

Recommendations of the Brazilian Society of Endocrinology and Metabology (SBEM) for the diagnosis and treatment of hypovitaminosis D

Recomendações da Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) para o diagnóstico e tratamento da hipovitaminose D

Sergio Setsuo Maeda¹, Victoria Z. C. Borba², Marília Brasília Rodrigues Camargo¹, Dalisbor Marcelo Weber Silva³, João Lindolfo Cunha Borges⁴, Francisco Bandeira⁵, Marise Lazaretti-Castro¹

ABSTRACT

Objective: The objective is to present an update on the diagnosis and treatment of hypovitaminosis D, based on the most recent scientific evidence. **Materials and methods:** The Department of Bone and Mineral Metabolism of the Brazilian Society of Endocrinology and Metabology (SBEM) was invited to generate a document following the rules of the Brazilian Medical Association (AMB) Guidelines Program. Data search was performed using PubMed, Lilacs and SciELO and the evidence was classified in recommendation levels, according to the scientific strength and study type. **Conclusion:** A scientific update regarding hypovitaminosis D was presented to serve as the basis for the diagnosis and treatment of this condition in Brazil. *Arq Bras Endocrinol Metab.* 2014;58(5):411-33

Keywords

Vitamin D; cholecalciferol; PTH; osteoporosis; deficiency; insufficiency; diagnosis; treatment

RESUMO

Objetivo: Apresentar uma atualização sobre o diagnóstico e tratamento da hipovitaminose D baseada nas mais recentes evidências científicas. **Materiais e métodos:** O Departamento de Metabolismo Ósseo e Mineral da Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) foi convidado a conceber um documento seguindo as normas do Programa Diretrizes da Associação Médica Brasileira (AMB). A busca dos dados foi realizada por meio do PubMed, Lilacs e SciELO e foi feita uma classificação das evidências em níveis de recomendação, de acordo com a força científica por tipo de estudo. **Conclusão:** Foi apresentada uma atualização científica a respeito da hipovitaminose D que servirá de base para o diagnóstico e tratamento dessa condição no Brasil. *Arq Bras Endocrinol Metab.* 2014;58(5):411-33

Descritores

Vitamina D; colecalciferol; PTH; osteoporose; deficiência; insuficiência; diagnóstico; tratamento

INTRODUCTION

Hypovitaminosis D is highly prevalent and represents a public health problem in the entire world. Studies show an elevated prevalence of this disease in several geographic regions, including Brazil. It can affect more than 90% of individuals, depending on the population studied (1).

Vitamin D is essential in functions related to bone metabolism, but it seems to be related in the patho-

physiology of many diseases. In children, vitamin D deficiency leads to growth retardation and rickets. In adults, hypovitaminosis D leads to osteomalacia, to secondary hyperparathyroidism and consequently, to an increase in bone resorption, favoring bone mass loss and the development of osteopenia and osteoporosis. Muscle weakness can also happen, which further contributes to elevating the risk of fall and bone fractures among patients with low bone mass (2,3).

¹ Endocrinology Division, Federal University of São Paulo (Unifesp), Escola Paulista de Medicina, São Paulo, SP, Brazil
² Department of Medicine, Paraná Federal University (UFPR), Curitiba, PR, Brazil
³ Department of Medicine, Univille Medical School, Joinville, SC, Brazil
⁴ Endocrinology Division, Brasília Catholic University (UCB), Brasília, DF, Brazil
⁵ Endocrinology Division, Agamenon Magalhães Hospital, University of Pernambuco (UPE), Escola de Medicina, Recife, PE, Brazil

Correspondence to:

Sergio Setsuo Maeda
 Rua Conselheiro Furtado, 847, ap. 93
 01511-001 – São Paulo, SP, Brazil
 ssetsuo@terra.com.br

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The correct diagnosis of this condition and the identification of improvement or worsening factors can help the elaboration of more efficient strategies for the treatment of risk populations, such as the elderly and post-menopausal women.

This document represents the efforts of the Department of Bone Metabolism of the Brazilian Society of Endocrinology and Metabology (SBEM) for the development of recommendations based on evidence available in the scientific literature regarding the diagnosis and treatment of this condition. The objective of this document is to respond daily questions and to be a guideline for endocrinologists and clinicians in the Brazilian context.

MATERIALS AND METHODS

The elaboration of this guideline was motivated by the SBEM within its Practical Guidelines program. The model applied followed the Guidelines Program of the Brazilian Medical Association (AMB) and the Federal Council of Medicine (CFM). After the selection of collaborators, with a significant role and relevant publications in the area, clinical questions to be approached were elaborated.

The publication search was performed using MedLine-PubMed and SciELO-Lilacs. We used the Oxford Classification, which evaluates the study design and considers the best available evidence for each question, to categorize the recommendation level or evidence strength of each article (4,5).

The levels of recommendation and evidence strength were reported as:

- A:** experimental or observational studies with better consistency.
- B:** experimental or observational studies with less consistency.
- C:** case reports (non-controlled studies).
- D:** opinion lacking critical evaluation, based on guidelines, physiological studies or animal models.

DEFINITION AND PHYSIOLOGY

1. What is vitamin D: a nutrient or a prohormone?

Although it is defined as a vitamin, conceptually it is a prohormone. In conjunction with the parathyroid hormone (PTH), it acts as an important regulator of calcium homeostasis and bone metabolism.

It can be obtained from food sources, such as cod liver oil and from other fat-rich fish (salmon, tuna, mackerel), or from endogenous cutaneous synthesis, which represents the most important source of this “vitamin” for the majority of human beings (2,3,6,7) (A). Table 1 shows some food sources of vitamin D (3).

Vitamin D can be found in the form of ergocalciferol or vitamin D₂ and cholecalciferol or vitamin D₃ (8). Vitamin D₂ can be obtained from some yeast and plants, being produced for commercial use, through irradiation of the ergosterol present in some mushrooms (8)(D).

Table 1. Vitamin D food sources

Food	Portion	Vitamin D content per portion
Wild salmon	100 g	~ 600-1,000 IU vitamin D ₃
Fish farming salmon	100 g	~ 100-250 IU vitamin D ₃
Canned sardine	100 g	~ 300 IU vitamin D ₃
Canned mackerel	100 g	~ 250 IU vitamin D ₃
Canned tuna	100 g	~ 230 IU vitamin D ₃
Cod liver oil	5 mL	~ 400-1,000 UI vitamin D ₃
Egg yolk	1 unit	~ 20 IU vitamin D ₃
Fresh mushroom	100 g	~ 100 IU vitamin D ₂
Sun dried mushroom	100 g	~ 1,600 IU vitamin D ₂

Adapted from ref. 3.

In the skin, the precursor is the 7-dehydrocholesterol (7-DHC) (8,9). During sun exposure, UVB photons (ultraviolet B, 290-315 nm) penetrate the epidermis and produce a photochemical fragmentation to originate pre-cholecalciferol. This intermediate is converted to vitamin D (or cholecalciferol) through a temperature-dependent isomerization (Figure 1).

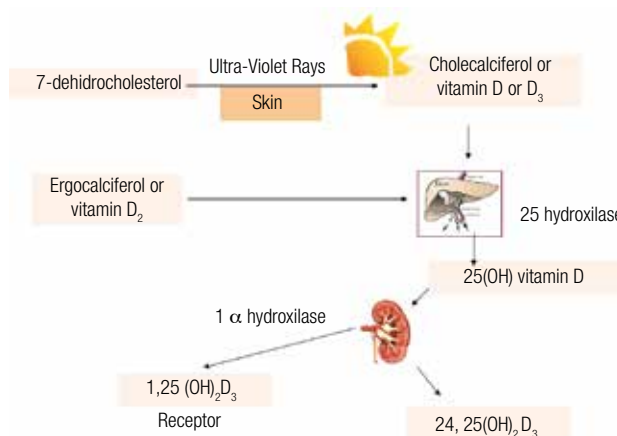


Figure 1. Photobiosynthesis of vitamin D.

Cholecalciferol is transported to the liver by DBP (vitamin D binding protein). In the liver, there is the hydroxylation of carbon 25 (CYP27B1), forming the 25-hydroxyvitamin D (25(OH)D), through a process which is not strictly regulated, since it happens without control, and depends on the combination of cutaneous and diet stocks of vitamin D (8).

After the liver step, 25(OH)D is transported to the kidneys by DBP, where it is converted to calcitriol or 1,25-dihydroxyvitamin D [1,25(OH)₂D] (Figure 1). This is the most active metabolite and it is responsible for stimulating intestinal calcium and phosphate absorption. The kidney hydroxylation is stimulated by PTH and suppressed by phosphate and FGF-23. Calcitriol production is strictly controlled by negative feedback, in a way to influence its own synthesis by the decrease of 1 α -hydroxylase. It is responsible for accelerating its inactivation through the conversion of 25(OH)D to 24,25(OH)₂D. This mechanism reflects a direct action of 1,25(OH)₂D in the kidneys, however there is still an inhibitory action on PTH production in the parathyroids (8,9). The 1 α -hydroxylase can also be found in other cells and tissues, such as the skin, prostate, breast, intestine, lungs, pancreatic β cells, monocytes and parathyroid cells. The 1,25(OH)₂D molecule can also be locally synthesized by these cells and tissues (8,9) (D).

