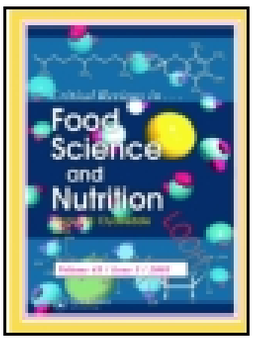

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REVIEW

Functional effects of vitamin D: From nutrient to immunomodulator

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ABSTRACT

Vitamin D can be obtained from the endogenous synthesis in the epidermis by exposure to UVB light, and from foods and supplements in the form of ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). The main metabolite used to measure vitamin D serum status is calcidiol [25(OH)D]. However, its active metabolite calcitriol [1α,25$(OH)_2$D] performs pleiotropic effects in the cardiovascular, neurological, and adipose tissue as well as immune cells. Calcitriol exerts its effects through genomic mechanisms modulated by the nuclear vitamin D receptor (VDR)/retinoid X receptor (RXR) complex, to bind to vitamin D response elements (VDRE) in target genes of several cells such as activated T and B lymphocytes, neutrophils, macrophages, and dendritic cells; besides of its genomic mechanisms, VDR performs novel non-genomic mechanisms that involve its membrane expression and soluble form; highlighting that vitamin D could be an immunomodulatory nutrient that plays a key role during physiological and pathological events. Therefore, the aim of this comprehensive literature review was to describe the most relevant findings of vitamin D dietary sources, absorption, synthesis, metabolism, and factors that influence its serum status, signaling pathways, and biological effects of this immunonutrient in the health and disease.

KEYWORDS

Calcidiol; calcitriol; absorption; metabolism; immunonutrient; VDR

Introduction

Several scientific studies have reported that vitamin D is more than a simple nutrient acquired from food; it is a nutrient that can act as a hormone synthesized endogenously, various biological effects are attributed it, including immunomodulatory effects about inflammation and exacerbated immune responses (Carlberg 2019; Chang and Lee 2019; Dankers et al. 2016).

The non-hydroxylated vitamin D metabolites ergocalciferol (vitamin D_2) obtained from vegetable food, and cholecalciferol (vitamin D_3) from animal foods, and its synthesis in the epidermis, circulate in the blood mainly bound to the vitamin D binding protein (VDBP), for its transport to the liver (Carlberg 2019; Chang and Lee 2019).

In liver, a first hydroxylation generates the synthesis of the primary circulating metabolite in serum, the calcidiol [25(OH)D], which is transported by VDBP to the kidney or other extrarenal sites, where the biologically active metabolite of vitamin D, the calcitriol or 1α,25-dihydroxyvitamin D [1α,25 $(OH)_2$ D_2 or 1α,25 $(OH)_2$ D_3] can also be synthesized (Carlberg 2019).

Calcitriol exert a plethora of pleiotropic genomic actions, through its binding to the vitamin D receptor (VDR) expressed in different organs, tissues, and cells such as the colon, osteoblasts, brain, heart, skin, prostate, breasts, and different immune cells (Carlberg 2019; Haussler et al. 2011). Likewise, non-genomic actions or rapid responses are linked to different signaling pathways modulated by the membrane vitamin D receptor (mVDR) expression, and the serum soluble VDR (sVDR) form have also been described in physiological and pathological conditions (Carlberg 2019; Hii and Ferrante 2016).

Due to its biological actions, vitamin D (calcidiol) serum deficiency has been described associated with an increased risk of different diseases that involve systems or organs where it exerts important actions, such as rickets, skin diseases, viral respiratory infections, cancer, cardiovascular, and autoimmune diseases (Charoenngam and Holick 2020). The calcidiol serum deficiency could be influenced by endogenous and exogenous factors such as vitamin D acquired from diet, diet composition, use of drugs that affect its absorption, limited sunlight exposition, use of sunscreen, latitude, obesity, color skin, as well as the presence of specific pathologies or genetic variations such as mutations and genetic polymorphisms (Borel, Caillaud, and Cano 2015; Chang and Lee 2019; Charoenngam and Holick 2020; Pilz et al. 2018).

Outstanding to the variety of literature available about this immunomodulatory vitamin, the present comprehensive literature review describes the most relevant findings for a better compression about its dietary sources, absorption,

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Table 1. Vitamin D metabolites.

	Vitamin D_2	Vitamin D_3	Generic denomination
Precursor	Ergosterol or pro-vitamin D_2	7-dehydrocholesterol or, pro-vitamin D_3	Pro-vitamin D
Isomer by irradiation	Pre vitamin D_2	Pre vitamin D_3	Pre-vitamin D
Isomer by thermoregulation	Vitamin D_2 or, ergocalciferol	Vitamin D_3 or, cholecalciferol	Vitamin D
Major form in serum circulation	25 (OH) D_2 or, 25-hydroxyvitamin D_2 or, 25-hydroxiergocalciferol	25 (OH) D_3 or, 25-hydroxyvitamin D_3 or, 25-hydroxycholecalciferol	25 (OH) D or 25-hydroxyvitamin D or calcidiol
Active form	1α,25 $(OH)_2$ D_2 or, 1α,25-dihydroxyvitamin D_2 or, 1 alpha,25-dihydroxyiergocalciferol	1α,25 $(OH)_2$ D_3 or, 1α,25-dihydroxyvitamin D_3 or, 1 alpha,25-dihydroxycholecalciferol	1,25 $(OH)_2$ D or 1,25-dihydroxyvitamin D or calcitriol

(Bikle, Adams and Christakos 2012; Masvidal Aliberch et al. 2012; Pierides 1981).

synthesis, metabolism, and factors that influence the vitamin D serum state, signaling pathways, and its biological effects of this important immunonutrient in physiological and pathological conditions.

Material and methods

A comprehensive literature search was performed in the following databases and search engines: PubMed, Europe PubMed Central, and Scielo. The most current and relevant information for each topic was included in this review. The following keywords were used to obtain information on the topics and subtopics: "vitamin D," "dietary vitamin D," "calcidiol," "calcitriol," "vitamin D AND foods," "vitamin D references," "vitamin D supplementation," "vitamin D AND food interactions," "vitamin D AND drugs interactions," "vitamin D absorption," "vitamin D metabolism," "vitamin D serum levels," "vitamin D functions," "vitamin D AND autoimmune diseases," "vitamin D deficiency AND autoimmune diseases," "genomic actions of vitamin D," "genomic pathway AND vitamin D," "non genomic actions AND vitamin D," "vitamin D receptor," "VDR" and "serum OR soluble VDR." Likewise, the methodology and the quality of the articles were carefully reviewed, and a complementary bibliography of each selected article to find more relevant information.

Dietary sources of vitamin D

Vitamin D is a fat-soluble compound, of which there are three significant molecules: the first is the vitamin D_2 (ergocalciferol), derived from ergosterol (pro-vitamin D_2) found in vegetable source foods. In animal source foods, the second molecule is presented in the form of vitamin D_3 (cholecalciferol), also synthesized from the 7-dihydrocholesterol (provitamin D_3) in mammalian skin exposed to ultraviolet B (UVB) radiation. Both (ergocalciferol and cholecalciferol) present the first hydroxylation in the liver, to generate the main metabolite in the blood, called calcidiol.

The third molecule is derived from dihydrotachysterol, a synthetic vitamin D analog, metabolized in the liver to generate the 25-hydroxytachysterol, which is the analog of calcitriol, and it does not undergo further hydroxylation by the kidney (Bikle, Adams, and Christakos 2012; Masvidal Aliberch et al. 2012; Pierides 1981) (Table 1).

Food sources of vitamin D

Vitamin D can be obtained 20% of diet (Pilz et al. 2018). The ergocalciferol found in vegetable sources, is synthesized from ergosterol by UVB radiation and could be found in yeast, sun-dried and ultraviolet irradiated mushrooms (Carlberg 2019; Chang and Lee 2019; Charoenngam and Holick 2020; Ovesen, Brot, and Jakobsen 2003; Schneider et al. 2014). The animal source is the cholecalciferol, found in fatty fish such as tuna (*Thunnus sp.*), sardines (*Sardina pilchardus*), salmon (*Salmo salar*), herring (*Clupea sp.*), and mackerel (*Scomber scombrus*). Moreover, cholecalciferol is found in cod liver, offal, cheese, and egg yolks. In order to cover the daily dietary reference intakes (DRI) to vitamin D, in several countries various foods are fortified with cholecalciferol or ergocalciferol, such as milk, orange juice, breakfast cereals, and margarine (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015; Borel, Caillaud, and Cano 2015; Carlberg 2019; Michael F. Holick 2007) (Table 2).

Notably, all animal food sources of cholecalciferol also contain calcidiol in varied low amounts, milk and fish contents $<0.1\ \mu g/100\ g$ of calcidiol, but it is higher in meat and offal (0.2-0.4 $\mu g/100\ g$), and egg yolk (up to $1\ \mu g/100\ g$). Calcitriol and 24,25-dihydroxycholecalciferol [24,25$(OH)_2$D], are also contained in animal products, but trace amounts, and could contribute very little to the biological vitamin D activity (Ovesen, Brot, and Jakobsen 2003).

Vitamin D supplements and requirements

In supplements, vitamin D can be available in the form of ergocalciferol synthesized by the UVB irradiation of ergosterol in yeast, and cholecalciferol by irradiation of 7-dehydrocholesterol from lanolin, and the chemical conversion of cholesterol (Borel, Caillaud, and Cano 2015). In the human population, the daily DRI of vitamin D could vary depending on disease presence and environmental factors such as the latitude at which the person lives.

The Institute of Medicine (IOM) has established the estimated average requirements (EARs) and the recommended dietary allowances (RDAs) of dietary vitamin D for all life stages; except for infants, where are reported adequate intakes (AIs). The AI for infants of 0-12 months is 400 IU/

Table 2. Dietary sources of vitamin D.

Food	Standard portion size	Vitamin D in Standard portion (μg)	Vitamin D per 100 grams (μg)
Dairy products			
Yogurt (various types)	8 oz	2.0-3.0	0.9-1.3
Milk (non-fat, 1% and 2%)	1 c	2.9	1.2
Whole milk	1 c	3.2	1.3
Cow cream[a]	1 tbsp	0.12	0.8
Cheese [a]	1 tbsp	0.35	2.7
Ricotta cheese[a]	1 tbsp	0.02	0.2
Mozzarella Cheese[a]	1 tbsp	0.04	0.4
Seafood			
Whitefish	3 oz	10.9	12.8
Tuna, canned	3 oz	5.7	6.7
Salmon	3 oz	11.1-14.5	13.1-17.1
Sardines	3 oz	4.1	4.8
Others			
Eggs	1 pc	1.1	2.2
Pork meat	3 oz	0.2-2.2	0.2-2.6
Mushrooms	1/2 c	1.4-1.7	5.1-5.3
Portobello mushrooms exposed to UVB light	1/2 c	7.9	13.1
Fortified food			
Breakfast cereals	1/3-1 1/4 c	0.2-2.5	0.8-8.6
Orange juice	1 c	2.5	1.0
Almond drink	1 c	2.4	1.0
Margarine	1 tbsp	1.5	10.7
Soy drink	1 c	2.9	1.2

1 μg is equivalent to 40 IU. oz: ounce. c: cup. tbsp: tablespoon. pc: piece (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015; Holick 2007).

day (10 μg/day); for children and adults of all age ranges, the EAR is 400 IU/day (10 μg/day), while the RDA is 600 IU/day (15 μg/day), except for adults older than 70 years old, where the RDA is 800 IU/day (20 μg/day) (Chang and Lee 2019; Charoenngam and Holick 2020; Holick et al. 2011; IOM (Institute of Medicine) 2011) (Table 3).

In exclusively breastfed infants, elderly persons, persons with insufficient sunlight exposition, patients with fat malabsorption, and pregnant women, a higher vitamin D intake is necessary (Borel, Caillaud, and Cano 2015). The American Geriatrics Society (AGS) and the National Osteoporosis Foundation (NOF), suggest an even higher dosage 800-1000 IU/day (20-25 μg/day) for adults >65 years old, to prevent falls and fractures (Chang and Lee 2019).

For women during pregnancy or lactation, the RDA from the IOM is 600 IU/day (15 µg/day), but for non-Caucasian women living at higher latitudes or pregnant during winter, this supplementation could increase to 1000 IU/day (25 μg/day) (Chang and Lee 2019), highlighting the role of ethnicity in the adequacy of the daily DRIs of each population.

Breast milk is the ideal food exclusively for the first six months of life of the newborn that provides several benefits. However, the breast milk contains minimal vitamin D (approximately 20–60 IU/L), that is not sufficient to maintain an optimal vitamin D serum levels in the infant, if exposure to sunlight is limited (Mulligan et al. 2010).

Due to vitamin D stores acquired from the mother are depleted by approximately eight weeks of age in breastfed infants (Mulligan et al. 2010), could be recommended supplementation of 400 IU/day (10 μg/day) of cholecalciferol, and in the presence of obesity or chronic medications, requirements maybe 2-4 fold more (Chang and Lee 2019).

