiency is also observed in patients with autoimmune Hashimoto's thyroiditis. Vitamin D deficiency is found in patients with systemic sclerosis, especially the diffuse form of the disease. It appears that optimal levels of vitamin D are important for immune function and for the prevention of autoimmunity in the human organism.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 13

Vitamin D Deficiency in Renal Disease

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Abstract

Vitamin D deficiency is highly prevalent in patients with renal disease. The abnormal vitamin D (VD) metabolism in chronic kidney disease (CKD) is a key factor for developing CKD-related mineral bone disease (CKD-MBD), which directly influences the survival of the CKD patients. The importance of VD is perhaps of greater value due to its pleiotropic effects that span beyond calcium-phosphorus metabolism (cancer protection, diabetes prevention, and renal protection). The aim of our chapter is to depict the clinical implications of VD deficiency in the setting of CKD, including VD pleiotropy in renal disease, and to propose the most adequate treatment suggested in the literature.

Keywords: vitamin D deficiency, chronic kidney disease, mineral bone disease, vitamin D pleiotropy, vitamin D supplementation

1. Introduction

Vitamin D (VD) deficiency is a growing problem worldwide [1]. Due to the wide distribution of the vitamin D receptor in human body, the effect of VD spans beyond calcium-phosphorus and bone metabolism—VD deficiency is associated with higher prevalence of hypertension, diabetes mellitus, and neoplasia [2]. As kidneys play an important role in the metabolism of VD, patients with chronic kidney disease (CKD) are at increased risk for VD deficiency. The aim of our chapter is to demonstrate the clinical implications of vitamin D deficiency in CKD and to outline the possible treatment options in this group of patients. In our review, a stress is laid on clinical trials due to their greater relevance to everyday clinical practice, compared to *in vitro* and animal models.

2. Definition: vitamin D deficiency, vitamin D sufficiency, chronic kidney disease

VD status is being evaluated via the serum level of 25-hydroxyvitamin D (25VD)—the metabolite formed in the first hydroxylation in the liver, due to its longer half-life (approx. 3 weeks), compared to the active metabolite 1,25-dihydroxyvitamin D (1,25VD) (4–6 hours). The best options for 25VD serum level evaluation methods are high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). There are reports, discussing the use of free 25VD as indicator of VD status. However, in renal disease,

the ratio between free and total 25VD remained unchained; therefore, total 25VD is the indicator of VD status in CKD [3].

Generally, 25VD level has reverse association with parathyroid hormone (PTH) levels. In addition, higher 25VD is associated with higher calcium intestinal reabsorption. However, at 25VD ≥ 75 nmol/l, no reduction in PTH levels and no increase in calcium intestinal reabsorption occurs [4, 5]. Therefore, 25VD ≥ 75 nmol/l is regarded as a cut-off value for vitamin D sufficiency.

No generally accepted definition for VD deficiency exists, as authors choose cut-off value either serum 25VD of 25 nmol/L [6] or serum 25VD of 50 nmol/L [7]. However, target levels of 75 nmol/L are recommended, taking into consideration the clinical importance of mild VD insufficiency (25VD between 50 and 74.9 nmol/L) [7, 8].

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health [one of the following: estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m^2,presence of proteinuria, structural abnormalities of the kidney detected on imaging, pathological findings detected on kidney biopsy, urine sediment pathology, electrolyte abnormalities due to tubular disorders, history of kidney transplantation] [9]. CKD patients are at increased risk for VD deficiency; therefore, they require VD screening [7].

3. Vitamin D metabolism in health and renal disease

3.1 VD metabolism in healthy subjects

VD is synthesized predominantly endogenously (approx. to 90% of the total VD in human body). In the skin, the ultraviolet light transforms 7-dehydroxycholesterol (provitamin D) to pre-vitamin D, which under the influence of body temperature spontaneously isomerizes to cholecalciferol (vitamin D3). Approximately, 10% of total body VD is taken orally (vitamin D2, ergocalciferol, and cholecalciferol). VD is transported via VD-binding protein to the liver, where it is hydroxylated to 25VD. The next step in VD activation is hydroxylation of 25VD by the enzyme 1α-hydroxylase (CYP27B1) to 1,25VD, which is the active VD metabolite. The process occurs predominantly in the renal tubules. In addition, non-renal CYP27B1 was detected in skin (basal keratinocytes and hair follicles), lymph nodes (granulomata), colon (epithelial cells and parasympathetic ganglia), pancreas (islets), adrenal medulla, brain (cerebellum and cerebral cortex), prostate epithelial cells, and placenta (decidual and trophoblastic cells) [10], indicating the wider significance of the VD metabolites. Finally, 1,25VD is inactivated by the enzyme 24-hydroxylaze.

1,25VD exerts its effect via the vitamin D receptor (VDR), which is detected in all human organs. 1,25VD binds to VDR, the complex forms a heterodimer with the receptor for retinoid X (RXR) within the nucleus. The 1,25VD-VDR-RXR complex binds to vitamin D reacting elements, modulating gene expression. The highest expression of VDR is detected in bones, small intestines, and parathyroid gland. VDR activation leads to influencing bone metabolism), increase of calcium and phosphate absorption, and PTH secretion suppression. However, due to the wider VDR distribution in human body, its activation is associated with effects beyond influencing calcium-phosphorus metabolism–renin-angiotensin system (RAS) suppression and cardiac hypertrophy prevention (myocardial VDR), diabetes mellitus control (pancreatic VDR), and immune system modulation (VDR in the immune cells).

The above-mentioned stages of VD metabolism form the so called VD axis.