The vitamin D receptor (VDR) belongs to the superfamily of nuclear receptors regulating the transcription factors of the steroid hormones, retinoic acid, thyroid hormones and vitamin D. After binding 1,25(OH)₂D to VDR, it interacts with the retinoic acid receptor, forming a heterodimeric complex (RXR-VDR), which then binds specific sequences of DNA, known as Vitamin D Responsive Elements (VDRE) (10,11). The main target organs for 1,25(OH)₂D are the intestine, bones, parathyroid glands and kidneys. However, its receptors have been found in several other tissues (10,11) (D).

SBEM recommendation: food sources are scarce in vitamin D and humans depend mainly on the skin production catalysed by UVB sunlight exposure (Evidence A).

2. What are the effects on bone metabolism?

SBEM recommendation: active vitamin D modulates PTH synthesis, increases intestinal calcium absorption, and improves bone mass and muscular function (Evidence A).

The best known and studied actions of vitamin D are related to bone metabolism, where it plays a crucial role. It participates in intestinal calcium absorption, muscle function, modulation of PTH secretion and bone cell function.

Parathyroid cells express the 1 α -hydroxylase enzyme, and can synthesize the active form of vitamin D (1,25(OH)₂D) inside the cells using the 25(OH)D serum pool (12) (B). In hypovitaminosis D, due to a minor intracellular synthesis, there is a secondary hyperparathyroidism, which is associated to an increase in bone resorption (2,13-16) (B), besides the fact that the circulating levels of 1,25(OH)₂D are, generally, normal. There is an inverse correlation between PTH and 25(OH)D, described in children (17) and the elderly (2). Several cut off values for 25(OH)D for the PTH normalization have been published, the majority, being around 28 and 40 ng/mL (70 to 100 nmol/L) (2,18-23) (C). Other causes of secondary hyperparathyroidism also have to be investigated, such as chronic kidney disease (creatinine clearance below 60 mL/min), Paget's disease, hungry bone syndrome and the calcium and vitamin D malabsorption syndromes (24).

Intestinal calcium absorption depends on the active vitamin D action in the duodenum, through a transcellular saturable process, whose stimulus leads to the synthesis of proteins such as calbindin-D_{9k} (CaBP-9k) and the epithelial apical channel TRPV6 (13,14) (D). However, there is evidence that the non-saturable transport, which happens with part of calcium absorption in the human ileum is also vitamin D sensitive (25). According to Heaney and cols., individuals with 35 ng/mL of 25(OH)D presented higher absorption than those with 25 ng/mL (26) (B). Increase of calcium absorption with increasing dose of vitamin D₃ or serum 25(OH)D was recently observed, but there is no evidence of what the minimum value of 25(OH)D to ensure calcium absorption from the intestine in the range of 16-52 ng/mL evaluated in the study (27).

Population studies correlated positively vitamin D concentration with bone mass, mainly of the hip, but with 25(OH)D cut off points varying from 12 to 36 ng/mL (30-90 nmol/L) (28-30) (C).

The muscle tissue expresses vitamin D receptors (13) and, clinically, muscle weakness and myopathy are observed in patients presenting severe vitamin D deficiency. Dhesi and cols., observed that the number of falls is higher among the elderly when they present the vitamin D deficiency (31) (C). The administration

of 800 IU of cholecalciferol for 12 weeks decreased in 49% the number of falls (32) (**B**). Cholecalciferol use is associated with the prevention of falls among the elderly with hypovitaminosis D, but not among those presenting normal serum values (33) (**B**).

In a meta-analysis of the main osteoporosis intervention studies; Bischoff-Ferrari and cols. indicated again 25(OH)D serum concentration above 30 ng/mL (75 nmol/L) to be the most beneficial for health in general (**A**). Bone health, here represented by a better bone mineral density (BMD), decreased risk of fall and femoral and non-vertebral osteoporotic fractures, seems to be benefited by 25(OH)D concentrations equal to or higher than 30 ng/mL (75 nmol/L), concentrations around 36 ng/mL (90 nmol/L) being suggested as the most advantageous (34-36). The same 25(OH)D values seem to benefit the muscle strength of lower limbs, which was evaluated by the TUG (Time Up and Go) test, where the individual is evaluated based on the time he needs to walk a distance equivalent to eight steps. Individuals presenting 25(OH)D in the range of 36 to 40 ng/mL (90 and 100 nmol/L) seem to perform with higher speed. Evidence also suggests that higher 25(OH)D values are associated with a lower risk for colorectal cancer and periodontal disease (36) (**A**).

Regarding the bone tissue, evidence suggests that 1,25(OH)₂D stimulates mineralization, through an indirect process that happens with the increase in intestinal absorption of the minerals which are incorporated into the bone matrix. Physiological concentrations of calcitriol promote calcium mobilization to the bones, while the administration of large doses promotes excessive bone remodeling. Osteoblasts present 1,25(OH)₂D receptor. This hormone modulates the gene expression of alkaline phosphatase and osteocalcin. Therefore, in the process of bone remodeling, 1,25(OH)₂D is important for bone formation and reabsorption (37).

Priemel and cols. evaluated 675 bone biopsies and correlated the histomorphometry findings with serum 25(OH)D concentration. The presence of bone mineralization defects was only found in individuals with concentrations below 30 ng/mL (75 nmol/L) (38) (**B**).

The role of vitamin D in non-bone related endpoints, such as mortality, cardiovascular risk, cancer and autoimmune diseases is still controversial (39,40).

DIAGNOSIS

3. How to define hypovitaminosis D?

SBEM recommendation: the analysis of the 25 hydroxyvitamin D metabolite (25(OH)D) should be used for the evaluation of the vitamin D status of an individual (Evidence A).

There is a consensus that 25(OH)D (calcidiol) is the most abundant metabolite and the best indicator for the evaluation of vitamin D status (**A**), the individuals being classified as: deficient, insufficient or sufficient in vitamin D (3,6,41,42). On the other hand, there is no consensus regarding the cut off value for the definition of “vitamin D sufficiency” (6,43,44).

The values discussed in the medical literature, based on populational studies, with emphasis on calcium homeostasis and bone health, vary from 20 to 32 ng/mL (50 to 80 nmol/L) (26,43-47). Several specialists agree that for correction of secondary hyperparathyroidism, reduction of the risk of fall and fractures and maximum calcium reabsorption, the best 25(OH)D cut off value is 30 ng/mL (75 nmol/L) (6,41,46). Thus, serum concentrations below 20 ng/mL (50 nmol/L) are classified as deficiency; those ranging from 20 to 29 ng/mL (50 to 74 nmol/L) as insufficiency and between 30 and 100 ng/mL (75 and 250 nmol/L) as sufficiency. Therefore, many consider 25(OH)D serum concentrations below 30 ng/mL as hypovitaminosis D (3,41-43,48,49). These values were recognized by the Endocrine Society guideline, although they differ from the ones accepted (20 ng/mL) by the Institute of Medicine (IOM) (50) (**B**). In general population, there is no evidence of benefit in the measurement of 25(OH)D due to the high cost, but according to the Endocrine Society, to maximize bone health; supplementation is recommended for children up to 1 year with at least 400 IU/day; between 1 and 70 years, at least 600 IU/day while over 70 years old, 800 IU/day (41).

SBEM recommendation: 25(OH)D concentrations above 30 ng/mL are desirable and should be the target for higher risk populations, because above this concentration, vitamin D benefits are more evident, especially regarding osteometabolic diseases and fall risk reduction (Evidence B).

4. What are the methodological implications for the plasma determinations of 25(OH)D?

SBEM recommendation: the methods based on chromatography are considered gold standard for the laboratory evaluation of 25(OH)D, although, automated immunometric assays can be used in the clinical practice taking into account the good correlation with the method of excellence, besides the practicality and lower cost. However, the clinician should be aware of the possible mistakes caused by several interfering conditions, possibly leading to diagnostic classification errors (Evidence B).

Circulating 25(OH)D level is the best method to evaluate the individual vitamin D status. Nevertheless, there are controversies regarding the best method for 25(OH)D determination. Some factors should be considered when the levels of this vitamin are evaluated, such as the lack of a precise physiological regulatory control (feedback), the variability of methods and standards, the inclusion of contaminant metabolites in the analysis, among others. Radioimmunoassays (RIA) used in the past underestimated the levels of 25(OH)D when the dominant levels were 25(OH)D₂. RIA have been replaced by automated chemiluminescent immunoassays, resulting in higher concentrations and by immunoenzymatic assays which measure total 25(OH)D, a combination of vitamin D₂ (25(OH)D₂) and vitamin D₃ (25(OH)D₃) (51) (B).