Vitamin D intoxication generally occurs after inappropriate supplementation. High doses of vitamin D $\geq$ 50000 IU/day (1250 μg/day) increase the calcidiol serum levels above 100-150 ng/mL, and is mostly associated with hypercalcemia and hyperphosphatemia (Chang and Lee 2019; Holick 2007). The vitamin D intoxication could generate symptoms such as confusion, polydipsia, polyuria, anorexia, vomiting, muscle weakness, and a chronic intoxication may lead to nephrocalcinosis, bony demineralization, and even pain (Chang and Lee 2019). Nevertheless, vitamin D toxicity has not been reported in adults chronically consuming up to 10000 IU/day of cholecalciferol (Pelajo, Lopez-Benitez, and Miller 2010).

The tolerable upper intake levels (UL) of vitamin D recommended for the IOM, are for infants up to 6 months 1000 IU/day (25 μg/day), for infants of 6-12 months are 1500 IU (38 μg/day), for children of 1-3 years old are 2500 IU (63 μg/day); for children of 4-8 years old are 3000 IU (75 μg/day), and for children of 9-18 years old, adults from 18 years old, pregnancy and lactation are 4000 IU (100 μg/day) (Chang and Lee 2019; IOM (Institute of Medicine) 2011). The recommended vitamin D supplementation by the Endocrine Society Guidelines on Vitamin D for treatment and prevention for vitamin D deficiencies varies according to age group, and certain special or pathologic conditions (Charoenngam and Holick 2020) (Table 3).

Dietary vitamin D absorption

Dietary vitamin D intestinal absorption

Once dietary forms of vitamin D have been ingested are de-esterified due to its fat-soluble nature, before its absorption in the small intestine (Hollis et al. 1996; IOM (Institute of Medicine) 2011; Weber 1981). The dietary vitamin D absorption is carried out by emulsification and solubilization in mixed micelles for diffusion and permeabilization through the enterocyte's membrane. First, in the stomach, where the

Table 3. Vitamin D intake in life stages, individuals at risk or with vitamin D deficiency recommended by the IOM and the Endocrine Society.

	IOM recommendations			For Individuals at risk for vitamin D deficiency		
Life stage	AI/EAR	RDA	UL	Daily DRI	UL	Treatment for patients with Vitamin D deficiency
Infants						
0-6 months	AI: 400 IU (10 μg)	–	1000 IU (25 μg)	400-1000 IU (10-25 μg)	2000 IU (50 μg)	2000 IU/d or 50000 IU/wk for at least 6 wk to achieve serum calcidiol >30 ng/mL maintenance therapy of 400-1000 IU/d.
6-12 months		–	1500 IU (38 μg)			
Children and adults						
1-3 years	EAR: 400 IU (10 μg)	600 IU (15 μg)	2500 IU (63 μg)	600-1000 IU (15-25 μg)	4000 IU (100 μg)	2000 IU/d or 50000 IU/wk for at least 6 wk to achieve serum calcidiol >30 ng/mL maintenance therapy of 600-1000 IU/d.
4-8 years			3000 IU (75 μg)			
9-18 years			4000 IU (100 μg)			
>18-70 years	EAR: 400 IU (10 μg)	600 IU (15 μg)	4000 IU (100 μg)	1500-2000 IU (37.5-50 μg)	10000 IU (250 μg)	6000 IU/d or 50,000 IU/wk for at least 6 wk to achieve serum calcidiol >30 ng/mL maintenance therapy of 1500-2000 IU/d.
>70 years		800 IU (20 μg)				
Pregnancy and lactation						
14-18 years	EAR: 400 IU (10 μg)	600 IU (15 μg)	4000 IU (100 μg)	600-1000 IU (15-25 μg)	4000 IU (100 μg)	–
19-50 years				1500-2000 IU (37.5-50 μg)	10000 IU (250 μg)	–
Obesity and malabsorption	–	–	–	4000-6000 IU (100-150 μg)	10000 IU (250 μg)	Dosage should be increased by 2-3 folds.

AI, Adequate intake; EAR, estimated average requirement; RDA, recommended dietary allowances; UL, tolerable upper intake level; DRI, dietary reference intakes. Calcidiol >30 ng/mL= 75 nmol/L. Treatment with D_2 (ergocalciferol) or D_3 (cholecalciferol); d: day; wk: week. (Charoenngam and Holick 2020; Holick et al. 2011; IOM (Institute of Medicine) 2011).

dietary vitamin D is not sensitive to the acidic pH, the pepsin may contribute to release the fraction of the dietary vitamin D associated with proteins, and the gastric lipase may partially hydrolyze the esters of vitamin D in order to be absorbable (Borel, Caillaud, and Cano 2015; Hollis et al. 1996) (Figure 1b).

In the small intestine, the dietary vitamin D absorption process requires the presence of fat in the intestinal lumen, which triggers the release of bile acids and pancreatic lipase (Hollis et al. 1996; IOM (Institute of Medicine) 2011; Weber 1981). Bile acids are essential for the normal absorption of fats, and contribute to the initiation of emulsification of lipids and support the formation of lipid-containing micelles, while pancreatic lipase hydrolyzes the triglycerides into monoglycerides and free fatty acids (Blomstrand and Forsgren 1967; Hollis et al. 1996; IOM (Institute of Medicine) 2011; G. R. Thompson, Lewis, and Booth 1966). In the duodenum, digestive enzymes, such as proteases, amylases, and lipases, continue to release the dietary vitamin D from the food matrix; vitamin D esters are hydrolyzed by bile salt stimulated lipase or carboxyl ester lipase (Borel, Caillaud, and Cano 2015; Lombardo and Guy 1980) (Figure 1c). Within the enterocyte in the intestinal wall, vitamin D and others lipids are packaged together into chylomicrons, and this is the main pathway of vitamin D that reaches the systemic circulation through the lymphatic pathway (Jones 2008; IOM (Institute of Medicine) 2011; Borel, Caillaud, and Cano 2015; Rautureau and Rambaud 1981) (Figure 1d).

The absorption efficiency of calcidiol and calcitriol by the enterocyte is around three-fold than ergocalciferol and cholecalciferol (Borel, Caillaud, and Cano 2015; Compston et al. 1981). Calcidiol is more polar than cholecalciferol and less dependent on bile acids, being partially water-soluble, and mainly absorbed via the portal vein (Borel, Caillaud, and Cano 2015; Maislos, Silver, and Fainaru 1981) while calcitriol absorption in the enterocyte is unaffected by the lack of biliary salts (Borel, Caillaud, and Cano 2015; Maislos and Shany 1987) (Figure 1e).

The main site of dietary vitamin D absorption in the small intestine in humans is not accurately known, but in the rat have been described that is the jejunum and ileum, mainly the latter (Borel, Caillaud, and Cano 2015; Hollander, Muralidhara, and Zimmerman 1978; Reboul and Borel 2011). Studies in human intestinal cell line Caco-2 and transfected HEK cells have shown that intestinal cell membrane receptors, such as SR-BI (Scavenger Receptor Class B type 1) or CD36 (Cluster Determinant 36) and NPC1L1 (Niemann-Pick C1-Like 1) are involved in the uptake of the non-hydroxylated vitamin D forms (cholecalciferol and ergocalciferol), and others fat-soluble nutrients at the apical side of the enterocyte (Borel, Caillaud, and Cano 2015; Reboul and Borel 2011) (Figure 1c).

Once in the circulation, vitamin D anchored to the chylomicrons are metabolized in peripheral tissues that express the enzyme lipoprotein lipase (LPL), mainly in adipose tissue and skeletal muscle, which are tissues rich in LPL. After lipolysis, the chylomicron remnants still contain a fraction of vitamin D bioavailable (Jones 2008; IOM (Institute of Medicine) 2011), which could remain in the bloodstream for its subsequent metabolism. Besides, a little fraction of recently absorbed vitamin D from the intestine is transported in the circulation with amino acids and carbohydrates into the portal system to reach the liver (Jones 2008; IOM (Institute of Medicine) 2011).

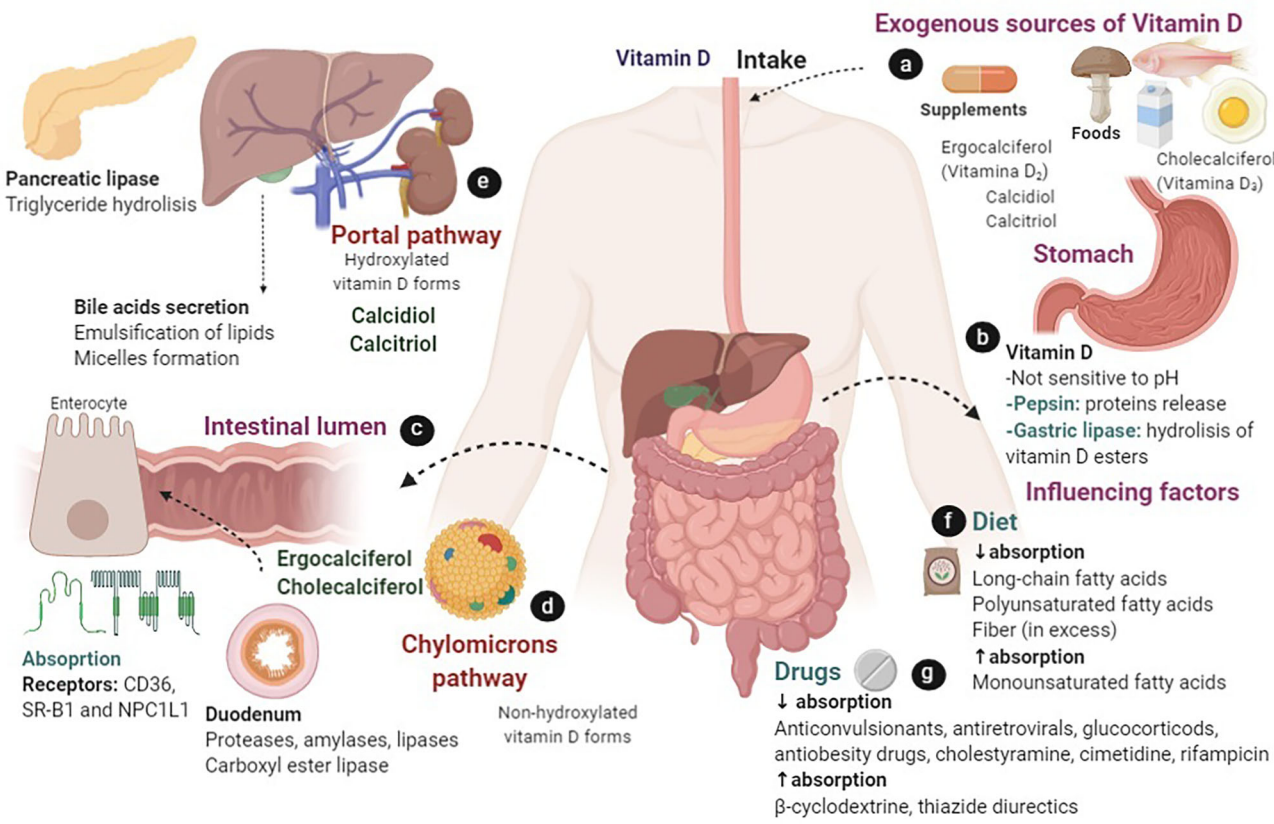

Figure 1. Vitamin D absorption. a) Exogenous sources of vitamin D: vitamin D can be obtained from the diet or supplements in form of ergocalciferol, cholecalciferol, calcidiol and calcitriol; b) Vitamin D digestion in stomach: pepsin may contribute to vitamin D dissociation from proteins, and the gastric lipase may partially hydrolyze the esters of vitamin D to be absorbable; c) Vitamin D intestinal absorption: in the duodenum, proteases, amylases and lipases enzymes, continue to release vitamin D from the food matrix; intestinal cell membrane receptors, such as SR-BI, CD36 and NPC1L1 are involved in the uptake of cholecalciferol and ergocalciferol by the enterocyte; d) Chylomicrons pathway: vitamin D is packaged into chylomicrons to reach the circulation through the lymphatic pathway; e) Portal pathway: vitamin D absorbed from the intestine is transported in the circulation with amino acids and carbohydrates into the portal system to reach the liver; f) Diet: long-chain fatty acids, a high polyunsaturated fatty acids diet, and an excess of dietary fiber may decrease the vitamin D absorption and bioavailability, conversely a high monounsaturated fatty acids diet may increases its absorption and bioavailability; g) Drugs: several drugs might reduce vitamin D absorption. **SR-BI:** Scavenger Receptor class B type 1; **CD36:** Cluster Determinant 36 scavenger receptor; **NPC1L1:** Niemann-Pick C1-Like 1.