3.2 VD metabolism in CKD

All levels of VD metabolism are significantly affected in renal disease.

Cholecalciferol synthesis is reduced in uremic skin. In addition, these patients are usually older, with reduced exposure to sunlight, which is additionally associated with poorer skin VD synthesis. What is more, oral VD intake is decreased due to protein intake reduction.

25VD is reduced in CKD due to reduced amount of its precursor, increased loss in nephrotic patients, and 25VD sequestration in adipose tissue due to higher rate of obesity in CKD patients [11, 12].

Even during the initial stages of CKD (eGFR below 60 ml/min/1.73 m^2) due to reduced phosphate tubular excretion, higher levels of fibroblast growth factor 23 (FGF-23) occur, which suppress CYP27B1 activity. Furthermore, phosphatemia and metabolic acidosis decrease enzyme activity too. In addition, 1,25VD levels in renal disease are reduced because of increased catabolism due to increased FGF-23 levels. Finally, the smaller number of functioning tubules is directly associated with lower 1,25VD production in advanced CKD.

VDR is affected in CKD too. Low 1,25VD downregulates of VDR expression [13]. In areas of nodular growth in the parathyroid gland reduced VDR content is detected. In uremia, there is significant decrease in VDR-RXR binding to vitamin D reacting elements, as well as reduced RXR content in the parathyroid glands, explaining increased PTH levels without the presence of hypocalcaemia and hyperphosphatemia [14]. Hypocalcaemia in advanced CKD increases the parathyroid levels of calreticulin, a cytosolic protein that binds the DNA-binding domain of nuclear receptors, thus blocking VDR-mediated transactivation. Higher levels of inflammatory cytokines were found to be associated with impaired binding of VDR-RXR to vitamin D

VD axis	Healthy subjects	In CKD
Cholecalciferol/ergocalciferol	Skin synthesis from UV light or oral intake	• Reduced skin synthesis (age, uremia, reduced UV exposure) • Reduced oral intake
25-hydroxyvitamin D(25VD)	Hepatic synthesis (25-hydroxylase)	• Reduced amount of its precursor • Increased loss in nephrotic patients • 25VD sequestration in adipose tissue
1,25-dihydroxyvitamin D(1,25VD)	Hydroxylation in renal tubules (1α-hydroxylase) and other organs	• Increased catabolism • Suppressed 1α-hydroxylase activity • Reduced synthesis in renal tubules in advanced CKD
Vitamin D receptor(VDR)	Widely spread in human body, esp. bone and parathyroid glands (calcium-phosphorus metabolism) In all other organs (pleiotropy)	• Downregulated expression (low 1,25VD, hypocalcaemia) • Impaired binding to VDRE (uremia, inflammatory cytokines, etc.) • Reduced VDR content in parathyroid glands (hypertrophy)

CKD, chronic kidney disease; VDRE, vitamin D reacting elements; UV, ultraviolet.

Table 1.
Vitamin D axis in health and in renal disease.

reacting elements, contributing to vitamin D resistance in patients on hemodialysis [15]. Finally, hypocalcaemia in CKD suppresses the calcium-sensing receptor (CaSR) in the parathyroid glands, which in turn downregulates parathyroid VDR expression; use of calcimimetics upregulated VDR expression in rat models [16].

The VD axis in health and renal disease is depicted in **Table 1**.

4. Vitamin D deficiency: clinical implications in renal disease

The major mechanisms for abnormal VD metabolism and VD deficiency were outlined in the section above. Of particular importance is hyperphosphatemia, caused by initial renal damage, leading to higher FGF-23 levels, which in turn suppresses CYP27B1 activity and increases 1,25VD catabolism. Suboptimal VD levels are the basis of the abnormal calcium-phosphate metabolism in renal disease. Currently, the term CKD-associated mineral bone disorder (CKD-MBD) is used to define the wide spectrum of CKD-related abnormalities in calcium-phosphate metabolism, as their importance spans beyond bone health.

4.1 CKD-associated mineral bone disorder (CKD-MBD)

CKD-MBD comprises of three major aspects—biochemical abnormalities, bone changes, and vascular calcifications [17].

4.1.1 Biochemical abnormalities in CKD-MBD

The biochemical abnormalities in CKD-MBD represent the laboratory aspect of the disorder. These include calcium level (ionized or total), phosphate level, PTH, alkaline phosphatase (total or bone specific), and VD status (25VD). Other indicators, such as 1,25VD and FGF-23 are not measured routinely. The earliest changes occur in PTH and VD metabolites—the abnormal values for PTH, 25VD and 1,25VD are detected in eGFR<60 ml/min/1.73 m^2, whereas abnormal calcium and phosphate levels are detected in eGFR below 40 and remain stable until eGFR<20 ml/min/1.73 m^2 [18].

According to the current guidelines, testing for calcium, phosphate, PTH, and alkaline phosphatase should be initiated in eGFR<60 ml/min/1.73 m^2; the frequency of laboratory evaluation should be based on the rate of CKD progression, the magnitude of abnormalities, and the evaluation of treatment's effectivity. Similarly, 25VD should be tested in patients with eGFR<60 ml/min/1.73 m^2 and frequency of testing depends on baseline values and therapeutic interventions [17]. The timing and frequency suggested by Kidney Disease: Improving Global Outcomes (KDIGO) are summarized in **Table 2**.

4.1.2 Bone disorders in CKD-MBD

Bone involvement in CKD [renal osteodystrophy (ROD)] is of pivotal importance, as it is associated with bone fractures (asymptomatic or symptomatic), bleeding, chronic disability, poorer life quality, and higher mortality in renal disease. In children with CKD, it leads to growth retardation and skeletal deformities [17].