The methods that do not employ direct immune detection are high performance liquid chromatography (HPLC) coupled to mass spectrometry (LC-MS), which can distinguish individual levels of 25(OH)D₂ and 25(OH)D₃. These are considered the gold standard for analysis and currently used as reference (52) (B). Both 1,25(OH)₂D and 25(OH)D circulate predominantly bound to proteins and their concentration can be determined. However, to evaluate the vitamin D status, 25(OH)D total serum level is used, including both D₃ and D₂ forms. The results can be reported in nanograms per milliliter (ng/mL) or nanomol per liter (nmol/L). For conversion, you just need to multiply the value obtained in ng/mL by 2.5, to obtain the value in nmol/L. Automated methods allow the use in clinical routines, they are fast and report 25(OH)D₂ and 25(OH)D₃ together, while LC-MS methods can distinguish between 25(OH)D₂ and 25(OH)D₃, being useful, then, for the evaluation

of the effectiveness of D₂ supplementation, versus endogenous D₃ production. These chromatographic methods, although more precise, are more laborious and expensive (53) (B).

SBEM recommendation: for conversion of 25(OH)D concentration from ng/mL to nmol/L, the multiplication factor 2.5 is applied.

The accuracy of the measurements varies widely between laboratories and between different assays, and even when testing identical samples, this variation can achieve 17 ng/mL (53). The immunoassay requires the development of selective antibodies for 25(OH)D₂ and 25(OH)D₃, which preferentially do not show cross reaction with any other metabolite. Matrix effects can still occur, caused by endogenous components that modify the binding of the antibody to the material to be analyzed. Metabolites with lower physiological potential end up being included in the quantification, such as the 3-epimer of the 25(OH)D, which can correspond to up to 5% of the total 25(OH)D. As its molecular weight is identical to 25(OH)D, these are not separated by LC-MS. Finally, 24,25 dihydroxyvitamin D (24,25(OH)₂D), considered an inactive metabolite, can correspond to up to 20% of the 25(OH)D measured, whereas some assays show 100% cross-reaction (51,54).

The use of a standard cut off value to evaluate vitamin D status is problematic if applied to all laboratories and all methods, considering there are still differences on vitamin D extraction from its binding protein, cross reaction between 25(OH)D₂, 25(OH)D₃ and other metabolites, besides the lack of standardization (52,53) and for this reason, quality control tools, such as DE-QAS (International Vitamin D External Quality Assessment Scheme) were created, as an attempt to decrease the variation in data analysis (55).

The most used methods nowadays are competitive assays, based on specific antibodies and non-radioactive markers, the improvement in the comparison between results obtained from different methodologies being necessary. Whatever the method employed is a precise definition of the normality range is fundamental (56). It is also important to highlight that the intra-individual variability can vary from 12.1 to 40.3% (57).

The clinical conditions that interfere with 25(OH)D serum concentrations are highly dependent on environmental factors and lifestyle, particularly UVB sun-

light exposure. Polymorphisms in CYP27B1, which codes for 1 α -hydroxylase, showed strong correlation with variations in 25(OH)D level. The vitamin D binding protein (DBP) is the main transporter for vitamin D metabolites, its phenotype helping predict 25(OH)D serum concentrations. Certain polymorphic forms can be more efficient for vitamin D binding, activation and metabolism, interfering with circulating levels. Genetic polymorphisms greatly contribute to the heterogeneity of clinical manifestations of hypovitaminosis D, especially among ethnic groups (51,58) (B).

EPIDEMIOLOGY

5. Which are the risk populations for hypovitaminosis D? What is the prevalence in Brazil?

SBEM recommendation: the analysis of 25(OH)D concentration is not recommended for the general population. It is recommended for specific groups belonging to populations at risk or for those with relevant clinical condition in which deficiency is suspected (Evidence A).

The Department of Bone and Mineral Metabolism from SBEM agrees with the guidelines published by the Endocrine Society, which does not recommend the 25(OH)D test for the general population considering the cost of this evaluation. The laboratory test is recommended for individuals under risk for hypovitaminosis D or for those with a relevant clinical condition. The candidates to be tested are the ones presenting the following conditions: rickets or osteomalacia, osteoporosis, history of falls and fractures in the elderly, obesity, pregnant and lactating women,

patients with malabsorption syndromes (cystic fibrosis, inflammatory bowel disease, Chron’s disease, bariatric surgery), renal or liver insufficiency, hyperparathyroidism, medications interfering in vitamin D metabolism (anticonvulsants, glucocorticoids, antifungal, antiretroviral, cholestyramine, orlistat), granulomatous diseases and lymphomas (41,59) (A). It is also useful for the evaluation of hypothesis of vitamin D intoxication.

Besides that, it is important to highlight that all the conditions that limit sunlight exposure can potentially cause hypovitaminosis D and can be added to the list of individuals in photoprotection regimen (60) (D) and religious garment users (veil, burqa, cassock and others) (61-63) (C).

Hypovitaminosis D is a world health problem and Brazil is part of this scenario, also presenting an elevated prevalence of hypovitaminosis D in the population (B). Table 2 presents some of the main Brazilian and international studies that included Brazil, published in the last decade. In general, in several regions of the country, the values indicate sub-optimum vitamin D concentrations, verifying high prevalence of hypovitaminosis D in several age groups. The majority of the studies included mainly the elderly and post-menopausal women which are the populations at risk for osteoporosis (C). However, three studies involving adolescents showed high prevalence of hypovitaminosis D in this age group of the Brazilian population (64-66). The factors that seem to favor the presence of higher serum concentrations in our population are: younger age (64,67-69), community life (70), the practice of outdoors physical activity (64,71), vitamin D oral supplementation (72), season of the year (spring, summer) (68-73), residence in sunny beach areas (74,75) and in lower latitudes (76).

Table 2. Prevalence of hypovitaminosis D in Brazil

Author, year	n	Population	Age (years) Average \pm SD	Local, Latitude	25(OH)D (ng/mL) Average \pm SD	25(OH)D (ng/mL) Cut off value	Prevalence (%)	Vitamin D intake or oral supplementation
Saraiva, 2005 (73) and 2007 (70)	420	Elderly, > 65 years		São Paulo, SP 23°S				7% with oral supplementation: daily dose 125-1.000 IU 4% with supplementation
	177	Institutionalized 125 W and 52 M	76 \pm 9		14.4 \pm 9.2	< 10 < 20 < 40	41 71 99	10% with supplementation
	243	From the community 168 W and 75 M	79 \pm 6		19.6 \pm 11.2	< 10 < 20 < 40	16 42 96	

continuation

Author, year	n	Population	Age (years) Average \pm SD	Local, Latitude	25(OH)D (ng/mL) Average \pm SD	25(OH)D (ng/mL) Cut off value	Prevalence (%)	Vitamin D intake or oral supplementation
Lips, 2006 (78)	151	Post-menopausal women with osteoporosis, > 41 years, in ambulatorial follow up	67	Vitória, ES 20°S	32.4	< 20 < 30	15 42	Values not mentioned
Maeda, 2007 (68)	121	Volunteers, 17-33 years, 72 W and 49 M	24 \pm 2	São Paulo, SP 23°S	31.2 \pm 13.2	< 28.8 < 40	50 75	Without supplementation
Silva, 2008 (72)	180	Endocrinology ambulatorial patients, 14-91 years, 165 W and 15 M	58	Belo Horizonte, MG 19°S	39.6 \pm 16.8	< 14 < 32	0.8 42	27% with oral supplementation: varied doses
Peters, 2008 (64)	136	Adolescents, 16-20 years, 72 W and 64 M	18	Indaiatuba, SP 23°S	29.2 \pm 0.8	\leq 10 \leq 30	0 62	Average daily intake 140 IU
Russo, 2009 (77)	251	Post-menopausal volunteers with low bone mass, 50-85 years	67 \pm 6	Rio de Janeiro, RJ 22°S	26 \pm 10.4	<10 < 20 < 30 < 40	2 27 67 92	Without supplementation
Kuchuk, 2009 (79)	1.486	Post-menopausal women with osteoporosis, 50-85 years	-	Latitude 15°S-23°S	28 \pm 7.6	< 10 < 20 < 30	0.5 12.5 66	Values not mentioned
Lopes, 2009 (80)	415	Post-menopausal women with and without fractures	Without fracture: 72.1 \pm 4.4 with fracture: 74.6 \pm 5.8	São Paulo, SP 23°S	Without fracture: 20.7 \pm 10.7 with fracture: 16.9 \pm 8.2	< 30	Without fracture: 82.3% with fracture: 93.65%	Supplementation users were excluded
Maeda, 2010 (71)	99	Physical exercise practitioners, 2 hours a week of outdoors physical activity, 52 W and 47 M	67 \pm 6	São Paulo, SP 23°S	31.6 \pm 12.4	< 10 < 20	3 19	7% with oral supplementation: 200-400 IU
Unger, 2010 (67)	603	Volunteers: employees and students from USP, 18-80 years, 485 W and 118 M	47 \pm 13	São Paulo, SP 23°S	Median post winter: 21.4 Post summer: increase of 10.6	< 30	77	Parameters not evaluated
Bandeira, 2010 (74)	93	Healthy post-menopausal women	65 \pm 7	Recife, PE 8°S	28.8 \pm 14.8	< 20	24	Parameters not evaluated
Neves, 2012 (75)	91	The elderly with SAH, > 60 years, 81 W and 10 M	69 \pm 7	João Pessoa, PB 7°S	Adequate: 44.8 \pm 12.5 Non-adequate: 24 \pm 3.5	< 20 < 30	4 33	Without supplementation
Santos, 2013 (66)	234	Adolescents 7-18 years	13.0 \pm 1.9	Curitiba, PR 25°	Varied from 20.8 to 22 in the studied tertiles	< 30 < 20	90,6 63,7	Without supplementation
Oliveira, 2013 (65)	160	Adolescents, 15-17 years, 71 W and 89 M	16	Juiz de Fora, MG 21°S	-	\leq 10 \leq 30	1,3 70,6	Average daily intake 88 IU