Bio-availabilities of dietary vitamin D metabolites and supplements

Difference between ergocalciferol and cholecalciferol bio-availabilities

Both forms (ergocalciferol and cholecalciferol) are absorbed with the same efficiency, but metabolic and biological activities of the hydroxylated metabolites generated from these are different (Borel, Caillaud, and Cano 2015) (Table 1). Has been reported that tissue level, there are differences in the way that ergocalciferol and cholecalciferol binds to the VDBP protein, and their metabolism due to the differences in the chemistry of their side chains (Armas, Hollis, and Heaney 2004; Bikle 2009; Masvidal Aliberch et al. 2012; Romagnoli et al. 2008), these differences are minor in the biologically active compounds generated from them, appear to be comparable at least concerning binding to VDR (Bikle 2009).

Single doses of ergocalciferol lead to lower calcidiol serum levels than single doses of cholecalciferol (Armas, Hollis, and Heaney 2004; Bikle 2009; Romagnoli et al. 2008), although daily administration of both metabolites maintains comparable calcidiol serum levels (Bikle 2009; Holick et al. 2008). Ergocalciferol and cholecalciferol increase the calcidiol serum levels in parallel, when these are recently administered, but as the days of their administration pass (14 and 28 days), ergocalciferol potency is less than one third that of cholecalciferol (Armas, Hollis, and Heaney 2004; Borel, Caillaud, and Cano 2015; Pelajo, Lopez-Benitez, and Miller 2010).

These differences could be not attributed to their first hydroxylation in the liver, because similar increases in calcidiol serum levels were observed after intravenous injection of both forms of vitamin D (Borel, Caillaud, and Cano 2015). Likewise, in other study after 60 days the cholecalciferol was almost twice more potent than ergocalciferol in increasing the calcidiol serum levels, due to a more rapid metabolism or clearance of ergocalciferol than cholecalciferol metabolites (Borel, Caillaud, and Cano 2015; Romagnoli et al. 2008).

Difference between calcidiol versus cholecalciferol and ergocalciferol bio-availabilities

The absorption and biological activity of calcidiol are better than cholecalciferol and ergocalciferol (Borel, Caillaud, and Cano 2015; Ovesen, Brot, and Jakobsen 2003). For an equivalent serum increase of calcidiol, a 10-fold higher amount of ergocalciferol or cholecalciferol is required than calcidiol (Borel, Caillaud, and Cano 2015; Stamp, Haddad,

and Twigg 1977). These differences could be explained by the absorption of cholecalciferol and ergocalciferol occurs via chylomicrons, while calcidiol absorption is by the portal pathway, and could happen that a fraction of absorbed cholecalciferol and ergocalciferol is quickly stored in adipose tissue (Borel, Caillaud, and Cano 2015; Jones 2008; IOM (Institute of Medicine) 2011).

Dietary vitamin D food sources or supplements?

Vitamin D needs to be extracted from its food sources or supplement matrix to become available for absorption. In different studies where compared the bioavailability of vitamin D between UV-B-irradiated button mushrooms *vs.* an ergocalciferol supplement (Outila et al. 1999), cholecalciferol fortified wheat, and rye bread *vs.* cholecalciferol supplement (Natri et al. 2006), have been reported that food matrix has no marked effect on vitamin D bioavailability. However, the supplement matrix effect has not yet been clarified (Borel, Caillaud, and Cano 2015). Moreover, studies performed in dairy products, such as fortified milk or cheeses, have been reported that these foods do not significantly affect the vitamin D bioavailability (Borel, Caillaud, and Cano 2015).

Interactions between vitamin D metabolites with other nutrients and pharmacological drugs

Lipids acquired from the diet and their influence in vitamin D absorption

Some characteristics of dietary lipids could influence the vitamin D absorption, such as the amounts, species of phospholipids and lipids emulsification. The amount of fat ingested has been described that it has not a significant effect on vitamin D bioavailability. The long-chain fatty acids are incorporated in micelles; this causes enlargement of the micelle size, and this slows their diffusion toward the enterocyte and hinders vitamin D absorption (Borel, Caillaud, and Cano 2015; Hollander, Muralidhara, and Zimmerman 1978) (Figure 1f). A clinical study reported that a high monounsaturated fatty acids diet might improve, and a high polyunsaturated fatty acids diet may reduce the effectiveness of cholecalciferol supplements in healthy older adults. However, more studies are needed to elucidate these facts (Borel, Caillaud, and Cano 2015; Niramitmahapanya, Harris, and Dawson-Hughes 2011) (Figure 1f).

Dietary fiber

Some dietary fiber sources can decrease the activity of pancreatic lipase and affect the fat-soluble nutrients absorption, the micelle formation, and its emulsification increases the viscosity of the chyme, and limits the diffusion of fat-soluble nutrients contained in the micelles to the brush border from enterocytes (Borel, Caillaud, and Cano 2015; Pasquier et al. 1996). However, there is little data to conclude about the fiber's effect on the bioavailability of vitamin D (Borel, Caillaud, and Cano 2015) (Figure 1f).

Drugs

Some anticonvulsants or antiretroviral agents (e.g., highly active antiretroviral therapy; HAART) can precipitate vitamin D deficiency by enhancing catabolism of calcidiol and calcitriol, while the antifungal drug Ketoconazole may further block 1α-hydroxylation (Chang and Lee 2019; Charoenngam and Holick 2020). A high-dose of glucocorticoids administration require more vitamin D, due to inhibition of intestinal vitamin D-dependent calcium absorption (Chang and Lee 2019; Charoenngam and Holick 2020), and the same is required for other drugs that activate steroid and xenobiotic receptor (Holick 2007) (Figure 1g).

Some anti-obesity drugs that diminish the absorption of triglycerides and cholesterol, such as the tetrahydrolipstatin (orlistat), a non-absorbed inhibitor of gastric and pancreatic lipases, and food additives as the olestra, a sucrose polyester used as a fat substitute, can also impair vitamin D absorption (Borel, Caillaud, and Cano 2015; Charoenngam and Holick 2020; Goncalves et al. 2011). Phytosterols, such as β-sitosterol used to diminish cholesterol absorption, compete with the cholecalciferol for its incorporation into mixed micelles, as well as for apical enterocyte uptake (Borel, Caillaud, and Cano 2015; Goncalves et al. 2011) (Figure 1g).

Other agents described hat can influence vitamin D levels are the rifampicin, plants like the St. John's Wort (*Hypericum perforatum*), drugs with lipid-lowering effects such as the cholestyramine (Charoenngam and Holick 2020), antituberculosis agents, theophylline, and cimetidine (Pelajo, Lopez-Benitez, and Miller 2010); while the β-cyclodextrin has been described that generates a 2.5-fold increase in vitamin D absorption, compared with vitamin D alone (Borel, Caillaud, and Cano 2015). Moreover, the thiazide diuretics may increase the calcidiol serum levels (Pelajo, Lopez-Benitez, and Miller 2010) (Figure 1g).

Endogenous vitamin D synthesis

Synthesis in the epidermis by exposure to UVB light

Vitamin D in the form of cholecalciferol is 80% synthesized endogenously in the keratinocytes (Pilz et al. 2018), where a photolytic cleavage occurs for the conversion of 15% of endogenous 7-dihydrocholesterol to pre-vitamin D_3, after exposure to UVB photons at a wavelength 290 to 315 nm (Bover et al. 2015; Chang and Lee 2019; Schneider et al. 2014). A sunlight exposition from 5 to 30 minutes can synthesize about 75 μg of cholecalciferol; however, this will depend on the time of day, season, latitude, and sensitivity of the exposed skin (Holick 2007; Wacker and Holick 2013) (Figure 2a).

The UVB radiation energy is used in a non-enzymatic reaction to open the β-ring of the 7-dehydrocholesterol, creating the thermodynamically unstable molecule, the pre-vitamin D_3 (Carlberg 2019). Subsequently, UVB light generates activation of the double bonds of pre-vitamin D_3 formed, and by thermal isomerization, the cholecalciferol is generated (Figure 2b), and passes into the bloodstream to binds to VDBP for its transport (Bover et al. 2015; Charoenngam