Several types of bone histological changes can be detected in CKD, according to three major histological indicators: turnover, mineralization, and volume. Bone turnover (T) is a parameter, corresponding to bone formation rate. It can be abnormally low, normal, or very high and is best assessed via bone biopsy and tetracycline

Indicator	CKD stage 3	CKD stage 4	CKD stage 5 and on dialysis (CKD 5D)
Calcium and phosphorus	6–12 months	3–6 months	1–3 months
PTH and alkaline phosphatase	Baseline	6–12 months	3–6 months
25-Hydroxyvitamin D	Baseline	Baseline	Baseline

CKD, chronic kidney disease; PTH, parathyroid hormone.

Table 2.
Suggested testing for biochemical indicators of CKD-MBD according to CKD stage.

labeling. Mineralization (M) is the second parameter. Normally, the osteoblasts lay down new collagen and direct mineralization of the matrix. This process is impaired in CKD, leading to thickened osteoid. Mineralization is measured by osteoid maturation time and mineralization lag time. The osteoid maturation time is the osteoid width divided by the distance between labels per day. The mineralization lag time is the osteoid maturation time adjusted for the percentage of osteoid surface that has a tetracycline label. Mineralization is classified as normal and abnormal. Bone volume (V) sums up bone formation and resorption rates. It is generally accepted that bone volume is expressed as bone volume per tissue volume and is classified as low, normal, and high bone volume.

According to the TMV classification of bone histology in CKD, the following ROD categories are recognized [19]:

1. Adynamic bone disease (AD)—low-turnover bone disease with normal mineralization. Volume can be low, but in some patients with normal mineralization and low turnover, it will be normal. AD is usually associated with PTH oversuppression, including overdose of VD analogs or calcitriol

2. Mild secondary hyperparathyroidism related bone disease (MHPTBD)—medium-to high bone turnover, any bone volume, normal mineralization

3. Osteitis fibrosa (OF)—represents a more advanced form of high-turnover disease, compared to MHPT, any bone volume and normal mineralization

4. Osteomalacia (OM)—low-turnover bone with abnormal mineralization. The bone volume may be low to medium, depending on the severity and duration of the process and other factors that affect bone health

5. Mixed uremic osteodystrophy (MUO)—represents features of the above mentioned variants; for example, a combination of high-turnover, normal bone volume, with abnormal mineralization

In addition, age related/postmenopausal osteoporosis can be detected. Measurement of bone mineral density [by using dual-energy X-ray absorptiometry (DXA)] is most informative in CKD stages 1–2; in these cases, low BMD is associated with osteoporosis and treatment is performed as in the general population. Patients with low BMD and CKD stages 3–5 are designated as having CKD-MBD with low BMD. Recent reports demonstrate that BMD testing can predict fracture risk in eGFR<60 ml/min/1.73 m^2. The current KDIGO guidelines broaden the indications for BMD testing in CKD stages 3–5D to assess fracture risk, if it will have effect on treatment. Finally, normal histology is also possible [17, 20].

Clinical presentation	HTMBD	LTMBD
	Arthralgia	Arthralgia
	Bone pain	Bone pain
	Calciphylaxis	Calciphylaxis
	Bone fractures	Bone fractures
	Muscle weakness, spasms, tetany, paresthesia, convulsions (hypocalcaemia)	Aluminum toxicity—anemia, dementia
	Vomiting, nausea, hypertension (hypercalcemia)	Vomiting, nausea, hypertension (hypercalcemia)
	Pruritus	Pruritus
	Myalgia	
Laboratory	**HTMBD**	**LTMBD**
Serum calcium	N in early stages ↓/N/↑ in advanced HTMBD	Early stages N/↑ Advanced stages—↑↑
Serum phosphate	N in early stages N to very high in advanced stages	Early stages N/↓ Advanced—↓/↑
BAP	N in early stages ↑ in advanced HTMBD	Early stages N/↓ Advanced—↓
PTH	N/↑ in early stages ↑↑↑ in advanced HTMBD	Early stages N/↓ Advanced stages ↓
Radiology	**HTMBD**	**LTMBD**
	Subperiosteal erosions—hands, clavicles, and pelvis	Fractures
	Vertebral osteosclerosis	Looser zones
	Brown tumors	Bone deformities
	Extraskeletal calcifications	Osteopenia and osteoporosis
Histology	HTMBD	LTMBD
	OF, MHPTBD	AD, OM

PTH, parathyroid hormone; BAP, bone specific alkaline phosphatase; N, normal; ↓, decreased; ↑, increased values; OF, osteitis fibrosa; MHPTBD, mild secondary hyperparathyroidism related bone disease; AD, adynamic bone disease; OM, osteomalacia.

Table 3.
Clinical presentation, laboratory, radiologic and histologic findings in low turnover mineral bone disease (LTMBD) and high turnover mineral bone disease (HTMBD).

It should be noted that histological findings differ according to renal replacement type. In patients on hemodialysis OF and MUO are the most common findings, in peritoneal dialysis–AD is detected in up to 50%, whereas in patients in CKD stages 3–5 not on dialysis the most common findings are OF and MUO. However, in the latter group, the highest percentage of normal histology is detected [20].

Bone biopsy is regarded as the golden standard for the precise diagnosis of the bone changes in CKD-MBD. Indications for bone biopsy are bone fractures, bone pain, unexplained hypercalcemia/hypophosphatemia, evaluation of the type of bone turnover (which may lead to treatment correction), and suspected aluminum toxicity. Planning antiresorptive treatment in eGFR<30 ml/min/1.73 m^2 is currently not an indication for bone biopsy, as no evidence exists, linking bisphosphonate use to higher AD prevalence in CKD [17]. The most widely recognized disadvantages

of the procedure are pain, laborious procedure, time-consuming, and expensive histological evaluation, as well as insufficient histopathological expertise [21].