continuation

Author, year	n	Population	Age (years) Average \pm SD	Local, Latitude	25(OH)D (ng/mL) Average \pm SD	25(OH)D (ng/mL) Cut off value	Prevalence (%)	Vitamin D intake or oral supplementation
Maeda, 2013 (69)	591	Volunteers, 17-100 years, 388 W and 203 M	Nursing homes: 76.2 \pm 9.0 Community: 79.6 \pm 5.3 Exercise: 67.6 \pm 5.4 young: 23.9 \pm 2.8	São Paulo, SP 23°S	Nursing homes: 15.0 \pm 11.9 community: 19.8 \pm 11.0 Exercise: 31.5 \pm 12.4 young: 34.5 \pm 14.0	< 10 < 20 < 30	19 47 73	6% with oral supplementation: 200-400 IU
Arantes, 2013 (76)	1.933	Post-menopausal women with low bone mass, 60-85 years	67 \pm 5	Latitude 8°S-33°S	27.2 \pm 8.4	\leq 30	68,3	Parameters not evaluated
Martini, 2013 (81)	636	Adolescents, adults and the elderly	-	São Paulo, SP 23°S	M: 16.7 W: 19.2	< 20	-	Intake varied from 108 to 140 IU/d
Cabral, 2013 (82)	284	Men, skin phototype evaluated	69.4 \pm 6.5	Recife, PE 8°S	28.0 \pm 13.6	< 20 < 30	31,5 66,7	2,5% took supplements

M: men; W: women; IU: international units.

TREATMENT

6. How to treat hypovitaminosis D in patients who are at high risk for the deficiency?

SBEM recommendation: generalized vitamin D supplementation is not indicated for the entire population. The benefits of the vitamin D treatment are more evident in populations presenting high risk for the deficiency (Evidence A).

Current evidence does not support the concept of general population supplementation (41) (A). As the adequacy of vitamin D concentration in our population is closely related to the cutaneous production, secondary to sunlight exposure, individuals with low exposure represent the main population of deficient individuals. Therefore, a simple interview can bring important information on the probability of vitamin D deficiency in a specific individual.

The complementation of the daily needs, as well as the treatment of the deficiency should be performed for individuals with hypovitaminosis D risk (see Epidemiology section) or those to whom sunlight exposure is prohibited, due to skin cancer, transplants or systemic lupus erythematosus (A).

The most available vitamin D form for treatment and supplementation is cholecalciferol or vitamin D₃ and this is the metabolite that has been shown to be the most effective one. Ergocalciferol or vitamin D₂ can also be used as a supplement, however the studies show that, as its half-life is a little shorter than the one of D₃, it should be used preferentially daily (83). Besides that, some laboratory methods that test 25(OH)D recognize only 25(OH)D₃, what can bring problems for the control of plasma levels when vitamin D₂ is used for supplementation. Therefore, although supplementation and treatment can be done with both vitamin D metabolites, preference should be given to vitamin D₃, due to the advantages on the maintenance of more stable concentrations.

The treatment doses vary according to the degree of deficiency and the target to be achieved. Apparently, 25(OH)D concentrations higher than 12 ng/mL would be sufficient to avoid rickets and osteomalacia, as well as to normalize intestinal absorption of calcium (84,85). However, to reduce fractures, concentrations above 24 ng/mL are necessary (86), while to avoid the development of secondary hyperparathyroidism, concentrations above 30 ng/mL are desirable (69). Therefore, especially during osteoporosis treatment, it

is recommended that plasma 25(OH)D is kept above 30 ng/mL.

As a practical rule, one can predict that, for every 100 IU supplemented, an increase of 0.7 to 1.0 ng/mL is gained in the concentration of 25(OH)D (41). However, other studies show that this dose-response curve is not linear.

In a study developed in Brazil, with an institutionalized population, showing high prevalence of hypovitaminosis D (40.4% with 25(OH)D < 20 ng/mL), supplementation with 7,000 IU/day, produced an average elevation of 7.5 ng/mL in 25(OH)D concentration after three months (87) and this elevation achieved a plateau around six weeks. However, as it has already been recognized by other authors, this increase was more evident among those individuals with lower initial values (< 20 ng/mL), in whom the average increase was 10.3 ng/mL after three months of treatment, while those showing 25(OH)D concentration above 20 ng/mL, increased on average only 5.18 ng/mL. Besides that, 45% of the individuals still kept insufficient (30 ng/mL) and 10% still kept deficient (< 20 ng/mL) at the end of three months of supplementation. This demonstrates that, for values lower than 20 ng/mL, doses higher than 1,000 IU/day will be necessary if the target to be achieved is 30 ng/mL (B).

In a similar institutionalized population, Moreira-Pfimer and cols. demonstrated, in a randomized, double blind prospective placebo controlled study, that an average dose of 3,700 IU/day of vitamin D₃ for six months was able to take the treated group to average concentrations of 34.6 (variation from 20.9 to 48.4) ng/mL, while the placebo group kept in 20.7 (variation from 9.4 to 41.2) ng/mL (p < 0.0001). There was a significant increase in calcemia for the treated group, but no patient developed hypercalcemia (88) (A).

Those institutionalized and bedridden are a population with elevated risk for deficiency. Mocanu and cols. evaluated the effect of the fortification of the bread roll with 320 mg of calcium and 5,000 IU of vitamin D on an institutionalized population for 12 months. It was possible to verify an effective increase in 25(OH)D (initial average 11.4 ng/mL and final average 50.0 ng/mL), with 92% of the individuals achieving concentrations higher than 30 ng/mL. No individual developed hypercalcemia ou hypercalciuria. PTH concentrations were reduced during treatment and there was a significant increase in BMD of the lumbar spine and proximal femur (89). However, when reevaluated, this same

population, three years after removal of supplementation, it was verified that the benefits gained with vitamin D supplementation had been lost (90).

In a population of post-menopausal women undergoing treatment for osteoporosis, followed in a specific outpatient clinic, Camargo (91) demonstrated that weekly doses higher than 7,000 IU (> 1,000 IU/day) are necessary to achieve vitamin D sufficiency (25(OH)D > 30 ng/mL), which is in accordance with the proposition of the Endocrine Society for the elderly at risk (Table 3). According to this Brazilian study, 73% of the patients, followed for at least three months in ambulatory directed to the treatment of osteoporosis were below the desired target concentration (> 30 ng/mL) (Figure 2). In the same study, a positive correlation was found between 25(OH)D concentrations and the femur BMD and a negative correlation with PTH (91).

Table 3. Vitamin D daily maintenance doses recommended for the general population and the population at risk for the deficiency

Age groups	General population (IU)	Population at risk (IU)
0 – 12 months	400	400 – 1,000
1 – 8 years	400	600 – 1,000
9 – 18 years	600	600 – 1,000
19 – 70 years	600	1,500 – 2,000
> 70 years	800	1,500 – 2,000
Pregnant women 14 – 18 years	600	600 – 1,000
Pregnant women > 18 years	600	1,500 – 2,000
Lactating 14 – 18 years	600	600 – 1,000
Lactating > 18 years	600	1,500 – 2,000

Adapted from the nutritional tables of the Institute of Medicine and the Endocrine Society.