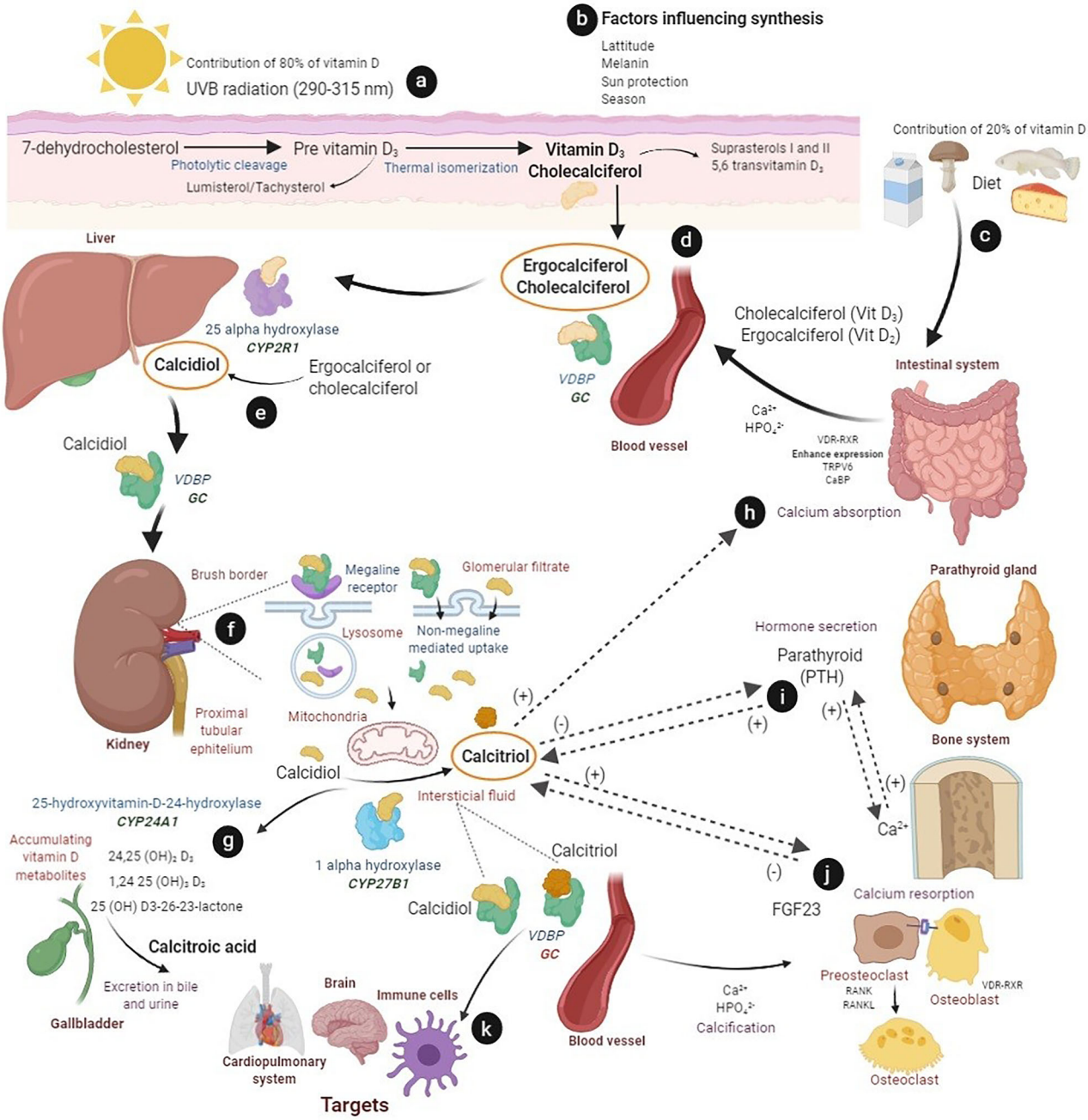

Figure 2. Metabolism of vitamin D. a) Endogenous synthesis: vitamin D is 80% synthesized in the keratinocytes, after exposure to UVB light (290 to 315 nm), to convert the 7-dihydrocholesterol to pre-vitamin D_3, and by thermal isomerization, cholecalciferol is generated; b) Factors influencing synthesis: melanin in the skin, extent of clothing, and topical sunscreen may generate less dermal vitamin D synthesis; c) Dietary sources**:** vitamin D can be obtained 20% from diet, cholecalciferol, also calcidiol and calcitriol in trace amounts are found in animal sources such as fatty fish, cod liver, offal, cheese and egg yolks, while ergocalciferol is found in yeast, sun-dried and ultraviolet irradiated mushrooms; d) Blood transport**:** ergocalciferol and cholecalciferol from diet or the cholecalciferol from endogenous synthesis passes into the bloodstream and binds to the VDBP for its transport; e) First hydroxylation**:** in the liver, ergocalciferol or cholecalciferol is metabolized by CYP2R1 to calcidiol, into circulation, calcidiol- VDBP is transported to the kidney; f) Second hydroxylation: calcidiol-VDBP complex is filtered through the glomerulus and endocytosed into the renal tubular epithelial cell, via megalin-mediated endocytosis or non-megaline-mediated uptake. In mitochondria, calcidiol is metabolized to the active form called calcitriol by CYP27B1 or is secreted directly to the interstitial fluid. Calcitriol can also be synthesized in other tissues where the CYP27B1 is expressed; g) Generation and excretion of others metabolites: stimulation of CYP24A1 produce inactive forms of vitamin D, such as 25(OH)D_3-26,23-lactone, 24,25$(OH)_2D_3$ from calcidiol, 12,425$(OH)_3D_3$ from calcitriol, and finally calcitroic acid form is excreted in bile or urine; h) Intestinal calcium absorption**:** via VDR, calcitriol enhances intestinal calcium absorption in the small intestine, through the enhance the expression of the epithelial calcium channel TRPV6 and calbindin 9 K (CaBP); i) Parathyroid gland**:** when calcium plasma level decreases, PTH stimulates the production of calcitriol from calcidiol in kidneys. Calcitriol inhibits the PTH production and decreases bone resorption, increasing the urinary calcium excretion and FGF23 production by the osteocytes, leading to increased urinary phosphate excretion; j) Bone system*:* on the bone, calcitriol induces the expression of osteocalcin, and in osteoblasts via VDR, increase the expression of RANKL, that induces the bone mineralization; k) Others vitamin D targets: calcitriol can exert genomic actions others targets that express VDR such as cardiopulmonary system, brain, and immune cells. **Vitamin D_2:** ergocalciferol; **Vitamin D_3:** cholecalciferol; **UVB:** ultraviolet B; **VDBP:** vitamin D-binding protein; **24,25$(OH)_2D_3$:** 24,25 dihydroxycholecalciferol; **12,425$(OH)_3D_3$:**1,24,25-trihydroxyvitamin D; **VDR:** vitamin D receptor; **TRPV6:** transient receptor potential cation channel, subfamily V, member 6; **CaBP:** calcium-binding protein; **PTH:** parathyroid hormone; **FGF23:** fibroblast growth factor 23; **RANKL:** receptor activator of nuclear factor-κB ligand; **RANK:** receptor activator of nuclear factor-κB; **Ca^{2+}:** calcium; **HPO_4^{2-}:** phosphorus. ***GC***, ***CYP2R1***, ***CYP27B1***, and ***CYP24A1*** are the respective genes that encode the different proteins/enzymes involved in vitamin D metabolism.

and Holick 2020; Schneider et al. 2014; Wacker and Holick 2013) (Figure 2d).

Toxic serum levels of cholecalciferol do not occur from prolonged sun exposure, because the thermal activation of pre-vitamin D_3 in the skin gives rise to multiple inert products, such as lumisterol and tachysterol (Holick, MacLaughlin, and Doppelt 1981; Masvidal Aliberch et al. 2012; IOM (Institute of Medicine) 2011; Webb, DeCosta, and Holick 1989), which formation is reversible and can be converted back to pre-vitamin D_3, if pre-vitamin D_3 levels fall (Bikle 2009). Besides, the vitamin D_3 photoisomerizes like suprasterols I/II and 5,6 trans-vitamin D_3 (Bikle 2009; Masvidal Aliberch et al. 2012), and melanin in the epidermis absorbs the UVB radiation limiting an excessive D_3 production (Bikle 2009) (Figure 2a).

Factors influencing vitamin D dermal synthesis

Skin color

Approximately one million years ago, human skin became highly pigmented, when modern humans' ancestors lost most of their body hair, to better sweat during physical endurance activity, such as hunting. The importance of endogenous cholecalciferol synthesis sparked a process of human evolution: their skin turned pale again, when some 50000 years ago, modern humans, such as Neanderthals, modern Europeans, and Asians migrated from Africa to Asia and Europe, due to the need for cholecalciferol synthesis in geographic regions with lower levels of UVB radiation (Carlberg 2019) (Figure 2b).

UVB radiation is more prevalent in the hours of 10 a.m. to 3 p.m. and during spring, summer, and autumn, 10-15 minutes of sun exposure (over arms and face, or arms and legs/hands) at this time can produce adequate cholecalciferol in light-skinned populations, while a more exposure is needed for the epidermal melanin of darker-skinned individuals. For example, persons from the Indian subcontinent require three-folds as much sun exposure, and Africans six to ten-folds more than Caucasians (Chang and Lee 2019; Charoenngam and Holick 2020). Notably, the tuberculosis rate of dark skin individuals living further away from the equator is higher than that of light-skinned people (Carlberg 2019).

Sun protection

Cutaneous vitamin D synthesis depends on the surface of skin exposed and sun exposure duration. The extent of clothing and using topical sunscreen may block an effective dermal synthesis. A sunblock of 30 SPF can reduce vitamin D production by 95% (Charoenngam and Holick 2020) (Figure 2b).

Environmental factors

The intensity of UVB radiation from sunlight varies according to season and latitude (Bikle 2009). The amount of UVB is higher at greater altitudes and sunny areas, while beyond a latitude of 33° can receive little UVB due to the angle and longer path of sunlight through the atmosphere, and in turn, the air pollution and cloud-shading may further limit sun exposure (Chang and Lee 2019) (Figure 2b).

Vitamin D metabolism

Metabolic pathway

After entering to the circulation, vitamin D acquired from the diet (ergocalciferol and cholecalciferol) or by the synthesis in the epidermis (cholecalciferol) (Figure 2a, c, and d) is transported by the VDBP to the liver, where is metabolized by the enzyme vitamin D-25-hydroxylase (CYP2R1) to calcidiol (Figure 2e), calcidiol passes into circulation and together with VDBP is transported to the kidney (Carlberg 2019; Chang and Lee 2019; Charoenngam and Holick 2020; Pierrot-Deseilligny and Souberbielle 2017).

The calcidiol-VDBP complex is filtered through the glomerulus and endocytosed into the renal tubular epithelial cell, via megalin-mediated endocytosis or non-megaline-mediated uptake. The complex is broken down inside the lysosomal compartment, and the calcitriol and VDBP are releases into the cytosol (White and Cooke 2000). In the mitochondria, calcidiol is metabolized to the active form calcitriol by hydroxylation at carbon 1 by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1), or it is either secreted directly to the interstitial fluid (Carlberg 2019; Chang and Lee 2019; Charoenngam and Holick 2020; Pierrot-Deseilligny and Souberbielle 2017; White and Cooke 2000) (Figure 1f). The proximal tubule cells of the kidneys are the main site of endocrine production of calcitriol. However, calcitriol can also be synthesized in the cytoplasm from 25-hydroxyvitamin D, and exerts its autocrine and paracrine functions in other tissues and cells where the CYP27B1 is expressed, such as parathyroid glands, breast, colon, keratinocytes, osteoblasts, microglia (Carlberg 2019; Chang and Lee 2019; Charoenngam and Holick 2020; Pierrot-Deseilligny and Souberbielle 2017), activated macrophages, monocytes, dendritic cells, (Carlberg 2019), T and B lymphocytes (Dankers et al. 2016), lymph nodes, placenta and alveolar macrophages (Chang and Lee 2019).

Key enzymes of vitamin D metabolism

Vitamin D binding protein (VDBP)

VDBP is encoded by the *GC* gene located on chromosome 4 (4q13.3) and is 63828 nucleotide base pair (bp) in size (Ahn et al. 2010). The main function of VDBP is the binding, solubilization and transport of vitamin D metabolites (Powe et al. 2013; Speeckaert et al. 2006). VDBP is a serum α2 globulin protein with a molecular weight of 52–59 kilodaltons (kDa). It is made up of about 458 amino acids in three domains and a leader sequence of 16 amino acids. Two binding regions within the VDBP sequence have been identified: a vitamin D binding domain between 35-49 residues and an actin-binding domain between 373-403 residues (White and Cooke 2000).

The VDBP hepatic synthesis, is estrogen-dependent and significantly increases during pregnancy and estrogen

therapy (Speeckaert et al. 2006), and it circulates at higher concentrations than the total amount of vitamin D metabolites, which might relate to the other functions of VDBP (Speeckaert et al. 2006; White and Cooke 2000). Nevertheless, VDBP has a short plasma half-life (2.5 days) (Speeckaert et al. 2006).

Vitamin D 25-hydroxylase (CYP2R1)

This enzyme is a protein of around 500 amino acids with a molecular weight of 50-55 kDa, encoded by the *CYP2R1* gene, located on chromosome 11 (11p15.2) of 16599 bp in size (Duan et al. 2018). The liver is the main site where the vitamin D 25-hydroxylase is synthesized,performs its 25-hydroxylation enzyme activity, and metabolizes the cholecalciferol and ergocalciferol to calcidiol. Besides the vitamin D 25-hydroxylase activity has also been described in the kidney and intestines (Carlberg 2019; Zhu and DeLuca 2012).

25 Hydroxyvitamin D_3 1-alpha hydroxylase (CYP27B1)

Corresponds to a cytochrome P450 protein of 507 amino acids of around 55 kDa, encoded by the *CYP27B1* gene, which is found on chromosome 12 (12q14.1) and its size is 6653 bp (Duan et al. 2018). This enzyme is predominantly expressed in the kidney, but it is also expressed in extrarenal sites, such as the colon, prostate, breast, brain, adipocytes, and immune cells (Duan et al. 2018; Holick 2007; Mutt et al. 2014) where is synthesize calcitriol from calcidiol.

In the proximal renal tubule, the CYP27B1 enzyme is stimulated by parathyroid hormone (PTH) and inhibited by the fibroblast growth factor (FGF)-23, and in nonrenal tissues, its regulation differs from that kidney, and it may be more substrate-dependent (Bikle 2009). CYP27B1 expression is also inhibited by calcitriol, through a negative vitamin D response element (VDRE) in its promoter to which vitamin D receptor (VDR) binds indirectly (Bikle 2009).

Vitamin D status and regulation

Metabolites and half-life in the blood

In the bloodstream, vitamin D (cholecalciferol or ergocalciferol) circulates 85-90% bound to VDBP, 10-15% bound to albumin, and <1% freely, for transport to the liver (Bover et al. 2015; Schneider et al. 2014). Also, VDBP binds the 88% of calcidiol with high affinity ($K_a = 5 \times 10^8\ M^{-1}$) and the 85% of calcitriol with a ten-times lower affinity ($K_a = 4 \times 10^7\ M^{-1}$) (Jones 2008; White and Cooke 2000).

The most stable, abundant, and main circulating vitamin D metabolite is calcidiol (Carlberg 2019), with a half-life of 2-3 weeks and whose concentration can be 1000 times greater than calcitriol, which is the functionally active form with an approximate half-life of 4-6 h (Chang and Lee 2019; Jones 2008).

Reference serum values

Currently, there is no definition of how much vitamin D is needed, and what serum calcidiol level is optimal for immune health status and other health benefits (Charoenngam and Holick 2020). However, as we shall see later different experts have proposed the definition of calcidiol optimal serum levels.

Calcidiol production is primarily substrate-dependent, so its serum levels are the best indicator of vitamin D status (Bikle 2009; Chang and Lee 2019), despite not being the active metabolite. The optimal calcidiol serum levels for skeletal or extra-skeletal health vary for different populations, and in adults, it is determined from studies of calcium homeostasis, bone mineralization, and PTH levels (IOM (Institute of Medicine) 2011). The IOM mentioned a calcidiol serum levels of 20 ng/mL as optimal for skeletal health (Chang and Lee 2019; IOM (Institute of Medicine) 2011), whereas the Endocrine Society (ENDO), the International Osteoporosis Foundation (IOF), the National Osteoporosis Foundation (NOF) and the American Geriatrics Society (AGS) established that at least 30 ng/mL is needed for disease prevention (Chang and Lee 2019).

Calcidiol serum levels <20 ng/mL (50 nmol/L) indicates a deficiency, serum levels between 21 to 29 ng/mL (52-72 nmol/L) indicate insufficiency, levels ≥30 ng/mL (≥75 nmol/L) are usually considered as sufficiency (Celikbilek et al. 2014; Holick 2007), whereas vitamin D intoxication is considered serum calcidiol levels ≥150 ng/mL (374 nmol/L) (Holick 2007; Pelajo, Lopez-Benitez, and Miller 2010).

The consensus for adequate calcidiol serum levels in children has not yet been established; however, different medical associations have established different cutoff values: the American Academy of Pediatrics (AAP) and Global Consensus classified as calcidiol sufficiency values >20 ng/mL (Chang and Lee 2019), while the Pediatric Endocrine Society established >30 ng/mL. There is no consensus on optimal calcidiol serum levels during pregnancy; however, 20 ng/mL is considered minimally acceptable (Chang and Lee 2019; IOM (Institute of Medicine) 2011).

The biologically active metabolite calcitriol is also measured in some studies, to assess the serum vitamin D status, and calcitriol could be found in blood circulation at concentrations between 15-60 pg/mL (40-140 pmol/L) (Avila, Barrera, and Díaz 2007; Ovesen, Brot, and Jakobsen 2003) or 16-80 pg/mL (39-193 pmol/L) (Balasuriya et al. 2019). However, more studies are necessary to evaluate its relevance in different pathologies and health, because the calcitriol does not have a clear pattern of linear increase or decrease with calcidiol serum levels in different conditions.