These limitations restrict the wide use of bone biopsy. Therefore, markedly elevated PTH and bone-specific alkaline phosphatase can be used in clinical practice to predict bone turnover in CKD-MBD. Thus, two types of mineral bone diseases are defined—high turnover mineral bone disease (HTMBD) and low turnover mineral bone disease (LTMBD). In cases, in which clinical and laboratory data are inconclusive of the type of bone turnover, bone biopsy should be considered [17]. The clinical, laboratory, radiological, and histological characteristics of the both entities are summarized in **Table 3**.

4.1.3 Vascular calcification in CKD-MBD

Vascular calcification (VC) is deposition of calcium phosphate in vascular tissues. It presents with calcification of arterial media, intima, valves, and rarely with calcific uremic arteriolopathy (calciphylaxis). Normally, this occurs with aging. However, the process is accelerated in CKD and leads to increased mortality and morbidity. Initially, it was regarded as a finding in patients with end-stage renal disease (ESRD), but currently, it is detected in early CKD stages in adults and in children with ESRD, thus depicting a more complicated picture [22].

Patients with VC are regarded as having the highest risk for cardiovascular events. The diagnosis is based on abdominal lateral radiograph (vascular calcifications), echocardiogram (valvular calcifications), or computer tomography [17].

The association of VC with VD status in CKD is not well defined as contradicting reports exist. Two report demonstrate, that lower serum 1,25VD is associated with increased risk for and demonstrated that 25VD has no association with VC [23, 24]. As arterial stiffening is regarded as related pathology to VC in CKD, it should be noted that two recent interventional studies demonstrated improvement of endothelial dysfunction in CKD patients after cholecalciferol supplementation [25–27]. Higher serum levels of 1,25VD and high doses of VD supplementation increased the risk for VC [23, 28], thus indicating the dual role of VD in vascular health.

Finally, the type of VD treatment may be important too. Generally, vitamin D analogs (VDA) demonstrate similar effectivity in reduction of PTH levels to calcitriol, with lower toxicity—lower rates of hypercalcemia and hyperphosphatemia, thus suggesting lower risk for supplementation-enhanced VC. Unfortunately, more trails are needed to evaluate the effect of different supplementation types on VC prevalence. In addition, new VDA are being evaluated in the treatment of CKD-MBD, with more expressed cardiac protection and less hypercalcemia and hyperphosphatemia than paricalcitol [29–31].

4.2 VD pleiotropy in renal disease

As mentioned above, the VDR and the enzyme CYP27B1 have wider distribution in the body and are being expressed in organs not involved in calcium-phosphate metabolism. This indicates a greater physiological importance of the VD axis, spanning beyond skeletal physiology. These extraskeletal properties are designated as pleiotropic effects of VD. In this sub-section, the current knowledge of VD pleiotropy in CKD patients will be presented.

4.2.1 VD pleiotropy in CKD: proteinuria and CKD progression

Poorer VD status was associated with higher proteinuria and faster progression of CKD. In addition, VDR activation was found to slow CKD progression in immune and non-immune renal diseases.

In IgA nephropathy, human studies associated poorer VD status with poorer clinical outcomes [32]. In addition, calcitriol supplementation suppressed proteinuria in IgA patients by activating the VDR and thus influencing cytokine and leukotriene metabolism [33].

Recent studies demonstrated the importance of VD axis in systemic lupus erythematosus (SLE) and lupus nephritis (LN). Poorer VD status correlated with higher SLE activity, whereas poorer VDR expression in renal tissue was linked to higher renal activity and more severe renal lesions in patients with LN [34, 35]. Podocyte autophagy is a key factor in renal involvement in LN. A recent study showed that poorer VD status correlated with higher podocyte autophagy activity and VD effectively suppressed it in LN, thus protecting podocytes from antibody-mediated injury [36]. VD and VDR affect autophagy via different mechanisms: activating calcium-dependent intracellular kinases, downregulating mTOR expression, or upregulation of the cyclin-dependent kinase inhibitor *p19INK4D* [37].

However, VD has renoprotective effect in non-immune glomerular diseases too. In diabetic nephropathy (DN), both animal models and clinical trials demonstrate the negative correlation between low VD and the risk for DN and DN progression, as well as the beneficial effect of VD/VD analogs in reducing albuminuria, renal fibrosis, thus retarding disease progression [38, 39]. The effect can be explained with RAS suppression, suppression of inflammatory mediators [nuclear factor-kappa B (NF-κB), transforming growth factor-β(TGF-β)], suppressing the Wnt/β-catenin pathway, which is involved in epithelial to mesenchymal cell transition (EMT) in high glucose milieu, as well as upregulation of nephrin expression [39–41].

The mechanisms mentioned in DN (suppression of RAS, inflammation, EMT) are the basis of renal protection of VD in other renal diseases–in animal models and human clinical trials [33]. Additionally, an inverse correlation between VD status and proteinuria and blood pressure control in autosomal polycystic kidney disease (ADPKD) was reported, as well as reduction of proteinuria and hypertension on treatment with VDR agonist in experimental PKD. However, the findings are to be evaluated prospectively in interventional study in patients with ADPKD [42].