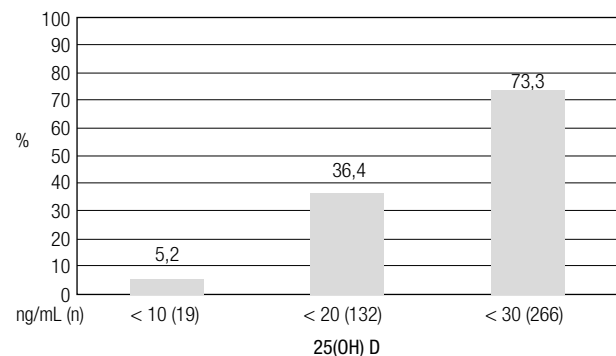


Figure 2. Percentage of non-adequacy of vitamin D according to the plasma concentrations of 25-hydroxyvitamin D (25(OH)D) in a population of individuals presenting osteoporosis at a medical school teaching ward (91).

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In children and adolescents, the doses, apparently, do not vary much from the ones in the adults, with the exception of the first year of life (Table 3). Winzenberg and cols., in a meta-analysis involving six studies that evaluated vitamin D supplementation in healthy children, were able to group 343 participants that received placebo and 541 participants that received vitamin D and the analysis suggested a benefit of the supplementation on the in lumbar spine bone mineral density and total body bone mineral content of those children that were previously deficient (92). Therefore, as already observed in other studies, the positive endpoints of the supplementation are always much more evident when the populations studied were initially deficient (A).

Vieth in a study done in Canada, tested two vitamin D₃ doses, compared to placebo. The first dose was 1,400 IU/week (or 200 IU/day), the same quantity recommended at current nutritional tables from the Ministry of Health in Brazil. The second dose was 14,000 IU/week, both groups being followed for 12 months. The group that received 1,400 IU/week did not present significant increase in 25(OH)D concentration, while the group receiving 14,000 IU obtained an increment of 15 to 30 ng/mL at the end of 12 months (93). Taking from that and similar studies, an alteration on the vitamin D daily recommendation tables was proposed in several countries.

In 2011, the Institute of Medicine, in the USA, an organ that regulates the reference tables for daily recommended intake (DRI) for the general population, increased the daily recommendation to 600 IU for individuals between the ages of 1 and 70, and to 800 IU for those older than 70 (Table 3) (50,94). However, the Brazilian nutritional table remains with the daily recommended intake of 200 IU, although several national studies have demonstrated that the typical meals of the Brazilian population are not a relevant vitamin D source (64,95), that we depend on cutaneous synthesis to obtain sufficiency and that the deficiency is present in all age and populational groups, especially among the elderly (70,74,76,80) (C).

Generally speaking, when 25(OH)D is lower than the target concentration (below 20 ng/mL), an attack dose is necessary to replenish the body stocks. The most used scheme currently is to administer 50,000 IU/week (or 7,000 IU/day) of vitamin D for six to eight weeks (41). In case the desired concentration of 25(OH)D is not achieved, a new cycle can be proposed. As there can be some individual variation in the

response to treatment, the reevaluation of the plasma values after each cycle is ideal, especially in the cases of more serious deficiencies, up to achieving the desired concentration. After this period, the maintenance dose should be defined and it varies according to the age group and concurrent conditions (Table 3). For the adults, maintenance doses vary between 400 and 2,000 IU, depending on the sunlight exposure and skin tone. For the elderly, the recommended doses vary from 1,000 to 2,000 IU/day or 7,000 to 14,000 IU/week. Obese individuals, presenting malabsorption conditions or in use of anticonvulsants might need doses that are twice or three times higher (41) (A).

SBEM recommendation: doses recommended for the maintenance of vitamin D sufficiency are indicated in table 3, varying for the different age groups. However, for deficiency correction, higher doses (attack doses) are necessary (Evidence A).

Due to the fact that it is a liposoluble substance, vitamin D is absorbed with fats and follows the enterohepatic circulation, meaning it is normally secreted through the gallbladder and reabsorbed in the small intestine. Thus, especially in cases of malabsorption, doses which are much higher than usual can be necessary in order to normalize 25(OH)D concentrations. Besides that, for supplementation studies and in the daily practice it is possible to notice some individual variation in blood concentrations of 25(OH)D reached in response to a same dose of vitamin D, suggesting individuals might present different competencies in intestinal absorption or its metabolization (96). It seems there is no difference considering vitamin D absorption in relation to fasting or meal type (97).

SBEM recommendation: the risk of intoxication with the defined doses in this document is almost null (Evidence A). Special care must be taken when pharmaceutical compounding is used (Evidence D) and with patients that have diseases with anomalous production of 1,25 dihydroxyvitamin D by the tissues, such as in granulomatous diseases and some lymphomas (Evidence B).

Vitamin D₃, when administered as described above is very safe. Doses of up to 10,000 IU per day for five months did not induce signals of toxicity, which can be translated as hypercalcemia and hypercalciuria (98) (A). Toxic concentrations of 25(OH)D (> 90 ng/mL) are

difficult to achieve with these routine doses (99). In rare clinical situations, such as in some cases of granulomatous diseases (sarcoidosis, tuberculosis and chronic fungal infections) and some lymphoma, activated macrophages can locally produce $1,25(\text{OH})_2\text{D}$ in excess and induce hypercalcemia and hypercalciuria (B). Children with Williams syndrome are more predisposed to hypercalcemia. Therefore, under these conditions, supplementation should be more criterious and follow frequent monitoring of plasmatic and urinary calcium (41).

7. What are the differences between vitamin D_2 and D_3 ?

SBEM recommendation: vitamin D deficiency treatment and supplementation can be done either with vitamin D_3 (cholecalciferol) or with vitamin D_2 (ergocalciferol), although the first metabolite presents some advantages over the second, due its commercial availability, for being more evaluated in clinical studies, for allowing more posologic formulations, promoting more effective increases and for being identified by all laboratory methods (Evidence B).

Vitamin D sources are: sunlight exposure, diet and supplementation. The main difference between vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) is the source. In summary, vitamin D_2 is the vitamin D from the plant sources, while the one from animal sources is in the form of vitamin D_3 . The D_2 and D_3 sources differ only due to the presence of an additional double bond and a methyl group incorporated to the long side chain of the biological form called D_2 (100). The two forms present equivalent biological power and are activated in equally efficient ways by hydroxylases in humans. However, there is controversy on the bioequivalence of these formulations for supplementation. A meta-analysis evaluating only controlled and randomized studies that use vitamin D_2 and D_3 showed that vitamin D_3 increased $25(\text{OH})\text{D}$ levels more significantly when compared to vitamin D_2 ($p = 0.001$), the single or in bolus dose of vitamin D_3 being better than D_2 ($p = 0.0002$). However, this advantage was lost in daily supplementation (101) (A). Previous studies had already shown a small superiority of vitamin D_3 when administered in single dose, to maintain $25(\text{OH})\text{D}$ levels for longer time (102).

When the two formulations were compared in daily use for 25 weeks, it was observed that those using vitamin D_2 presented $25(\text{OH})\text{D}$ average concentrations lower when compared to those that received D_3 , although, without altering PTH levels (103) (A).

A recent study did not demonstrate difference in effectiveness when higher $25(\text{OH})\text{D}$ levels were found and also in sustained serum concentration of $1,25(\text{OH})_2\text{D}_3$, after 11 weeks of supplementation with 1,000 IU of vitamin D_2 or D_3 per day (104) (A).

The same was observed in the treatment of children with rickets and controls, where there was a similar increase in $25(\text{OH})\text{D}$ and $1,25(\text{OH})\text{D}$ levels with both formulations (105) (A). It is possible to conclude that both forms are equivalent in relation to daily supplementation and that vitamin D_3 presents superiority in relation to the maintenance of the $25(\text{OH})\text{D}$ levels for single dose supplementation.

8. What is the difference between vitamin D and calcitriol?

SBEM recommendation: vitamin D active forms, such as calcitriol or alfacalcidol, should not be used when the objective is supplementation, or in the treatment of vitamin D deficiency, because of their higher risk of side effects (Evidence A).

Calcitriol or $1,25(\text{OH})_2\text{D}$ is an active hormone, a final product of two vitamin D hydroxylations. Its endocrine action starts with renal production, finely controlled by the activity of the 1α -hydroxylase enzyme. This enzyme, present in the epithelial cells or the proximal convoluted tubules is stimulated mainly by the PTH and is inhibited by FGF-23, among other less important regulators. Circulating calcitriol itself also deviates its synthesis to an inactive product, the $24,25(\text{OH})_2\text{D}$ and, this way, protects the organism from its excess. The 1α -hydroxylase enzyme was identified in different tissues, what makes us believe there is some local production of calcitriol, with autocrine and paracrine actions. Opposite to renal cells, where calcitriol production is rigorously controlled, in these other tissues it is believed that production only depends on the presence of substrate (14,41). While all the systems of the organism are fully functioning, the recommendation is for the supplementation and treatment of the deficiency to be done with vitamin D itself, allowing tissues to produce their necessities, controlled by local or hormonal mechanisms, in the case of renal tubular cells. The use of calcitriol should be reserved for special situations, such as in chronic kidney insufficiency, in type 1 and type 2 vitamin D dependent rickets and in hypophosphatemic rickets, or in cases of extreme malabsorption. The use of calcitriol presupposes a much more rigorous

control of calcemia and calciuria, because hypercalcemia can frequently occur (14,41) (A).