Regulation axis

Regarding calcitriol, its production is regulated by calcium, phosphorus, and PTH serum levels, which increases the conversion from calcidiol to calcitriol, and their levels in adults have a negative correlation with calcidiol serum levels; nevertheless, this relationship is weak in children (Chang and Lee 2019; Holick 2007). When parathyroid gland calcium receptors detect a decrease of ionized calcium plasma level, the PTH is secreted, which stimulates the production of calcitriol from calcidiol in kidneys (Chang and Lee 2019; Holick 2007)

(Figure 2i). High calcitriol serum levels increase the calcium transport within intestines (Figure 2h), bones, and kidneys, and regulates the osteoblast and osteoclast activity (Figure 2j); when plasma calcium levels normalize, further secretion of PTH decreases (Chang and Lee 2019).

Another regulator is the FGF23 produced primarily by bone, by osteoblasts and osteocytes, which causes the internalization of the sodium-phosphate cotransporter in the cells of the kidney and small intestine, and suppresses the synthesis of calcitriol (Bikle 2009; Holick 2007; Shoenfeld et al. 2018) (Figure 2j), and this feedback loop like that for PTH secretion maintains a balance in the levels of these crucial hormones (Bikle 2009).

Once calcitriol completes these actions, the stimulation of the enzyme 25-hydroxyvitamin-D-24-hydroxylase (CYP24A1) produce inactive forms of vitamin D, such as $25(OH)D_3$-26,23-lactone, 24,25 dihydroxycholecalciferol [$24,25(OH)_2D_3$] from calcidiol, 12,425-trihydroxyvitamin D [$12,425(OH)_3D_3$] from calcitriol, and finally is excreted in bile or urine in form of calcitroic acid (Holick 2007; Ovesen, Brot, and Jakobsen 2003; Shoenfeld et al. 2018) (Figure 2g).

In the immune system, after the activation of macrophages via toll-like receptors (TLRs), CYP27B1 is induced, whereas PTH and FGF23 do not influence this regulation, possibly to lack of their cognate receptors. Macrophages may express in the cytoplasm a nonfunctional alternatively spliced form of CYP24 that potentially interferes with substrate access to the mitochondrial CYP24, and this reduces calcidiol and calcitriol catabolism (Bikle 2009).

In keratinocytes, calcitriol production can be stimulated by the CYP27B1 induction via specific TLRs, as well as by TNF-α and interferon (IFN) stimulation. Besides that, the expression of a fully functional CYP24 and its induction by calcitriol limits its levels in the epidermis (Bikle 2009).

Although sufficient serum calcidiol levels are considered necessary for the calcitriol production homeostasis and serum calcium levels (Chang and Lee 2019), have also been reported that high calcitriol serum levels do not rise in response to increased exposure to sunlight or increased intake of vitamin D, due to renal regulation of its production (Holick 2007; Ovesen, Brot, and Jakobsen 2003) as well as, in a serum calcidiol insufficient state, calcitriol levels are often normal or even elevated, due to local calcitriol synthesis in several organs and tissues, to regulate the genes related with cellular proliferation and other healthy essential functions (Holick 2007).

Although calcidiol is considered the best indicator of serum vitamin D status, its deficiency could not reflect the calcitriol serum status. Therefore, the evaluation of calcitriol, calcium, and PTH serum levels, are necessary to elucidate in a better way the complex biological regulation of the vitamin D serum status in the health and disease.

Host-related factors that influence vitamin D status

Nutrient status of the host

The host's vitamin D status can influence vitamin D absorption. It was reported that intestinal absorption of cholecalciferol and its hydroxylation in the liver were higher in vitamin D-deficient rats than in vitamin D-treated ones, because vitamin D absorption in the intestine and its 25-hydroxylation in hepatocytes may decrease when the cholecalciferol levels increases (Apukhovskaia et al. 1990; Borel, Caillaud, and Cano 2015). People with vegetarian diets or restrictive eating disorders are more likely to be deficient in vitamin D due to an unbalanced diet (Chang and Lee 2019) if they do not have adequate supervision and supplementation.

Age

Age-related functional alterations of gastrointestinal may modify the efficiency of vitamin D absorption and other vitamins. However, different studies have suggested no significant effect of the aging on vitamin D absorption efficiency; therefore, the lower vitamin D status in the elderly than in young subjects may be due to other factors related to dietary intake, sun exposure (Borel, Caillaud, and Cano 2015), efficiency in skin synthesis or its hydroxylation in the liver (Borel, Caillaud, and Cano 2015; Chang and Lee 2019), and more insufficient response of target tissues (Chang and Lee 2019).

Diseases

Some diseases or conditions that impaired fat absorption, such as obstructive jaundice/bile insufficiency, pancreatic insufficiency, cystic fibrosis, celiac disease, gastric bypass surgery, inflammatory bowel disease, as well as nephrotic syndrome, liver, and renal insufficiencies, may contribute to reducing vitamin D absorption or production (Borel, Caillaud, and Cano 2015; Chang and Lee 2019; Charoenngam and Holick 2020). The negative effect of these diseases on vitamin D bioavailability could be partially corrected by sun exposure or calcidiol administration. This hydroxylated vitamin D form is absorbed directly via the portal vein and can have a well-preserved absorption (Borel, Caillaud, and Cano 2015).

Obesity

A higher body mass index has been related to lower calcidiol serum levels (Mutt et al. 2014). Subjects with obesity have lower vitamin D serum levels, because the vitamin D could be deposited in the adipose tissue due its fat-soluble characteristic; hence, low serum levels are not caused by a lower skin synthesis or bioavailability (Borel, Caillaud, and Cano 2015; Chang and Lee 2019; Charoenngam and Holick 2020).

Calcidiol serum deficiencies

Nowadays, humans tend to stay most of the time indoors and cover their skin by textile, and this generates insufficient UVB exposure, which could influence in low endogenous cholecalciferol production, that together with a low vitamin D dietary consumption may contribute to serum calcidiol

subclinical deficiencies, which are common around the world (estimated more than a billion individuals), both in healthy and unhealthy populations (Carlberg 2019).

Severe calcidiol deficiency may cause rickets in infants or children and osteomalacia in adults, not common diseases in most developed countries. In contrast, its subclinical deficiency is more prevalent, and may be associated with osteoporosis and a higher incidence of falls or fractures (Chang and Lee 2019).

Calcidiol serum deficiency has been linked with the incidence and severity of many diseases such as proximal muscle weakness, rickets (osteomalacia, osteoporosis, and osteoarthritis), skin diseases (epidermolytic ichthyosis), respiratory infections, sepsis, hypertension, cardiovascular disease, type 2 diabetes, neuropsychiatric disorders (schizophrenia, major depressive and neurodegenerative disorders), allergic diseases (asthma, wheezing disorders, urticarial and atopic dermatitis), cancers (breast, colon, and prostate) (Charoenngam and Holick 2020), and notably in autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, multiple sclerosis, vitiligo, psoriasis, and psoriatic arthritis), highlighting the role of vitamin D in the immune homeostasis (Charoenngam and Holick 2020; Dall'Ara et al. 2017). Infants and adolescents are potential populations at risk for low calcidiol serum levels, because of rapid skeletal growth after birth and puberty (Chang and Lee 2019).

Vitamin D genomic pathway

Calcitriol is the active metabolite

Calcitriol acts as an endocrine hormone with a high-affinity ligand (K_D 0.1 nM) to the VDR (Carlberg 2019). In the absence of ligand, VDR only binds to some 200–2000 sites per cell type, and after its binding to calcitriol, the number of VDR sites increases on average by a factor of 2.5 (Carlberg 2019). The genomic responses generally take several hours of days to be fully apparent, and this response could be blocked by inhibitors of transcription and translation (Haussler et al. 2011).

Calcitriol can be presented in 6-*s-cis* and 6-*s-trans* conformers, resulting from 360° rotation around the 5,6 carbon-carbon bonds at a rate of millions of times per second. The nuclear VDR ligand (nVDR), calcitriol, is in a bowl-shaped twisted 6-*s-trans* conformation with the A-ring and side-chain both 30° above the C/D ring (Haussler et al. 2011; Norman et al., 1997).

Vitamin D receptor (VDR)

The VDR is a member of the steroid/thyroid hormone receptor superfamily. Functionally VDR is a transcription factor regulated by ligand binding and possibly by phosphorylation events. VDR is a soluble protein located mainly in the nucleus and cell cytoplasm, from where it is translocated to the nucleus through the microtubule system after interaction with its ligand, calcitriol (Dankers et al. 2016). VDR is a 427 amino acid phosphoprotein, of 48 kDa, consisting of different regions or functional domains (Álvarez-Hernández et al. 2007; Ortega-Domínguez, Herrera-Ramírez, and Tecalco-Cruz 2015).

Domain A/B: Located in the amino-terminal portion of the protein, generally contains a constitutively active transcriptional activation function, called ligand-independent activation function-1 (AF-1) and is the most variable in size and sequence. Post-translational modifications, such as phosphorylation, can generate several isoforms (Jurutka et al. 2000; Sunn et al. 2001).

Domain C: contains the DNA-binding domain (DBD), made up of two zinc fingers that are located in a central sequence of 66 amino acids and consisting of a tetrahedral complex formed by 4 cysteines with a zinc molecule that creates an amino acid loop to stabilize the union with contacts of DNA phosphate (Dankers et al. 2016; Khorasanizadeh and Rastinejad 2001).

D domain or hinge: Gives the ligand-binding domain (LBD) and DBD flexibility to adopt different conformations without generating steric problems. It contains an NLS (nuclear localization signal) that contributes to its translocation to the nucleus when VDR interacts with calcitriol (Shaffer, McDonnell, and Gewirth 2005).

E/F domain: located in the carboxy (C)-terminal. Contains the LBD with high affinity for calcitriol (Choi et al. 2001) that is a sandwich-like structure of at least 12 α-helices (Rochel et al. 2000) that presents VDR surfaces for heterodimerization with the RXR (retinoid x receptor), predominantly helices 9 and 10 and the loop between helices 8 and 9 (Haussler et al. 2011). In the LBD pocket, the residues 237 of serine and 274 of arginine directly interact via hydrogen bridge with the 1α-OH molecule of calcitriol, and the histidines 305 and 397 interact with the 25-OH; while amino acids 239-259 and 317-401 are essential for recruitment and dimerization with the RXR (Choi et al. 2001; Haussler et al. 2011).

The AF-2 located in the helices 12 between amino acids 416-427 (Choi et al. 2001; Haussler et al. 2011) presents a structural change after ligand binding and for its activation requires the binding of motor proteins (Choi et al. 2001). Coactivator interfaces in VDR consist of portions of helices H3, H5, and H12 (Haussler et al. 2011).

VDR isoforms

Three isoforms of the VDR has been described. The most common form is the isoform VDRA of 427 amino acids and 48 kDa, with a start site in exon 2. The second is a long isoform VDRB1 of 477 amino acids, and 54 kDa, with 50 more amino acids in the N-terminal domain by an ATG start site in exon 1d, which has been described in the human kidney as well as intestinal and renal epithelial cell lines (Sunn et al. 2001; Zenata and Vrzal 2017). The third is a shorter VDR isoform of 424 amino acids and greater transactivation capacity as a transcription factor, caused by the presence of the single nucleotide polymorphism called *FokI* in the exon 2; the *FokI* site dictates which of two potential translation initiation sites will be used in the *VDR* gene, to generate a

reading frame shift at the translation start codon from the exon 1d to exon 2 (Zenata and Vrzal 2017).

VDR cell expression

Almost all nucleated cells express the VDR at variable levels (Chang and Lee 2019). VDR is found in various organs and cell types, such as colon, osteoblasts, beta islet cells, brain, heart, skin, gonads, prostate, breasts, and different immune cells, such as activated T and B lymphocyte, neutrophils, macrophages, and dendritic cells (Chang and Lee 2019; Dankers et al. 2016; Shoenfeld et al. 2018). The few cells or tissues with low or absent VDR expression include red blood cells, mature striated muscle, and some highly differentiated brain cells, such as the Purkinje cells of the cerebellum (Eyles et al. 2005).

Vitamin D response elements (VDREs)

Calcitriol-VDR-RXR heterodimer can recognize docking sequences named VDREs located in the genomic DNA sequence of vitamin D-regulated genes, by the combined DNA-binding domain (DBD) zinc fingers of the two receptors, and their C-terminal extensions (CTE) (Haussler et al. 2011).

VDREs consist of a direct repeat of two hexanucleotide half-elements with a spacer of three nucleotides (DR3, the most common) or an everted repeat of two half-elements with a spacer of six nucleotides (ER6) motif, often in multiple copies dispersed up to 100 kb 5′ or 3′ of the transcription start site in vitamin D-target genes. In positive DR3 VDREs, VDR occupies with high affinity the 3′ half-site PGTTCA, where P is a purine base, and the RXR residing with high affinity the 5′ half-site PGGTCA (Haussler et al. 2011).