4.2.2 VD pleiotropy in CKD: autoimmunity, inflammation, and infection

The presence of VDR and CYP27B1 has been well recognized in the immune cells, which modulate their differentiation and proliferation. VD suppresses B-cell proliferation, modulates T-helper proliferation, favoring the T-helper type 2 subtype, thus suppressing inflammatory cytokine synthesis (IL17, IL21) and stimulating the production of anti-inflammatory ones (IL10). In addition, VD enhances the production of cathelicidin and β-defensin 2, as well as influences autophagy and apoptosis [33, 43]. All these properties of VD and VDR demonstrate the importance of VD axis in protective and pathological immunity.

4.2.2.1 VD and autoimmunity/inflammation

The role of VD in LN has been already discussed. Other studies, evaluating the VD–SLE association demonstrate that poorer VD is related to higher SLE activity in adults and children [44]. Different supplementation regimens also demonstrated improvement in inflammatory markers, disease activity indices, decrease in T-helpers types 1 and 17 [44]. However, despite the different papers, reporting beneficial effect from VD, a recent study demonstrated increase of SLE activity after exposing patients to UV radiation, despite improvement in VD status [45]. Thus, the SLE-VD correlation still remains to be clarified.

The rheumatoid arthritis (RA) and the inflammatory bowel disease (IBD) are diseases that influence kidney health by causing AA amyloidosis, which in turn progresses to ESRD. VD status was inversely associated with RA disease activity and RA-associated complications [46]. However, despite some reports, indicating beneficial effect of VD supplementation on T-helper 17 function, the results for VD supplementation currently are inconsistent [46, 47].

Several studies demonstrated lower VD status in patients with more aggressive IBD. However, the findings may be attributed to lower absorption of VD due to the active intestinal inflammation, especially in Crohn's disease [48, 49]. Interventional studies also demonstrated the beneficial effect of VD supplementation in suppressing pro-inflammatory markers in IBD. Yet, the importance of VD in IBD remains to be clarified [50, 51].

4.2.2.2 VD and infection

Infection is a well-recognized leading cause for death in CKD patients, especially those on dialysis. It was already mentioned that the VD axis plays a role in immunity by enhancing cathelicidin production. In dialysis patients, low cathelicidin levels were detected, which were associated with higher risk for infection and also modest correlation to 1,25VD levels was discovered [52]. Interventional studies also demonstrated the decreased risk for infection in dialysis patients. VD supplementation reduced significantly the risk for hospitalization due to acute respiratory infections in hemodialysis (HD) patients; in another study, VD supplementation decreased the risk for peritonitis in patients on peritoneal dialysis (PD) [53, 54]. A more recent study also demonstrated decrease in infection rates in dialysis patients treated with VDR analogs [55]. In contrast to these findings, Yildirim et al. failed to demonstrate a significant relation between VD status and inflammatory markers in CKD patients [56].

4.2.3 VD pleiotropy in CKD: neoplasia

An already mentioned paper demonstrated reduced malignancy-associated mortality in VDA-treated dialysis patients. However, the study did not evaluate the specific localization of the neoplasia, nor its histology [55]. Therefore, in this subsection, the most common malignant diseases will be reviewed and their association with VD. Unfortunately, the exact association between VD and neoplasia in CKD patients is not well evaluated, as CKD patients are usually excluded from large trials and registries [57].

4.2.3.1 Breast cancer

The reports evaluating the association between VD deficiency and breast cancer are inconsistent. The inverse association between breast cancer and VD, detected in several studies was not supported by more recent prospective studies. In addition, a controversy exists about the beneficial effect of VD supplementation on breast cancer risk and survival [44]. Unfortunately, the cited studies did not demonstrate data for CKD patients.

4.2.3.2 Colorectal cancer

Several studies demonstrate an inverse correlation between colorectal cancer and 25VD levels [58, 59]. The findings do not correspond to the results of a larger

study, detecting no significant association between colorectal cancer and VD status [60]. Unfortunately, the data from interventional trails with VD supplementation also have conflicting result for the effect of VD on colorectal cancer prevention and survival improvement [44, 61]. Similarly to breast cancer, the data for the association between colorectal cancer and VD status in renal patients is limited.

4.2.3.3 Other neoplasms

The results about the influence of VD on the risk for other malignancies are controversial. A large study (n = 70,563) evaluated the association of 25VD levels on the risk for prostate cancer, breast cancer, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer, and neuroblastoma. 25VD concentrations did not correspond to the risk for any of the mentioned neoplasias. Therefore, the authors do not support the regular VD screening as an attempt for cancer prevention [62]. In addition, a large multicenter study in patients on hemodialysis also did not demonstrate significant association between total cancer prevalence and VD deficiency [63].

Multiple myeloma (MM) is a plasma cell disease that often presents with kidney manifestations. Several studies reported high rates of VD deficiency in MM patients and specific alleles for VDR were associated with higher risk for MM [64, 65]. Lauter and Schmidt-Wolf established that lower VD levels were associated with more expressed plasma cell infiltration of the bone marrow. In this study, VD supplementation improved anemia, led to higher white blood cell and lower platelet count [66]. Yet, the risk for hypercalcemia should be taken into consideration when VD supplementation is applied in MM patients.

4.2.4 VD pleiotropy in CKD: diabetes mellitus, cardiovascular disease (CVD)

4.2.4.1 VD and diabetes mellitus

Basic science studies demonstrated improvement in glucose metabolism due to immunomodulatory effect (especially in diabetes type 1), improvement of β cell function by increase in insulin secretion, and decreasing insulin resistance by stimulating insulin receptor expression via activating the VDR. Other mechanisms such as RAS suppression and anti-inflammatory effect of VD were also taken into consideration. However, observational studies do not establish clear relationship between VD status and DM prevalence, as some studies support the association, whereas others do not [44]. Interventional trials also have conflicting results. Two recent prospective studies showed improvement in glucose metabolism after vitamin D supplementation was applied [67, 68]. Unfortunately, the studies did not evaluate patients with renal disease. Two interventional studies with paricalcitol failed to detect improvement in insulin resistance in patients CKD stages 3 and 4 and in dialysis [69, 70].