9. How to do supplementation in special cases?

a) In chronic kidney disease

The patient with chronic renal disease presents higher risk for vitamin D deficiency. In renal disease, PTH concentrations also correlate with circulating 25(OH)D levels (A). Therefore, it is believed that vitamin D deficiency contributes to the development of secondary hyperparathyroidism in chronic renal patients, independent on the renal calcitriol production. Nowadays it is known that several tissues, such as macrophages and osteoblasts have the capacity of producing active vitamin D (calcitriol) and that this synthesis depends on the substrate, therefore, it is not strictly regulated as the renal synthesis. Because of that, the treatment of the deficiency and the adequacy of the circulating levels of 25(OH)D is always recommended each time plasma concentrations are lower than 30 ng/mL (106-109). According to the opinion of the committee responsible for writing the treatment guidelines for osteomineral disease in chronic renal patients in Brazil, 25(OH)D quantification is recommended at the end of each cycle of attack doses, until the target concentration is achieved, and from then on, every six months (110) (D).

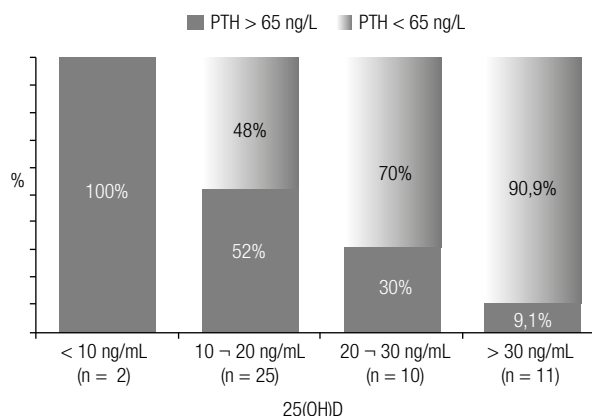
b) In the treatment of osteoporosis

A good part of vitamin D benefits on the risk of fractures observed in the literature has been associated with the concomitant use of calcium. Therefore, the adequacy of calcium intake, either through diet, or through the use of calcium salts, is part of any protocol for osteoporosis treatment. Recommended vitamin D doses are those capable of taking and maintaining plasma concentration to 30 ng/mL or above, avoiding, this way, the secondary hyperparathyroidism and the increase in bone resorption (111-113) (A). The non-adequacy of vitamin D concentration is considered one of the potential failures in medicinal treatment of osteoporosis (significant BMD loss and fractures).

SBEM recommendation: for patients with osteoporosis and increased risk of fractures, it is recommended that the 25(OH)D concentrations are maintained above 30 ng/mL for full benefits, for the prevention of secondary hyperparathyroidism, decreased risk of fall and improvement of BMD. To this end, daily doses between 1,000 and 2,000 IU are necessary (Evidence A).

c) In obesity and post-bariatric surgery

Obese patients present lower vitamin D concentration when compared to non-obese and are considered a population at risk for the deficiency (41) (A). Nowadays, bariatric surgery is a very used alternative to induce weight loss in these individuals, possibly further aggravating this deficiency. Therefore, it is advisable to correct this condition prior to surgery. Santos and cols. demonstrated that women submitted to bariatric surgery at least three years before, presented lower 25(OH)D values when compared to normal paired controls, 77.1% of them presenting vitamin D insufficiency/deficiency and 41.7% presenting secondary hyperparathyroidism (Figure 3) (114). 25(OH)D concentrations correlated inversely with PTH ($r = -0,57$, $p < 0,05$) and directly with bone remodeling markers (CTX and osteocalcin) which, together, can justify the increased risk for fracture observed in this population by other researchers (C). Depending on the surgical technique used and the degree of disabsorption promoted, some individuals might have a lot of difficulty normalizing 25(OH)D and PTH concentrations, being necessary to follow these parameters and to consider a new attack dose or even higher maintenance doses that should be individually adjusted (D). In some cases, the orientation for frequent sunlight exposure and use of parenteral vitamin D are resources that can be necessary (115-117).



25(OH)D: 25-hydroxyvitamin D. Significance level of the chi-square test ($p < 0.025$).

Figure 3. Prevalence of secondary hyperparathyroidism in a population of women submitted to bariatric surgery at least three years prior to the study enrollment. Different ranges of circulating 25(OH)D (114).

SBEM recommendation: obese people are a population at risk for deficiency and after bariatric surgery it is further aggravated, leading to a secondary hyperparathyroidism and an increased risk for fractures (Evidence A). 25(OH)D test in this situation is very useful for the titration of the daily vitamin D doses, which can be as high as ten times the routine ones (Evidence D).

d) Pregnancy

It is a critical period, because women are oriented to avoid sunlight exposure. Vitamin D deficiency in pregnant women was associated to low birth weight of the newborn, besides some late endpoints, such as low bone mass and cardiovascular risk markers in school age children. In a recent meta-analysis, Aghajafari and cols. analyzed 31 studies, including 18,869 individuals and they concluded that the serum levels of 25(OH)D are related to gestational diabetes, preeclampsia, low birth weight newborn and bacterial vaginosis (118). In another meta-analysis, vitamin D supplementation showed positive effect on the low birth weight reduction (119) (A). The doses recommended for supplementation in this period of life can be found in table 3.

When vitamin D deficiency is suspected, the treatment with higher doses is still indicated, but daily doses are preferable. The 25(OH)D concentration in the newborn shows high correlation with the one found in the mother. The placenta presents the 1 α -hydroxylase enzyme and therefore, has the capacity of converting 25(OH)D to calcitriol. Apparently, this production is not strictly controlled as that what happens in renal tubules, and depends only on the amount of substrate (41) (C). For this reason, the recommendation during pregnancy is to avoid weekly or monthly doses.

SBEM recommendation: for pregnant women presenting deficiency risk, treatment brings benefits for the mother (Evidence B) and the newborn (Evidence A). Daily doses of vitamin D are recommended during pregnancy and weekly or monthly higher doses should be avoided, because the placental production of calcitriol is substrate-dependant (Evidence C).

10. When should active vitamin D analogues be used?

SBEM recommendation: vitamin D analogues can be used to suppress PTH concentrations in patients with hyperparathyroidism secondary to chronic kidney disease (CKD), the indication and the dose depending on the CKD stage and the PTH level (Evidence A). There is no superiority of the analogues currently available in Brazil in comparison with cholecalciferol and calcitriol for the prevention of fractures (Evidence B). The analogues are not recommended for the prevention of cancer (Evidence B).

Active vitamin D analogues are synthetic substances that directly bind the vitamin D receptor (VDR). They present different selectivity to the parathyroid cells, depending on its chemical structure, calcitriol (1,25(OH)₂D₃) shows smaller selectivity, promoting more side effects such as hypercalcemia, hyperphosphatemia and vascular calcifications. More selective compounds such as paricalcitol (19-nor-1 α ,25(OH)₂D₂), maxacalcitol (22-oxa-1 α ,25(OH)₂D₃) and doxercalciferol (1 α (OH)D₂) and eldecalcitol (1 α ,25(OH)₂-2 β -(3-hydroxypropyloxy)D₃) and doxercalciferol (1 α (OH)D₂) and eldecalcitol (1 α ,25(OH)₂-2 β -(3-hydroxypropyloxy)D₃) promote less adverse effects. Doxercalciferol and the alfacalcidol require liver 25-hydroxylation to become active (120) (A).

a) Use in secondary hyperparathyroidism

The analogues are classically used to suppress PTH levels in patients with secondary hyperparathyroidism (SHPT) and chronic kidney disease (CKD). In CKD there is an increase in PTH levels, secondary to an alteration of the regulation of the fibroblast growth factor (FGF-23) in the PTH-vitamin D axis and the decrease of calcitriol production due to CKD itself. The suppression of PTH levels in patients in stages 3-4 of CKD is more than 40% in 90% of the patients (121,122) (A).

The dose used is variable depending on the CKD stage, if the patient is undergoing dialysis or not and also on the serum PTH concentration. In CKD stages 3-5, the ideal PTH levels are not defined yet, other PTH increasing factors having to be discarded. Hypocalcemia, vitamin D deficiency and hyperphosphatemia should be corrected initially. If PTH levels remain elevated and progressively increasing, the use of analogues such as calcitriol, should be considered. In CKD stage 5D with elevated and sustained PTH levels, the recommendation is to maintain PTH levels between two and

nine times the upper limit of normality. There is no consensus on the doses of doxercalciferol and paricalcitol, some studies calculated the dose in relation to the initial PTH value dividing it by 80 to 120, to minimize the excessive suppression of PTH or hypercalcemia and hyperphosphatemia (106) (B).