Variations in the VDREs sequences induce unique conformations in the VDR-RXR complex, and promotes its association with distinct subsets of co-modulators to permit various biological actions (Haussler et al. 2011; J. Zhang et al. 2011). Some genes possess multiple VDREs for maximal induction by calcitriol, whereas that individual VDREs act synergistically to attract coactivators and basal factors for transactivation. VDREs are juxtapositioned with more proximal VDREs via DNA looping in chromatin, and this allows supporting the transcription machine (Haussler et al. 2011).

Vitamin D target genes

Lipid sensing and signaling via key nuclear receptors have an essential role in the differentiation and epigenetic programming of immune cells. VDR, together with others receptors such as retinoic acid receptor (RAR), peroxisome proliferator-activated receptors (PPARs), and liver X receptor (LXR), act as sensors of the respective micro- and macronutrients in order to adapt gene expression profiles in metabolic organs and immune system cells (Carlberg 2019).

Ligand-activated VDR binds genome-wide varies from 5–20000 sites within accessible chromatin and generates some changes of the transcriptome (Carlberg 2019). The recruitment of co-repressor (with histone deacetylase activity) or activators (with histone acetylase activity) molecules lead to the inhibition or activation of transcription of vitamin D target genes (Dankers et al. 2016; Ferrer-Mayorga et al. 2019).

The VDRE-containing in genes are related with important physiological processes such as: a) Bone health (bone metabolism: *rBGP*, *mSPP1*; bone anabolism: *mLRP5*; bone resorption: *mRANKL)*; b) Mineral regulation (mineral homeostasis: *cPTH*; intestinal Ca^{2+}: *hTRPV6*; renal phosphate reabsorption: *hFGF23*, *hklotho*); c) Detoxification (1,25 D detoxification: *hCYP24A1*; xenobiotic detoxification: *hCYP3A4*); d) Cell life (proliferation, differentiation, migration, and death; cell cycle control: *hp21*, *hFOXO1*; mammalian hair cycle: *hSOSTDC1*, *rPTHrP*); e) Metabolism (amino acid, lipid, and carbohydrate; homocysteine clearance: *hCBS*) (Haussler et al. 2011), and e) Innate and adaptive immunity regulation: *THEM4* (Thioesterase Superfamily Member 4), *SMAD7* (SMAD Family Member 7), *IL-17A*, *FOXP3* (forkhead box P3) (Dankers et al. 2016; Ferrer-Mayorga et al. 2019), *ACVRL1* (activin A receptor like type 1), *CAMP* (cathelicidin antimicrobial peptide), *CD14*, *CD93*, *CEBPB* (CCAAT enhancer binding protein beta), *FN1* (fibronectin 1), *MAPK13* (mitogen-activated protein kinase 13), *NINJ1* (ninjurin 1), *LILRB4* (leukocyte immunoglobulin like receptor B4), *LRRC25* (leucine rich repeat containing 25), *SEMA6B* (semaphorin 6B), *SRGN* (serglycin), *THBD* (thrombomodulin), *THEMIS2* (thymocyte selection associated family member 2) and *TREM1* (triggering receptor expressed on myeloid cells 1) (Koivisto, Hanel, and Carlberg 2020).

Mechanism of the genomic pathway

Initiation of the genomic pathway

Due to its fat-soluble nature, calcitriol can pass the plasma membrane through a passive diffusion process (Figure 3a). Calcitriol binds to nVDR-LBD in the cytoplasm (Figure 3b), leading to dissociation of proteins, such as the heat shock protein 90 (HSP90) chaperone (Figure 3c), which keeps the nVDR in an inactive state (Ortega-Domínguez, Herrera-Ramírez, and Tecalco-Cruz 2015; Pierrot-Deseilligny and Souberbielle 2017). The calcitriol-VDR complex is translocated to the nucleus through the nuclear import process regulated by α and β importins, that recognize the nuclear localization signal (NLS) present in the VDR-D domain (Ortega-Domínguez, Herrera-Ramírez, and Tecalco-Cruz 2015; Pierrot-Deseilligny and Souberbielle 2017) (Figure 3d).

In the nucleus, the calcitriol-nVDR complex through the second zinc finger of the nVDR-DBD, like other nuclear receptors, forms a heterodimer with the RXR, which gives nVDR greater affinity for binding to DNA (Figure 3e). Through the first zinc finger of the nVDR-DBD, the complex binds to VDREs in target genes (Carlberg 2019; Dankers et al. 2016; Pierrot-Deseilligny and Souberbielle 2017) (Figure 3f). Subsequently, the binding of the nVDR-RXR heterodimer to DNA can influence histone

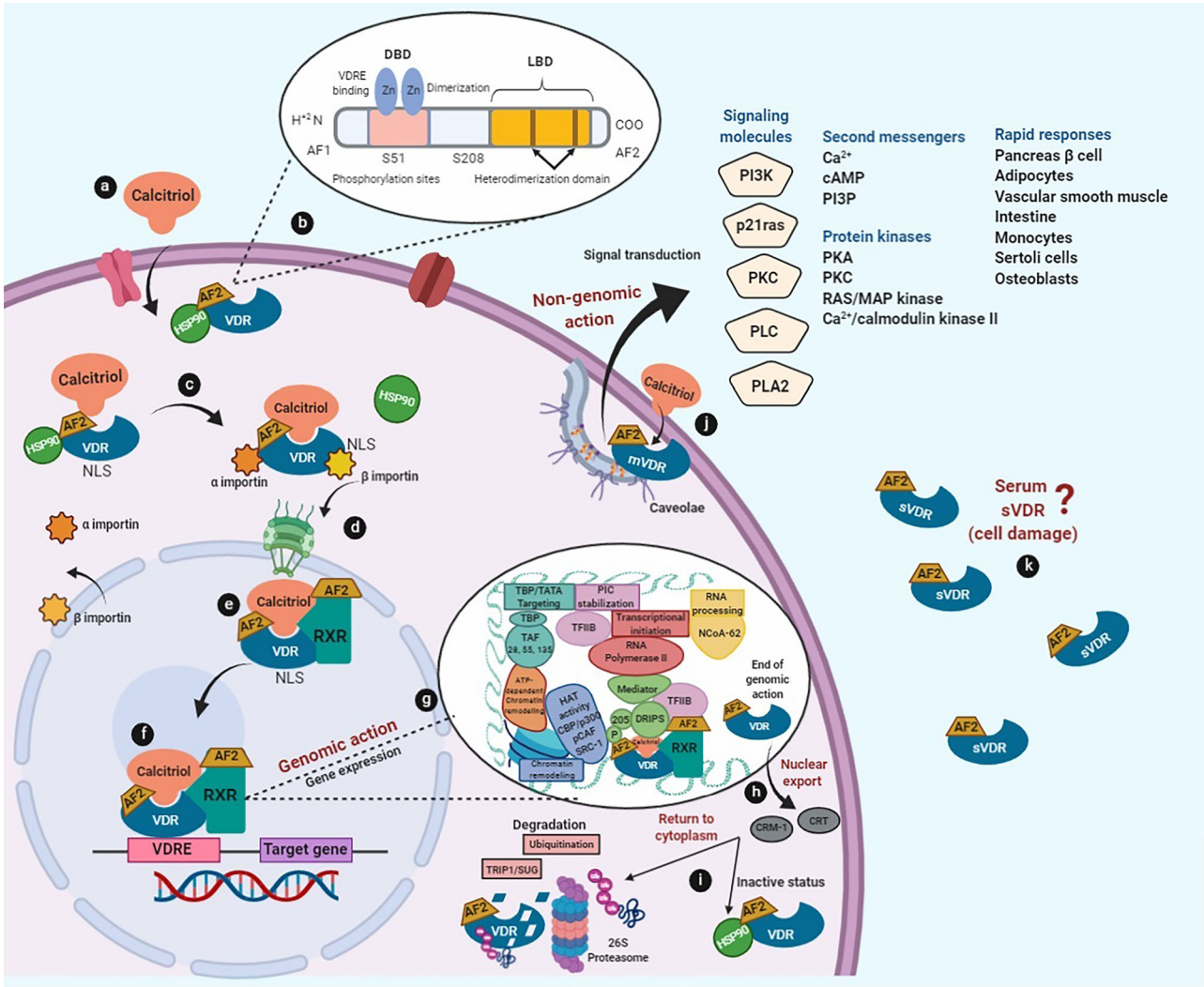

Figure 3. Genomic and non-genomic pathways of vitamin D. a) Entry of calcitriol into the cell: calcitriol pass the plasma membrane through a passive diffusion process; b) VDR structure: phosphoprotein that consists of different functional domains: the DBD, the LBD, and the AF2; c) Binding of calcitriol to VDR: in cytoplasm, calcitriol binds to the LBD of the VDR, leading to dissociation of the HSP90 chaperone which keeps the VDR in an inactive state; d) Translocation to the nucleus: calcitriol-VDR complex is translocated to the nucleus through the nuclear import process regulated by α and β importins that recognize the NLS present in the VDR D domain; e) Heterodimerization: in nucleus, calcitriol-VDR complex through the second zinc finger of the VDR-DBD forms a heterodimer with the RXR; f) Binding to VDRE in target genes: through the first zinc finger of the DBD, VDR binds to VDREs in target genes and may influence in changes in histone modification that may modifies the chromatin accessibility; g) Initiation of genomic action: transcription control by VDR requires the recruitment of coregulators, such as DRIP that couples the trans-activators to the C-terminal tail of RNA polymerase II, and recruits CREB, and others co-activator proteins with HAT activity such as CBP/P300, PCAF, and methyltransferases to the VDR, resulting in a multi-subunit complex. VDR can also interact with TAFs, with basal transcription factors such as TFIIB and with the NCoA-62, which may be involved in the RNA splicing by the spliceosome; h) Nuclear export: after acts as a transcription factor, VDR returns to cytoplasm trough the nuclear export process mediated by the CRM-1 or the CRT joined to DBD-VDR domain; i) Return to cytoplasm: VDR may remain inactively attached to HSP90 in the cytoplasm or interacts with TRIP1 that facilitates its ubiquitination and subsequent degradation by the 26S proteasome; j) Non-genomic action: calcitriol may exerts non-genomic actions, downstream of nVDR and or/mVDR complexed to caveolin1, such as the activation of signaling molecules (PLC, PLA2, PI3K and p21ras), the rapid generation of second messengers (Ca^{2+}, cAMP, fatty acids and 3-phosphoinositides such as PIP3), the activation of protein kinases (PKA, src, MAP kinases, PKC and Ca^{2+}/calmodulin kinase II), and the opening of Ca^{2+} and Cl^{-} channels. k) sVDR: VDR, is found mainly in the cytoplasm and nucleus, its presence in soluble form could be indicative of a high rate of cell damage or apoptosis. **VDR:** vitamin D receptor; **DBD:** DNA-binding domain; **RXR:** retinoid X receptor; **VDREs:** vitamin D response elements; **LBD:** ligand-binding domain; **AF1 or AF2:** ligand-independent activation function 1 or 2; **HSP90:** heat shock protein 90; **NLS:** nuclear localization signal; **DRIP:** vitamin D receptor-interacting protein complex; **CREB:** cAMP response element-binding; **HAT:** histone acetyltransferase; **PCAF:** P300/CBP-associated factor; **TAFs:** TATA-binding protein-associated factors; **NCoA-62:** nuclear coactivator-62 kDa; **CRM-1:** chromosomal Maintenance 1 or exportin 1; **CRT:** calreticulin; **TRIP1:** thyroid hormone receptor-interacting protein 1; **mVDR:** membrane vitamin D receptor; **PLC:** phospholipase C; **PLA2:** phospholipase A2; **PI3K:** phosphatidylinositol-3 kinase; **cAMP:** cyclic adenosine monophosphate; **PIP3:** phosphatidylinositol 3,4,5 trisphosphate; **MAP:** mitogen-activated protein; **PKA:** protein kinase A; **PKC:** protein kinase C; **sVDR:** soluble vitamin D receptor.

modification changes that may modify the chromatin accessibility (Koivisto, Hanel, and Carlberg 2020) (Figure 3g).

Activation of gene transcription

Transcription control by nVDR requires the additional recruitment of coregulators (Bikle 2009). The high-affinity binding of calcitriol to the LBD-pocket causes a conformational change in the helix 12 at the nVDR C-terminus, bringing it to the "closed" position that will allow AF-2 to binds with coactivator complexes (Bikle 2009; Haussler et al. 2011; P. W. Jurutka et al., 1997; Masuyama et al. 1997).

nVDR after ligand binding can interact with the steroid receptor coactivator 1 (SRC-1 or nuclear coactivator NCOA1), comprised of the p160 family of SRC1, SRC2, and

SRC3 coactivators (Bikle 2009; Carlberg 2019; Haussler et al. 2011), which has histone acetyltransferase (HAT) and methyltransferase activity that destabilizes the interaction between DNA and the histone core, allowing transcription (Bikle 2009), as well as with the nVDR interacting protein complex (DRIP) or mediator complex that does not have HAT activity, but couples the trans-activators to the C-terminal tail of RNA polymerase II (Arriagada et al. 2007; Bikle 2009; Barletta, Freedman, and Christakos 2002; Haussler et al. 2011) (Figure 3g).