4.2.4.2 VD and cardiovascular disease (CVD)

CVD is a leading cause for mortality in CKD. As VDR and 27CYPB1 are expressed in the cells of the cardiovascular system, it could be expected that the VD axis can play a beneficial role in cardio-vascular health. A possible cardioprotection can be explained with RAS suppression and anti-inflammatory effects; VDR activation can improve cardiac muscle cells contractility and modulate their proliferation and growth. In addition, VD can improve peripheral vascular health by affecting endothelial and smooth muscular cells too [71].

Even though the association between VD deficiency and CVD has been recognized in the general population and in CKD, large randomized interventional studies failed to demonstrate improvement from VD supplementation in cardiovascular outcomes, cardiac structure, or function in CKD patients [72]. An improvement in blood pressure control and endothelial function (as measured by brachial artery flow-mediated dilatation) was observed after VD supplementation in the general population; however, in CKD patients, no significant antihypertensive effect was detected [73]. Similarly, the results for the effect on VD supplementation on arterial stiffness and endothelial function are conflicting [72, 73]. Therefore, the use of VD in renal disease in order to improve cardiovascular events is currently not recommended [72].

4.2.5 VD pleiotropy in CKD: muscle health, cognitive function, peritoneal fibrosis, and anemia

4.2.5.1 Muscle health and cognitive function

In hemodialysis (HD) patients, a positive relationship between muscle strength and VD levels was detected, with optimal handgrip strength in 25VD above 30 ng/ml (75 nmol/L). In ESRD patients, suboptimal VD was associated with lower quadriceps mass and increased risk for falls [74]. In addition, in PD patients, low 25VD was found to be the independent factor for global cognitive impairment due to the antioxidant and neuroprotective role of 1,25VD. This is of crucial importance, as PD is a home-based renal replacement therapy that needs adequate cognition and self-monitoring [75].

4.2.5.2 Anemia and peritoneal fibrosis

Treatment with VDA led to the improvement of anemia in ESRD patients. A possible mechanism is suppression of inflammation and direct stimulation of erythropoiesis. In addition, paricalcitol reduced PD-associated thickening of the peritoneum and prevented peritoneal fibrosis in animal models. However, recent study in PD patients did not detect any benefit from paricalcitol supplementation in preventing peritoneal remodeling. In contrast to these results, Kerschbaum et al. demonstrated protective effect of oral active VD against peritonitis in PD patients [33, 54, 76].

5. Vitamin D deficiency after kidney transplantation

Suboptimal 25VD is a commonly detected problem after kidney transplantation (KT) with prevalence above 80% of the kidney transplant recipients (KTRs). As KTRs are CKD patients, all mentioned factors, predisposing to impaired VD metabolism are valid for this cohort of patients, especially considering the fact that more than 50% of the KTRs have GFR<60 ml/min/1.73 m^2. In addition, transplant-specific factors influence VD synthesis: sun exposure avoidance due to the increased risk for skin malignancies, use of sun-protecting cosmetics (limiting further UV exposure), proteinuria after KT (increased loss in urine), higher prevalence of obesity after KT (reduced bioavailability), the presence of new onset diabetes after transplantation (NODAT) (by reducing intestinal absorption), use of steroids (increased 1,25VD catabolism), and calcineurin inhibitors (impaired 25VD liver synthesis) [77, 78]. Similarly to CKD in native kidneys, the VD-associated clinical implications after KT are categorized as post-transplant mineral bone disorder (PTMBD) and VD pleiotropy.

5.1 Post-transplant mineral bone disorder

Similarly to native kidneys, PTMBD consists of three aspects—biochemical abnormalities, bone involvement, vascular pathology and is associated with higher risk for fractures and death.

5.1.1 PTMBD: biochemical abnormalities

Significant changes occur in the biochemical indicators of calcium-phosphorus metabolism after KT. In the early post-transplant period, the fluctuations in the parameters are more pronounced as the graft function is rapidly changing. Due to the presence of functioning renal tubules and still high levels of PTH and FGF-23, hypophosphatemia and mild hypercalcemia are common. These changes usually normalize within months after KT as graft function stabilizes. Therefore, the current KDIGO guidelines recommend at least weekly testing for calcium and phosphorus immediately after KT [17]. FGF-23 and PTH rapidly decrease; however, PTH may be significantly elevated years after successful KT due to parathyroid cell hypertrophy. VD levels are low in the early post-transplant period; yet, suboptimal levels are very common later after KT [78].

In the late post-transplant period, current guidelines recommend the testing for calcium, phosphorus, PTH, and alkaline phosphatase to be performed according to the magnitude of the abnormalities, rate of progression of post-transplant CKD, and the presence of medical treatment. Practically, the timing is similar to patients with CKD stage 3–5 with native kidneys (see **Table** 2). 25VD should be tested at baseline and repeated testing should be performed according to the initial level and the presence of medical interventions. In our center, 25VD levels are monitored twice annually, taking into consideration its seasonal variations in the general population. Thus, a significant deterioration of VD status in the winter/fall was detected, allowing adequate seasonal supplementation to be initiated.