The use of vitamin D analogues minimizes bone loss in CKD due to the suppression of PTH levels and prevents bone remodeling reduction, due to the effect on the differentiation of normal osteoblasts and inhibition of osteoclastogenesis. However, the concern with the excessive suppression, which would lead to adynamic bone disease persists (122) (B).

Several studies have demonstrated benefit on the survival of patients undergoing dialysis treated with calcitriol or vitamin D analogues. Besides that, there is a smaller risk of progression for terminal renal disease and an increase in the survival in patients with CKD stages 3-4 (123,124) (B). There is doubt in relation to the benefit of vitamin D active analogues, compared to placebo, in relation to fractures, quality of life, hospitalizations, muscle function and fall in these patients (125,126) (A).

The most selective analogues, compared to calcitriol, demonstrate lower mortality, lower number of hospitalizations and lower duration of each hospitalization per year (124) (B). Doxercalciferol, similar to paricalcitol, presents higher benefit in relation to survival, when compared to calcitriol. A dose-response was observed in the benefit when the levels of PTH were adjusted according to vitamin D (127) (B).

The use of analogues to avoid the evolution of nephropathy in diabetic patients is discussed, although the results are still conflicting, using microalbuminuria and albuminuria as markers. Results from VITAL study are awaited for a more definitive observation (120,128,129) (B).

b) Fracture prevention

The relationship between vitamin D levels with falls and fractures has been described, also as a significant linear predictor of major osteoporotic fractures in ten years (130) (B). A recent meta-analysis demonstrated a modest decrease in the prevention of fractures with the use of $1,25(\text{OH})_2\text{D}_3$ (calcitriol) and 1α -hydroxyvitamin D_3 (alfacalcidol), similar to that obtained using doses higher than 700 IU/day of vitamin D_3 (35) (A).

Eldecalcitol ($1\alpha,25$ -dihydroxy- 2β -[3-hydroxypropiloxy] vitamin D_3) is a new analogue of vitamin D

active form, recently approved for the treatment of osteoporosis in Japan. Eldecalcitol presents a strong inhibitory effect on bone resorption and significantly increases bone mineral density. This drug showed a 26% decrease in the incidence of new vertebral fractures in three years and 71% decrease in the risk of wrist fracture, in comparison to alfacalcidol, but showed no benefit against hip fracture. An increase in serum calcium was observed, although hypercalcemia was only seen in 0.4% of patients (131) (A). Eldecalcitol compared to alfacalcidol promoted better quality of life and decreased severity of vertebral fractures (125,126) (A).

NON-BONE ACTIONS OF VITAMIN D

11. What is the evidence for extra-skeletal effects of vitamin D?

SBEM recommendation: although observational studies show an association between low vitamin D concentration and alterations in several extra-skeletal systems, in the moment it is still not possible to prove a cause-effect relationship (Evidence B).

Traditionally, vitamin D was associated only with calcium metabolism functions. The possibility of existence of extra-skeletal effects occurred after the discovery of vitamin D receptor (VDR) in tissues not involved with calcium metabolism (e. g. skin, placenta, breast, prostate, and colon cancer cells) and the identification of the enzyme 1α -hydroxylase in extra-renal tissues. The question to be discussed is the real biological meaning of the presence of VDR and 1α -hydroxylase in different tissues (132).

Nagpal and cols. (133) reported that $1,25(\text{OH})_2\text{D}_3$ through its transcriptional activity was able to directly or indirectly regulate at least 200 genes. These genes are involved in the control of proliferation, apoptosis and angiogenesis in several tissues. The etiological connection between vitamin D deficiency and specific extra-skeletal diseases still needs to be identified in humans. Findings using animal models, regarding the beneficial effects of $1,25(\text{OH})_2\text{D}_3$ suggest mechanisms that involve similar human signaling pathways (134,135) (B). The main non-skeletal effects studied in the literature will be described here.

a) Vitamin D and cardiovascular disease

Vitamin D deficiency was included as a new risk factor for cardiovascular diseases (CVD) based on obser-

vational studies that demonstrate a strong association between vitamin D deficiency and mortality due to cardiovascular disease, the mechanism being unclear in the literature (136) (**B**). Potential hypothesis include the action in the regulation of genes involving renin production, proliferation of cardiac and vascular muscle cells, negative regulation of reactive C protein and other involvement in other proinflammatory processes. Fiscella and Franker (137) showed that black individuals, presenting calcitriol levels in the lower quartile presented 40% increase in the risk of death from coronary arterial disease (CAD) or stroke (**B**).

Serious vitamin D deficiency in patients with stabilized CVD is related to 50% more deaths from stroke and three to five times more sudden death (138). On the other hand, another study, demonstrated that very high levels are associated with an increase in the risk of ischemic myocardial disease (139). An increased risk of systemic arterial hypertension and metabolic syndrome has been demonstrated when sub-optimum vitamin D levels are detected (140). Observational and cohort studies led to the potential vitamin D use as an anti-hypertensive agent. Some studies demonstrated decreased levels of systolic blood pressure upon supplementation. However, larger studies were not able to prove these positive effects. Two prospective studies did not show reduction in cardiovascular mortality upon vitamin D supplementation (131). A meta-analysis presented 8% reduction in the mortality from all causes with modest doses (141). Nonetheless, a recent meta-analysis including 51 studies, concluded that supplementation did not have a significant effect in the mortality (RR 0.96), CAD incidence (RR 1.02) and stroke (RR 1.05) (136). So far, there is no strong evidence for the screening of vitamin D deficiency in patients under risk for CVD, as well as patients with previous CVD. Good prospective studies are necessary for a better understanding of the efficacy of supplementation in the risk reduction of cardiovascular disease (132-134) (**B**).

b) Vitamin D and diabetes

Epidemiological and observational studies demonstrate a potential involvement of vitamin D in the pathogenesis of the inflammatory process and in the prevention and control of both *diabetes mellitus* type 1 and type 2 (DM1 and DM2). Studies performed in animals and humans suggest that vitamin D can be a potential modifier of these diseases (142) (**A**).

Animal studies demonstrate that immunomodulatory and antiinflammatory actions of vitamin D reduce autoimmune insulinitis in DM1. It seems to suppress the antigen capacity of macrophages, inhibit the maturation of dendritic cells, modulate the development of CD4 lymphocytes and inhibit the production of cytokines such as interferon (IFN) and interleukin-2 (IL-2). These cytokines are known for activate macrophages and cytotoxic T cells, that lead to the destruction of pancreatic cells (143).

In DM2, vitamin D acts reducing insulin resistance and increasing its secretion, through the modulation of the immune and inflammatory process. DM2 is associated to an increase in the levels of tumor necrosis factors α and β , C-reactive protein (CRP), plasminogen activating factor and interleukin-6 (142).

Epidemiological studies demonstrate that children with vitamin D deficiency present 2.4 times higher risk of developing DM1. In the EURODIAB study there was as a reduction in the risk of developing DM1 in 33% among supplemented children (144) (**B**). The same way the maternal supplementation demonstrates a protective effect to the newborn. A meta-analysis concluded that, childhood supplementation seems to be protective against the development of DM1 (130). In adults with the disease, a reduction in the insulin dose was seen with the calcitriol supplementation (145) (**B**).

In rats with vitamin D deficiency, after supplementation there was improved insulin secretion (146). Two large studies evidenced that the combined use of calcium and vitamin D reduced the risk of DM2. In a dose-response analysis, DM2 risk was reduced in 4% at each increment of 4 ng/mL in the concentration of 25(OH)D (147) (**B**). In a meta-analysis, it was concluded that the insufficiency of calcium and vitamin D can negatively influence glycemia and that supplementation of both can be beneficial for the optimization of glucose metabolism (146-147) (**B**).

There is evidence suggesting that vitamin D has a role in the prevention and treatment of DM1 and DM2, through its action on the immune system, insulin secretion and resistance. However, further studies, using larger populations, are necessary to better elucidate the mechanisms of action and the doses necessary to present the best benefits (132-134,148) (**A**).

c) Vitamin D and cancer

Epidemiological studies demonstrated a correlation between sunlight exposure and mortality due to some

types of cancer, as well as skin color seems to be related to an increase in the prevalence of colorectal, breast and prostate cancer (149) (B). The risk of development and death due to neoplasia is more elevated in places of higher latitude and this can be related to lower sunlight exposure. Women presenting vitamin D insufficiency show a higher risk of developing colorectal cancer when compared to women presenting sufficient vitamin D levels, although no benefit was observed in the use of vitamin D for prevention (150,151).