This recruit of cAMP response element-binding (CREB) protein-binding protein (Bikle 2009), and others coactivator proteins with HAT activity such as CBP/P300, P300/CBP-associated factor (PCAF) (Carlberg 2019; Haussler et al. 2011), and methyltransferases to the nVDR, resulting in a multi-subunit complex (Bikle 2009).

The attraction of a coactivator to the helix-3, −5, and −12 platform stabilizes the nVDR-RXR heterodimer on the VDRE and induces the nVDR-LBD to migrate to the 5′ site of the RXR-LBD, and the RXR-LBD rotates 180° employing the force of the ionic and hydrophobic interactions with the helices 9 and 10 in nVDR (Haussler et al. 2011), and RXR may not bind 9-*cis* retinoic acid when heterodimerized to liganded nVDR (Haussler et al. 2011; P. D. Thompson et al. 2001).

These conformational changes convert the nVDR into a more efficient substrate for protein kinases (Haussler et al. 2011; Peter W. Jurutka et al. 2002). The casein kinase II (CK2) phosphorylates the serine 208 in nVDR, and this event potentiates the transcriptional activity of the VDR-RXR heterodimer (Haussler et al. 2011; Jurutka et al. 1996), probably by enhancing interactions with coactivators such as DRIP205, which is a subunit of the mediator complex (Arriagada et al. 2007; Barletta, Freedman, and Christakos 2002; Haussler et al. 2011).

In the transactivation process, the nVDR can also interact with other factors such as TATA-binding protein-associated factors (TAFs, especially TAFs 28, 55, and 135) (Haussler et al. 2011; Lavigne et al. 1999), with basal transcription factors such as TFIIB (Blanco et al. 1995; Haussler et al. 2011) and with the nuclear coactivator-62 kDa (NCoA-62) (Haussler et al. 2011; C. Zhang et al. 2003).

After the transcription machine has moved away from the promoter, with DRIP205/Mediator dissociating from the nVDR-RXR heterodimer replaced by the thyroid hormone receptor-interacting protein 1 (TRIP1), NCoA-62 also dissociates and may be involved in the RNA splicing by the spliceosome (Haussler et al. 2011) (Figure 3g).

nVDR can also regulate some genes through post-transcriptional mechanisms or indirect mechanisms through the induction of microRNAs or their interaction with some signaling pathways or with other transcription factors such as the nuclear factor of activated T-cells (NFAT), nuclear factor kappa B (NF-κB), and the SMAD family factors (Boonstra et al. 2001).

The pioneer factors purine-rich box 1 (PU.1) and CCAAT/enhancer-binding protein alpha (CEBPA), work closely together with the nVDR in hematopoietic differentiation into monocytes and granulocytes. The PU.1, CEBPA, and GABPA (GA binding protein transcription factor subunit alpha) help the nVDR to find enhancer regions close to vitamin D target genes (Carlberg 2019).

Within the epigenome, calcitriol can influence the modulation of the three-dimensional organization of chromatin. At some 1300 genomic sites, calcitriol may affect the binding profile of the transcription factor CCCTC binding factor (CTCF), which is a crucial protein in organizing the human genome into loops, denominated topologically associated domains (TADs) (Carlberg 2019).

Suppression of gene transcription

Some corepressors, such as nuclear corepressor 1 (NCOR1) and silencing mediator of retinoic acid and thyroid receptor (SMRT) (Bikle 2009; Carlberg 2019) in the absence of ligand, link the nVDR to chromatin modifiers of the histone deacetylase family (HDAC) (Carlberg 2019). However, this block of the nVDR-mediated transcriptional activity is displaced when calcitriol binds to nVDR and recruits the coactivators (Bikle 2009).

About the function of the nVDR in the repression of genes, different factors are involved. HDAC and demethylases catalyze the restructuring of chromatin. The binding in reverse polarity on negatives VDRE generates an allosteric transformation on the conformation of the nVDR, which favors the binds corepressor rather than coactivator to their overlapping docking sites in helices 3–6. This altered nVDR conformation may also be more prone to modification by protein phosphatase than protein kinase, and this also favors corepressor attraction (Haussler et al. 2011).

Another corepressor described is hairless (Hr) found primarily in the brain, epidermis, hair follicles, and other epithelia, with trace levels of expression elsewhere (Bikle 2009).

End of the genomic pathway

Finally, after acts as a transcription factor, like other nuclear receptors, nVDR returns to cytoplasm trough the nuclear export process, which can be mediated by nuclear export proteins such as chromosomal Maintenance 1 (CRM-1 or exportin 1) or calreticulin (CRT) joined to nVDR-DBD (Ortega-Domínguez, Herrera-Ramírez, and Tecalco-Cruz 2015) (Figure 3h). nVDR may remain inactively attached to HSP90 in the cytoplasm, or it may interact with the thyroid hormone receptor-interacting protein 1 (TRIP1), that facilitates its progressive ubiquitination and subsequent degradation by the 26S proteasome (Haussler et al. 2011; Masuyama and MacDonald 1998) (Figure 3i).

Skeletal effects of vitamin D

Calcitriol via nVDR promotes bone mineralization passively by increasing intestinal phosphate absorption to 80% (Charoenngam and Holick 2020; Holick 2007) and enhances intestinal calcium absorption in 30 to 40% of the small intestine through the enhance the expression of the

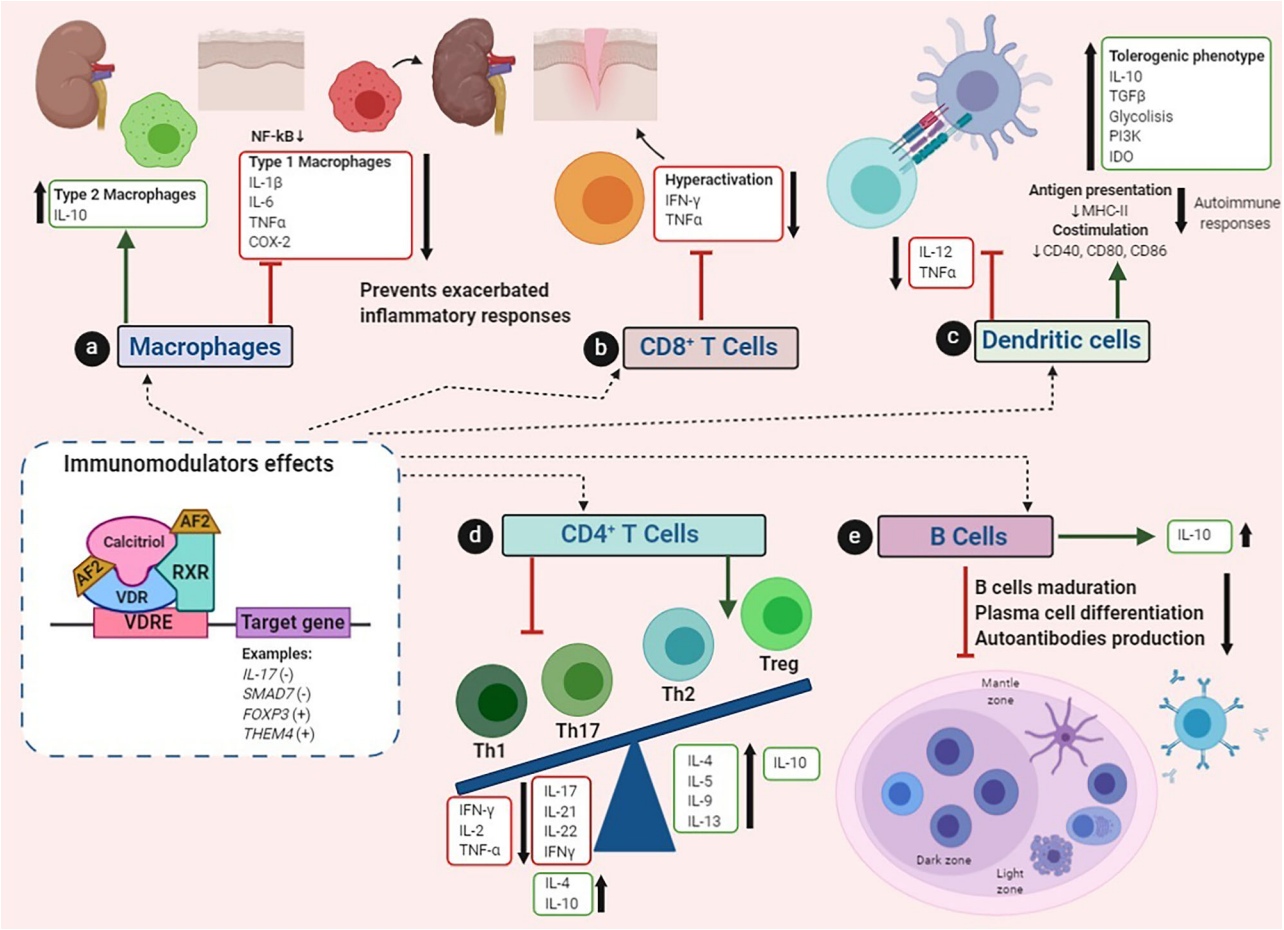

Figure 4. Calcitriol immunomodulatory effects. a) Macrophages: calcitriol promotes macrophages M1 phenotype switching to M2 via the VDR-PPARγ pathway, and IL-10 expression. Exerts an anti-inflammatory effect in macrophages through *THEM4* that inhibits the binding of NFκB to the COX-2 locus, and inhibits IL-6 and TNFα expression; b) $CD8^+$ T Cells: calcitriol prevents the hyperactivation of CD8+ T cells, generates a high secretion of IL-5 and TGF-β and low secretion of IFNγ and TNFα; c) Dendritic cells: calcitriol induces a tolerogenic phenotype via expression decrease of MHC class II genes, and CD40, CD80, and CD86, downregulating the activity other immune system cells, the antigen presentation, and production of IL-12, and increases the IL-10 production; d) $CD4^+$ T Cells: calcitriol suppresses the proliferation of T lymphocytes, and modulates cytokine production, and promotes a shift from Th1 and Th17 to Th2 immune profile by suppressing the expression of Th1 (IL-2, IFN-γ, TNF-α) and Th17 (IL-17, IL-21) cytokines, while inducing Th2 cytokines (IL-4, IL-5, IL-9, IL-13), and promote differentiation of Treg cells; e) B Cells: calcitriol may induce apoptosis of activated B cells and plasma cells, promotes B-cell anti-inflammatory cytokines production (IL-10, CCR10), suppresses the differentiation from mature B cells to plasma cells, class-switched memory B cells, and reduction of autoantibody production. **THEM4:** thioesterase superfamily member 4; **NF-κB:** nuclear factor kappa B; **COX-2:** Cyclooxygenase 2; **TNF-α:** Tumor necrosis factor-α; **TGFβ:** Transforming growth factor-beta; **IFN-γ:** interferon-gamma; **MHC:** major histocompatibility complex; **PI3K:** phosphatidylinositol 3-kinase; **IDO:** Indoleamine 2,3-dioxygenase; **CD:** Cluster of differentiation; **SMAD7:** SMAD Family Member 7; **FOXP3:** forkhead box P3; **Th:** T helper cell; **Treg:** T regulatory cell.

epithelial calcium channel [transient receptor potential cation channel, subfamily V, member 6 (TRPV6)] and calbindin 9 K, a calcium-binding protein (CaBP) (Figure 2j), and renal tubular calcium reabsorption that help maintain an adequate calcium-phosphate product that crystallizes in the collagen matrix (Charoenngam and Holick 2020; Holick 2007). On the bone, calcitriol induces osteocalcin expression (Charoenngam and Holick 2020). In osteoblasts via nVDR, increase the expression of the receptor activator of nuclear factor-κB ligand (RANKL), which binds to RANK on preosteoclasts, and this induces preosteoclasts to become mature osteoclasts. Mature osteoclasts remove calcium and phosphorus from the bone to maintain adequate calcium (Ca^{2+}) and phosphorus (HPO_4^{2-}) levels in the blood, and promote bone mineralization (Holick 2007).

Besides, calcitriol directly inhibits PTH production (Bikle 2009; Charoenngam and Holick 2020), leading to a decrease in bone resorption and increased urinary calcium excretion, and induces FGF23 production by the osteocytes, leading to an increase in urinary phosphate excretion (Charoenngam and Holick 2020) (Figure 2i and j).

Immunological effects of vitamin D

Calcitriol may exert effects on various immune cells that express the nVDR, through different mechanisms depending on the cell type. Besides, some immune cells can convert calcidiol into calcitriol, such as monocytes, dendritic cells, macrophages, B cells, and T cells (Dankers et al. 2016) (Figure 4).

In the early stages of infection, calcitriol is necessary for adequate pathogen clearance (Dankers et al. 2016). Calcitriol produced by monocytes and macrophages stimulates the efficient recognition of bacterial pathogens via TLRs (Carlberg 2019; Holick 2007), generates a shift from pro-inflammatory to tolerogenic immune status (Charoenngam and Holick 2020), and promotes M1 phenotype switching to M2 via the nVDR-PPARγ pathway, and via upregulation of

the expression of IL-10 (X. Zhang et al. 2015). Also, the calcitriol has an anti-inflammatory effect in macrophages, through its target gene thioesterase superfamily member 4 (THEM4), that inhibits the binding of NFκB to the COX-2 locus and inhibits IL-6 and TNFα expression (Dankers et al. 2016) (Figure 4a).