5.1.2 PTMBD: bone disease

Post-transplant bone disease is commonly observed after KT and encompasses ROD, osteoporosis, bone fractures, and osteonecrosis. Deterioration in BMD occurs mainly during the first 12 months, though BMD loss persists at lower rates after the first post-transplant year. The etiology is multifactorial: pre-existing CKD-MBD, duration of dialysis and transplantation, poor graft function, hypogonadism, higher rates of diabetes after KT, suboptimal VD levels, and use of immunosuppressive agents. Of these, steroids are of particular importance as their cumulative and mean dose is associated with decreased bone formation and bone density. Some reports indicate that calcineurin inhibitors can rise PTH levels and increase the risk of osteoporosis, but the findings are not uniformly accepted [17, 79].

In KTRs, not only fracture prevalence is significantly increased compared to the general population, but also fracture-associated complications, including mortality, are more common in the post-transplant setting. Major fracture sites are the hip (usually osteoporosis-associated) and the ankle/foot (atypical for osteoporosis), which demonstrate that both ROD and osteoporosis play an important role in bone fragility [80].

The use of DXA–BMD measurement is getting more attention in native CKD, as a growing body of evidence demonstrates that it can predict fracture risk across CKD categories. To date, only one retrospective study, total hip DXA measurement after KT was associated with increased fracture risk. In addition, a recent prospective study had similar findings [81, 82]. Despite the insufficient evidence for KTRs,

KDIGO suggests the use of BMD testing in all stages of post-transplant CKD in KTRs with high risk for osteoporosis, if the measurement will have effect on treatment. Bone biopsy can be considered to guide treatment [17].

5.1.3 PTMBD: vascular calcifications

VC is common after KT and is usually associated with pre-transplant uremia. In addition, most studies are semi-quantitative, thus making post-transplant VC progression assessment difficult. However, there are studies demonstrating a stop in progression or even improvement in VC in KTRs [83, 84]. Recognized risk factors for VC after KT are statin use, low 25VD levels, male sex, older age, and higher phosphate levels [85]. The data for the effect of immunosuppressive agents are conflicting. Mycophenolates proved to have protective effects against calcification, especially compared to steroids and calcineurin inhibitors; rapamycin suppressed smooth muscle cell proliferation, whereas everolimus impaired the vasoactive and antithrombotic function of the endothelium [86]. Therefore, more studies are needed in order to evaluate the effect of KT on VC.

5.2 VD pleiotropy after kidney transplantation

The graft survival at the tenth year after KT is significantly lower than the survival during the first 12 months. The explanation for these unsatisfactory results is poorer patient survival due to neoplasia, CVD, NODAT, calcineurin toxicity. It could be hypothesized that VD can improve graft and patient survival due to its pleiotropy. However, the trials in KTRs are small in number and in size, thus further research in this sphere is warranted.

5.2.1 VD pleiotropy after KT: proteinuria and renal protection

Observational studies linked poor VD status to poorer graft outcomes [86]. Our results also demonstrated that higher VD is associated with lower post-transplant proteinuria [87]. However, interventional studies did not fully support the VD–graft function association. Cholecalciferol supplementation failed to demonstrate renoprotection in prospective study [88]. However, in a recent prospective placebo-controlled study, paricalcitol ameliorated proteinuria in KTRs [89].

5.2.2 VD pleiotropy after KT: rejection

Observational studies demonstrated higher rates of acute rejection in VD deficient KTRs [90]. Unfortunately, interventional studies did not find protective role of cholecalciferol supplementation on rejection prevalence [88]. Therefore, the role of VD in rejection prevention after KT is not fully understood and is under debate.

5.2.3 VD pleiotropy after KT: infection

Infection is a major cause for death after KT. Several recent reports established negative correlation between VD status and infection risk, especially for cytomegalovirus and BK virus infections in KTRs [91, 92]. However, our observational study showed no association between VD status and prevalence of urinary tract infections after KT [93]. Furthermore, the VITA-D study did not establish positive effect of cholecalciferol supplementation on infection risk [88]. A probable explanation for the discrepancies in the studies are the different types of infection evaluated (e.g. in the VITA-D study the total infection risk was assessed). Probably, a more specific

approach should be chosen and the infection risk for specific etiological agent and its association to VD should be analyzed.

5.2.4 VD pleiotropy after KT: malignancies

Despite the anti-neoplastic properties of VD *in vitro* and in animal models, the evidence for anti-malignancy effect of VD in CKD patients and KTRs is insufficient. Observational studies report conflicting results for the association between post-transplant malignancies and VD status [86]. A recent study in our transplant center showed that VD-deficient KTRs had higher prevalence of non-cutaneous cancers [94]. A single center study established beneficial effect from active VD supplementation on post-transplant neoplasia rates [95]. The larger prospective, multicenter, double-blind, randomized, controlled study VITALE is currently being performed, which will evaluate the effect of cholecalciferol supplementation on malignancy risk after KT [96].

5.2.5 VD pleiotropy after KT: NODAT

The major risk factors for NODAT are use of steroids and calcineurin inhibitors. The data for VD-NODAT association are insufficient. The trial VITALE is to assess the effect of high and low doses cholecalciferol in VD–insufficient KTRs on NODAT prevalence [96].

5.2.6 VD pleiotropy after KT: cardiovascular disease

Marchal et al. established a significant association between lower 25VD and vascular calcification after KT [85]. However, a more recent study did not find a relationship between VD status and post-transplant hypertension and major CV events [97]. One of the aims of the already mentioned trial VITALE is to assess the effect of cholecalciferol supplementation on blood pressure control and CVD rates in KTRs [96].