In tissues where 25(OH)D is available, there is paracrine production of 1,25(OH)₂D₃, which through binding to its receptor, VDR, regulates transcription of target genes, that act in the differentiation of normal and tumor cells. Epidemiological and pre-clinical studies suggest the action of vitamin D in cancer prevention and treatment. Polymorphisms in the VDR gene are associated with an increased risk for the development of neoplasias (150). Local production of 1,25(OH)₂D₃ does not have any function in the control of calcium metabolism, but it presents autocrine and paracrine effects. *In vitro*, it is possible to observe a decrease in 1 α -hydroxylase (CYP27B1) and in vitamin D receptor (VDR) as the tumor progresses, associated to an increase in 24-hydroxylase (CYP24A1), which is inactivating. *In vitro* and *in vivo* studies demonstrate the direct or indirect effect of 1,25(OH)₂D₃ and its analogues on proliferation, differentiation, apoptosis, angiogenesis, invasion and inflammation of malignant cells. Microarray studies show that 1,25(OH)₂D₃ influences the transcription of a great number of genes, mainly related to apoptosis control (149,152) (B).

Low vitamin D levels make the tissues more sensitive to pro-carcinogenic events. Vitamin D analogues are not able to eradicate tumor cells, however they can be used as adjuvants in cancer treatment. It is believed that high doses of these substances are necessary for a real benefit to be observed, although it increases adverse effects (153) (B).

A double-blind placebo controlled study determined that the use of 1,25(OH)₂D₃ in pre-leukemia showed promising results at the beginning, although it determined an increase in calcemia during the blastic crisis (154). In prostate cancer, the administration of 2,000 IU/day resulted in PSA levels reduction, though the presence of severe hypercalcemia determined the end of the study (134).

Some non-hypercalcemic calcitriol analogues are associated with good prognosis in patients that present

elevated VDR expression. However, the use of calcitriol and analogues for the treatment of cancer patients is so far uncertain. Most of the clinical studies were conducted in patients with prostate cancer and patients with advanced cancer that do not respond to traditional therapies. Laboratory evidence indicates that calcitriol generates a biological response that results in the inhibition of the neoplastic progress. However, large scale clinical studies are necessary to confirm the benefits of vitamin D use in neoplasias (155,156) (B).

d) Vitamin D and autoimmune disease

Vitamin D action on the immune system seems to be mediated by B and T lymphocytes. VDR is present in these cells. The molecule 1,25(OH)₂D₃ inhibits the proliferation of T cells, suppresses the synthesis and proliferation of immunoglobulins, prevents the formation of IFN- γ (interferon- γ) and IL-2 (interleukin-2); besides increasing the activity of suppressor T cells (TH2). In humans, there is epidemiological evidence of the importance of vitamin D in the immune system (157) (C).

Sunlight or vitamin D are environmental factors in the etiology of multiple sclerosis and can have a role together with class II MHC inherited factors. Epidemiological studies suggest that adults with elevated vitamin D levels present lower risk of developing multiple sclerosis. Women with high vitamin D intake have 42% less chance to develop this disease (134,158) (B).

Preliminary studies suggest that activated vitamin D can be an efficacious treatment for rheumatoid arthritis. Treated mice showed decreased activity of the cell responsible for this disease. In humans, epidemiological studies also confirmed a negative association between the levels of vitamin D and the prevalence of the disease. Other autoimmune diseases that are being associated with vitamin D are: autoimmune encephalitis, systemic lupus erythematosus, intestinal inflammatory disease and autoimmune thyroiditis. Additional studies are necessary for the confirmation of data, and the use of vitamin D for the prevention and treatment of autoimmune diseases (155,156).

e) Vitamin D and innate immunity

Recent studies suggest that vitamin D can modulate innate immunity. Hypovitaminosis D can present a negative impact in infectious diseases. It has been observed that 1,25(OH)₂D₃ has an antimicrobial activity, including *Mycobacterium tuberculosis*, through the stimula-

tion of cathelicidin production (protein that acts on the destruction of pathological agents) (159).

A study that used elevated vitamin D doses, 600,000 IU, in tuberculosis patients, demonstrated higher body weight increase and less residual disease in those who received the vitamin, in comparison to the controls. The patients that were deficient when they were enrolled in the study (25-hydroxyvitaminD < 20 ng/mL) showed a more significant increase in *Mycobacterium tuberculosis* induced IFN- γ (160) (A).

A research with post-menopausal women who received 2,000 IU of vitamin D per day, showed a 90% reduction in upper respiratory tract infections, when compared to those who received 400 IU per day (B). Some studies also demonstrate that lower vitamin D levels can be a risk factor for sepsis. Studies for bacterial vaginosis, skin infection and of the oral cavity are being developed (155).

f) Vitamin D and psoriasis

The active form of vitamin D is a powerful inhibitor of keratinocytes proliferation and can be safely used in non-malignant hyperproliferative diseases of the skin, such as psoriasis. Data from controlled randomized studies demonstrated that the active form is an effective and well-tolerated treatment in patients with chronic initial or moderate psoriasis plaques. The topic application of 1,25(OH) $_2$ D $_3$ or its analogue calcipotriol can be used as a first line treatment against psoriasis (161).

g) Vitamin D and respiratory diseases

In children with asthma, the level of 25(OH)D seems to positively correlate with the control of the disease and the pulmonary function; and negatively with the use of corticoids. Few intervention studies, evaluating vitamin D supplementation with asthma exists in the literature (155,156,162). One of them demonstrated that 1,200 IU per day in children was associated to 83% reduction in the risk of disease exacerbation. It is believed that the immunomodulating effects of vitamin D and the effects on the pulmonary function can be useful for the treatment of respiratory diseases (163).

h) Vitamin D and physical and cognitive function of the elderly

In large populational studies, low vitamin D levels are associated with mobility reduction, worsening in muscular function and this way, an increase in the risk of

fall (132,156,164). Vitamin D receptors present high concentrations in several areas of the central nervous system. Epidemiological studies demonstrated that low ingestion of vitamin D is associated with a cognitive decline, an increased risk for Alzheimer disease and depression. The mechanism suggested for this association includes the formation and aggregation of β -amiloid fibers, a deregulation of the gabaergic system and an increase in the calcium influx in the neurons (165).

Vitamin D seems to be involved in physiological and possibly pathologic changes that follow aging. If the supplementation can have a positive impact in the aging process is still uncertain and long-term intervention studies are necessary (133,134).

i) Vitamin D and obesity

Obesity is associated with a higher prevalence of vitamin D deficiency, interpreted as a sequestration by the adipose tissue. In fact, when compared with non-obese individuals, the necessary dose for the reposition of vitamin D is higher among the obese. Recent data suggest that low concentrations of 25(OH)D could predict an acceleration in the increase of fat mass and this way would be involved with an increase of obesity incidence (135-137). Sergeev and cols., are investigating the mechanism through which 1,25(OH) $_2$ D $_3$ regulates the apoptosis of adipocytes. Preliminary studies in rats suggest that the supplementation with elevated doses of calcium and vitamin D reduce the weight and fat mass in obese rats. Studies in humans are necessary for the evaluation of the efficiency of vitamin D in the treatment of obesity (166).

In recent meta-analyzes and systematic reviews, it was observed an association between 25-hydroxyvitamin D and several non-skeletal outcomes in observational studies, but that was not seen in randomized controlled trials (RCTs) (40,151). The effects of vitamin D in other tissues is still controversial.

CONCLUSIONS

Vitamin D food sources are scarce and humans depend mainly on cutaneous synthesis. Hypovitaminosis D is very frequent in our country. Laboratorial evaluation should be performed measuring 25(OH)D and the following should be considered individuals at risk for vitamin D deficiency: the elderly, osteoporosis patients, patients with history of falls and fractures, obese people, pregnant and lactating women, patients making use of medications that interfere with vitamin D me-

tabolism (glucocorticoids, anticonvulsants, antifungal drugs), patients with malabsorption syndromes, primary hyperparathyroidism, renal or liver insufficiency, granulomatous diseases and lymphomas.

The most adequate normality value taking bone benefits into consideration is 30 ng/mL. The factors that seem to favor the presence of higher serum concentrations in our population are: younger age, community life, practice of outdoor physical exercises, oral vitamin D supplementation, season of the year (spring, summer), residence in sunny beach areas and in lower latitudes.

The most available vitamin D form for treatment and supplementation is cholecalciferol or vitamin D₃. For patients with osteoporosis and increased risk of fractures it is recommended that 25(OH)D concentrations are kept above 30 ng/mL for full benefits on the prevention of secondary hyperparathyroidism, decreased risk of fall and better impact on BMD. For this purpose, maintenance doses between 1,000 and 2,000 IU are necessary. Vitamin D active forms, such as calcitriol or alfacalcidol should not be used when the objective is supplementation or in the treatment of vitamin D deficiency, because of their higher risk of adverse effects. Special considerations regarding pregnant and lactating women, patients with chronic kidney disease, obese people and those submitted to bariatric surgery.

Nowadays there is special interest in the research of extra-skeleton effects of vitamin D, due to observational studies that demonstrated association between low vitamin D concentration and several endpoints with mortality, cardiovascular complications, diabetes, cancer, autoimmune diseases, cognitive function, among others. However, in the moment it is still not possible to prove a cause-effect relationship.

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