In addition, calcitriol via nVDR binding, could favor macrophage phagocytic activity, decrease antigenic presentation in macrophage-lymphocyte cooperation and the expression of prostaglandin E2, as well as could antagonize pro-inflammatory transcription factors, such as NFAT, AP-1, and NF-κB, in T cells, which results in the decreased expression of cytokines, such as IL-12, IL-1, IL6, IL-17 and TNF-α (Carlberg 2019; Charoenngam and Holick 2020; Ferrer-Mayorga et al. 2019; Shoenfeld et al. 2018).

$CD8^+$ T cells have a high expression of nVDR, thus targeting calcitriol to control the autoimmune response. Calcitriol prevents the hyperactivation of $CD8^+$ T cells (Dankers et al. 2016) because it generates a greater secretion of IL-5 and TGF-β and less secretion of IFNγ and TNFα by activated $CD8^+$ T cells (Lysandropoulos et al. 2011) (Figure 4b).

Calcitriol induces immature and tolerogenic antigen-presenting cells (APCs) via decreased expression of genes for the major histocompatibility complex (MHC) class II and co-stimulatory molecules the cell surface such as CD40, CD80, and CD86. The generation of a tolerogenic dendritic cell (tDC) phenotype promotes polarization of regulatory T cells, downregulating the activity other immune system cells, the antigen presentation, production of IL-12, and increases the IL-10 production (Carlberg 2019; Charoenngam and Holick 2020). Induction of tDC phenotype involves the reprogramming of their glucose metabolism via the up-regulation of the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (*PFKFB4*), and the glycolytic enzyme fructose-bisphosphatase 1 (*FBP1*), both vitamin D target genes (Koivisto, Hanel, and Carlberg 2020) (Figure 4c).

Calcitriol suppresses the monocytes' TLR expression, and in the natural killer (NK) cells is still unclear if it induces or inhibits its function. Once T lymphocytes are stimulated and activated, express both nVDR and the CYP27B1 enzyme that generates calcitriol from local calcidiol, and this stimulates activation of the nVDR (Charoenngam and Holick 2020).

Calcitriol suppresses the proliferation of T lymphocytes, and modulates cytokine production and differentiation of different T helper (Th) cells subgroups: promotes a shift from Th1 and Th17 to Th2 immune profile by suppressing the expression of Th1 (IL-2, IFN-γ, TNF-α) and Th17 (IL-17, IL-21) cytokines, while inducing the expression of Th2 cytokines (IL-4, IL-5, IL-9, IL-13), to limit inflammatory processes and autoimmune reactions (Charoenngam and Holick 2020; Shoenfeld et al. 2018) (Figure 4d). Also, promote differentiation of regulatory T cells directly and indirectly via its interaction with APCs, resulting in suppressing the pro-inflammatory state (Charoenngam and Holick 2020) (Figure 4d).

In a hyperactive state, calcitriol may inhibit cytokine-mediated B-cell activation by acting on Th cells; induce apoptosis of activated B cells and plasma cells, directly promotes B-cell anti-inflammatory cytokines production (IL-10, CCR10), and suppresses the differentiation from mature B cells to plasma cells and class-switched in memory B cells, helping to reduce the autoantibody production; and therefore, the risk of autoimmune diseases such as systemic lupus erythematosus (Charoenngam and Holick 2020; Shoenfeld et al. 2018) (Figure 4e).

Others physiological effects of vitamin D

Vitamin D has other effects on different organs and systems: in the pancreas and other tissues contributes to an increase in insulin secretion and glucose absorption; in the cardiovascular system exerts an antihypertensive action, acts on the vascular wall, the heart muscle, and on the renin-angiotensin system; in the skin, promotes the differentiation of keratinocytes and wound repair, and in the central nervous system contributes to development and neuroprotection (Bikle 2009; Carlberg 2019; Kajikawa et al. 1999).

Also, vitamin D exert antiproliferative actions on tumor cells because calcitriol stimulates the expression of cell cycle inhibitors p21 and p27, the expression of E-cadherin, and inhibits the transcriptional activity of β–catenin (Bikle 2009). In adipose tissue, calcitriol inhibits adipogenesis antagonizing the trans-acting activity of PPARγ and downregulating mRNA expression and protein levels of CCAAT/Enhancer-binding protein (C/EBP β) (Mutt et al. 2014).

Vitamin D non-genomic pathways

Calcitriol is conformationally flexible secosteroid hormone that can also exert many biological effects via rapid response or non-genomic pathways (Figure 3j), generated within 1–2 to 15–45 minutes. Calcitriol and the $1\alpha,25(OH)_2$-lumisterol (JN) locked in a 6-*s-cis* shape are optimal ligands for the VDR conformer that signals rapid responses (Haussler et al. 2011; Norman et al., 1997).

VDR in the cellular membrane

A distinct membrane VDR (mVDR) was initially proposed on the basis that some vitamin D analogs were incapable of binding to nVDR, to initiate the rapid responses of calcitriol (Hii and Ferrante 2016; Norman 2006). mVDR could be found around the perinuclear area, and at the plasma membrane is localized in caveolaes where it binds caveolin-1 and phospholipase A2 (Hii and Ferrante 2016) (Figure 3j). mVDR receptor was named membrane-associated rapid response steroid (MARRS) binding protein, and it was later identified by others names such as thioredoxin-like protein or GRP58 (for glucose-responsive protein, 58 kDa), an endoplasmic reticulum protein 57/60 kDa (ERp57 or ERp60), and protein disulfide isomerase family A, member 3 (Pdia3) (Doroudi, Schwartz, and Boyan 2015; Fleet 2004; Hii and Ferrante 2016).

Signaling pathways

Calcitriol also exerts non-genomic actions, downstream of nVDR and/or mVDR complexed to caveolin1, such as the activation of signaling molecules [phospholipase C (PLC) and phospholipase A2 (PLA2), phosphatidylinositol-3 kinase (PI3K) and p21ras], the rapid generation of second messengers [Ca^{2+}, cyclic adenosine monophosphate (cAMP), fatty acids and 3-phosphoinositides such as phosphatidylinositol 3,4,5 trisphosphate or (PIP3)], the activation of protein kinases [protein kinase A, src, mitogen-activated protein (MAP) kinases, protein kinase C (PKC) and Ca^{2+}/calmodulin kinase II] and the opening of Ca^{2+} and Cl^{-} channels (Doroudi, Schwartz, and Boyan 2015; Fleet 2004; Hii and Ferrante 2016) (Figure 3j).

While the existence of an mVDR and its role in the non-genomic actions of calcitriol are well-accepted, there has been some debate about if nVDR participates in the calcitriol non-genomic actions. Employing RNA interference (RNAi) to knockdown either nVDR or Pdia3 was reported that calcitriol-mediated activation of src and phospholipase A2 requires mVDR (Pdia3) and nVDR in MC3T3-E1 osteoblasts (Chen et al. 2013; Hii and Ferrante 2016). Studies using C2C12 murine myoblasts in which nVDR was knocked down by RNAi support the argument that nVDR is involved in calcitriol-stimulated activation of p38 and ERK1/ERK2, src, and PI3K/Akt (Buitrago, Pardo, and Boland 2013; Hii and Ferrante 2016).

In the caveolae of MC3T3-E1 osteoblasts, nVDR has been reported to colocalize with mVDR (Pdia3) and caveolin-1, where nVDR interacts with caveolin-1. In contrast, in human skin fibroblasts, nVDR interacts with ERp57 or Pdia3, and the Pdia3 binds caveolin-1 and PLA2 activation protein, whereas nVDR binds caveolin-1 and src, but both receptors are essential for the activation of src and PLA2 (Fleet 2004; Hii and Ferrante 2016).

Rapid or non-genomic responses

Some calcitriol/mVDR complex modulating the rapid responses, have been described in processes of rapid hormonal stimulation of calcium absorption (Norman et al., 1997); insulin secretion from rat pancreatic β cells (Kajikawa et al. 1999); the vascular smooth muscle cell migration via activation PI3 kinase in cell culture (Rebsamen et al. 2002); the opening of voltage-gated Cl^{-} channels, and exocytosis in mouse osteoblast cells culture subject to 'patch clamping' (Haussler et al. 2011; Zanello and Norman 2004), and the rapid opening of Ca^{2+} and Cl^{-} channels in Sertoli cells (located in the seminiferous tubules of the testis) (Haussler et al. 2011; Menegaz et al. 2010) (Figure 3j).

Serum soluble VDR, possible biomarker of cellular damage

Few studies have focused on serum soluble VDR (sVDR) roles; in certain diseases that have an autoimmune or neurological component, have been reported that sVDR form could be implicated in the pathophysiology of these diseases.

A study realized in Turkish ankylosing spondylitis (AS) patients, reported elevated serum sVDR levels in patients with activated AS (Bath Ankylosing Spondylitis Disease Activity Index: BASDAI >4= clinical disease activity), and in turn AS patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) presented higher BASDAI scores and sVDR levels compared to those using biological agents and control groups. Higher sVDR levels were associated with peripheral joint involvement, and the presence of enthesitis, as well as the sVDR levels were positively correlated with the BASDAI scores, and systemic inflammatory markers such as C reactive protein (CRP), and erythrocyte sedimentation rate (ESR) (Kültür et al. 2019). The authors propose to sVDR as a new inflammatory marker and acute phase reactant to indicate clinical activity for AS, apart from others markers which are found to be increased and dysregulated in this disease, such as IL-22, IL-17, TNF-α, IL-6, IL-10, TLR-4, and IL-23, about which, the VDR is effective in its regulation of expression and function (Kültür et al. 2019; Yin and Agrawal 2014).

In the case of other autoimmune diseases like multiple sclerosis (MS), conversely, was found lower serum calcidiol levels in patients with MS than in control subjects, but no significant differences were observed about sVDR and VDBP levels between the groups (Aktürk et al. 2019).

In a study focused on determining the contribution of sVDR levels, serum calcidiol levels, and other clinical parameters to colorectal cancer (CRC) risk, suggested the sVDR levels as a diagnostic marker for CRC because the CRC patients showed lower serum calcidiol levels and very low concentration of sVDR compared to controls subjects (Al-Ghafari, Balamash, and Al Doghaither 2020). Authors hypothesize that these findings were due to changes in physiological conditions, because in human cancer cells, the expression of VDR and CYP27B1 increases in the initial stage of tumorigenesis, but when the tumor progresses and becomes more aggressive, the expression decreases (Al-Ghafari, Balamash, and Al Doghaither 2020; Lopes et al. 2010; Matusiak and Benya 2007).

In another study, in Caucasian migraine patients reported lower calcidiol ($p = 0.012$), and sVDR ($p = 0.038$) serum levels in these patients compared to controls subjects (Celikbilek et al. 2014). This evidence could indicate a differential pattern of the sVDR expression according to the nature of the disease studied, and its potential role as a biological marker of damage (Figure 3k).

Conclusion and perspectives

In summary, this comprehensive literature review showed the biological mechanisms about the sources, absorption process, metabolism, action mechanisms, and functions of vitamin D, which highlight as an immunomodulator nutrient. Therefore, is necessary to ensure the vitamin D sufficiency serum levels in the health and disease conditions.

The main effects of its active form, calcitriol, are carried out through genomic mechanisms due to its interaction with the nVDR, found mainly in the cytoplasm and nucleus.

Several studies, besides evaluating the calcidiol serum levels, have been interested in evaluating the sVDR serum levels in cancer and autoimmunity.

The sVDR presence in serum could be an indicator of a high rate of cell damage or apoptosis, characteristic of autoimmune diseases. However, more studies are required to elucidate this hypothesis. Likewise, a better knowledge of its non-genomic mechanisms involving a membrane receptor will be required to elucidate its non-canonical pathways.

Disclosure statement

The authors declare that they have no conflict of interest or competing for financial interest.Figures were created with BioRender software, ©biorender.com.

Abbreviations

25 (OH) D	25-hydroxy vitamin D, 25-hydroxy cholecalciferol or/and 25-hydroxy ergocalciferol
25 (OH) D_2	25-hydroxy ergocalciferol or calcidiol
25 (OH) D_3	25-hydroxy cholecalciferol or calcidiol
1α,25$(OH)_2D_3$	1 alpha, 25-dihydroxy cholecalciferol or calcitriol
1α,25$(OH)_2D_2$	1 alpha, 25-dihydroxy ergocalciferol or calcitriol
mVDR	membrane vitamin D receptor
nVDR	nuclear vitamin D receptor
Pro-vitamin D_3	7-dihydrocholesterol
RXR	retinoids x receptor
sVDR	soluble vitamin D receptor
UVB	ultraviolet B
VDBP	vitamin D binding protein
VDRE	vitamin D response elements
Vitamin D_2	ergocalciferol
Vitamin D_3	cholecalciferol

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