6. Treatment of vitamin D deficiency in renal disease

6.1 Treatment with cholecalciferol, ergocalciferol, calcitriol, VDA

Supplementation with cholecalciferol (vitamin D3), ergocalciferol (vitamin D2), or treatment with the active vitamin D calcitriol or VDA suppresses PTH in secondary hyperparathyroidism in CKD. As a first step, a correction of hypocalcemia, hyperphosphatemia, and suboptimal 25VD levels should be performed. In more advanced CKD-related secondary hyperparathyroidism calcitriol and VDA can be initiated. It should be noted that over-suppression of the parathyroid gland due to overdose of the VD treatment is a major cause for AD. Therefore PTH, as well as serum phosphate and calcium, should be regularly monitored. It should be noted that the optimal PTH values for dialysis patients are two times up to nine times the upper normal limit, whereas for patients not on dialysis the optimal range is not established. If trend for lowering/rising values is present, changes in treatment should be changed so that the negative trends be reverted [17]. Similarly, in KTRs cholecalciferol/calcitriol/VDA treatment should also take these trends into consideration.

6.1.1 Supplementation with cholecalciferol/ergocalciferol

In the general adult population, supplementation doses of VD at least 600 IU daily; however, if improvement in VD status is needed, doses of at least

1500–2000 IU per day should be prescribed. The maximal dose VD without medical supervision should be 4000 IU daily. Cholecalciferol and ergocalciferol are equally effective [98]. The KDIGO guidelines recommend VD deficiency in CKD patients and in KTRs to be treated as in the general population. It is the initial therapeutic step, together with correction of hypocalcemia and hyperphosphatemia, as it effectively suppresses PTH in CKD stages 3–5 not on dialysis. More aggressive approach is to be avoided as modest PTH elevation is regarded as adaptive mechanism in declining GFR [17].

6.1.2 Treatment with calcitriol and VDA

Calcitriol is naturally existing 1,25VD, whereas VDA are synthetic derivatives of vitamin D2 (paricalcitol, doxercalciferol) and vitamin D3 (alfacalcidol, falecalcitriol, 22-oxacalcitriol). Despite the existing trials demonstrating positive effect of calcitriol/VDA on biochemical abnormalities, more recent studies do not demonstrate improvement in patient-centered end-points, such as left ventricular mass index and heart function; however, hypercalcemia was observed. Taking into account that modest PTH elevation is a possible adaptive response in CKD, the lack of significant clinical effects and higher risk for hypercalcemia, the use of calcitriol and VDA is not routinely recommended in CKD stages 3–5 not on dialysis. Their use is advocated in cases of severe and progressive secondary hyperparathyroidism and eGFR below 30 ml/min/1.73 m^2 or dialysis patients [17].

The data for hypercalcemia rates in calcitriol and VDA are conflicting. Zand et al. demonstrated lower hypercalcemia prevalence in patients treated with paricalcitol; other reports established no difference in hypercalcemia between calcitriol and paricalcitol [29, 99].

6.1.3 Other therapeutic measures

Other measures, recommended by KDIGO that optimize VD treatment in CKD-MBD are avoidance of hypercalcemia, reduction of phosphate serum levels, including phosphate dietary restriction, limitation of the use of calcium-based phosphate binders, calcium dialysate concentrations within the range of 1.25 and 1.50 mmol/l [17].

6.1.4 Novel agents

6.1.4.1 Calcifediol

Calcifediol is an oral 25-hydroxyvitamin D3. A study demonstrated reduction in PTH levels without changes in phosphate, calcium, and FGF-23 levels in CKD patients. In this chapter, patient-related outcomes were not assessed [100]. Further trials are needed to clarify the use of calcifediol in renal disease.

6.1.4.2 Vitamin K

Vitamin K plays a crucial role in vascular health. It serves as a cofactor for γ-glutamyl carboxylation, which converts glutamate into γ-carboxyglutamate (Gla). In vitamin K insufficiency, which is common in CKD, higher levels of desphosphorylated-uncarboxylated matrix Gla-protein (MGP) are established, which are associated with VC, as MGP serves as calcification inhibitor. Vitamin K supplementation increases MGP carboxylation. What is more, VD supplementation upregulates MGP synthesis, whereas vitamin K suppressed 1,25VD-associated calcinosis [101, 102].

Therefore, it can be hypothesized that vitamin K supplementation in CKD can be an adjuvant treatment to VD supplementation and will decrease its adverse effects. Further research of the efficacy and safety of vitamin K supplementation in CKD is needed.

7. Conclusion

VD deficiency in renal patients has been a burning issue in nephrology for many years. Yet, many questions remain unanswered. Of particular interest are the effect on VD treatment on clinical outcomes, especially death and cardiovascular events; VD-associated adverse events in CKD; VD pleiotropy in renal disease (randomized controlled prospective interventional studies are needed); the use of novel therapeutic agents should be further evaluated (vitamin K, new VDA, calcifediol). In addition, new biomarkers, evaluating bone health in CKD and new techniques, evaluating BMD and fracture risk may guide VD treatment more accurately. Therefore, new diagnostic and therapeutic strategies can be expected in the future.

Conflict of interest

The authors declare no conflict of interest.

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Vitamin D is the topic for many discussions in the scientific community. Nowadays, a different interpretation of this secosteroid hormone is needed. Today the term "vitamin" may be considered outdated. This compound may be correctly be called a vitamin only when it is administered to humans or animals that suffer from its deficiency. This book attempts to clarify the role of Vitamin D deficiency in many pathological processes in the whole organism. Chapters in this book cover such issues as the earliest clinical and preclinical investigations of the consequences of Vitamin D deficiency for cognitive, cardiovascular, metabolic, immune, and renal disorders.

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