HIGHLIGHTED ARTICLE

Major Meta-Analysis, Randomized Clinical Studies, and International Consensus on Serum Levels and Importance of Supplementing Vitamin D:State of the art

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Abstract: Introduction: Hypovitaminosis D is highly prevalent and constitutes a public health problem worldwide. It can affect more than 90% of individuals, depending on the population studied. **Objective:** To make a broad analysis of the world literature to compose the State of the Art on serum vitamin D levels and its adequate supplementation, to prevent and mitigate various diseases, based on randomized clinical studies, analysis, and latest international conferences and consensus. **Methods:** The present study followed a literary review of randomized clinical studies, meta-analysis, and the latest international consensus. Cochrane instrument was adopted to assess the quality of the included studies between 2015 and 2020. **Major considerations and conclusion:** Laboratory evaluation should be performed by measuring 25(OH)D, and the main groups of individuals at risk for vitamin D deficiency are the elderly, patients with osteoporosis, history of falls and fractures, obese, pregnant women, and infants. For patients with osteoporosis and increased risk of fractures, recommended that concentrations of 25(OH)D remain above 30 ng/mL for full benefits on the prevention of secondary hyperparathyroidism, decreased risk of falls. Special considerations must have taken to pregnant women and infants, in patients with chronic renal failure, obese patients, and those undergoing bariatric surgery. Several clinical studies and current meta-analysis have shown significant results with vitamin D supplementation in cardiovascular complications, diabetes, cancer, autoimmune diseases, cognitive function, among others, with doses above 30 ng/mL, reaching up to 70 ng/mL, and maintaining serum dosage at 50 ng/mL.

Keywords: Vitamin D, Hypovitaminosis, Immunity, Disease trigger, COVID-19.

Introduction

Hypovitaminosis D is highly prevalent and constitutes a public health problem worldwide. Studies show a high 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 also seems to be related to the pathophysiology of several diseases. In children, vitamin D deficiency leads to growth retardation and rickets [2]. In adults, hypovitaminosis D leads to osteomalacia, secondary hyperparathyroidism, and, consequently, increased bone resorption, favoring bone loss, and the development of osteopenia and osteoporosis. Muscle weakness can also occur, which contributes to further increase the risk of falls and bone fractures in patients with low bone mass [3].

Deficiency of vitamin D (serum 25-hydroxyvitamin D (25 (OH) D) <50 nmol/L or 20 ng/mL) is common than is thought in the majority of the world population. It occurs in <20% of the population in northern Europe, in 30-60% in western, southern, and eastern Europe and up to 80% in Middle Eastern countries [4]. Severe deficiency (serum 25 (OH) D <30 nmol/L or 12 ng/mL) is found in > 10% of Europeans. The European Society of Calcified Tissues (ECTS) recommends that the measurement of serum 25 (OH) D be standardized by a Vitamin D Standardization Program. Risk groups include young children, adolescents, pregnant women, the elderly (mainly institutionalized), and non-Western immigrants [2].

The consequences of vitamin D deficiency include mineralization defects and lower bone mineral density, causing fractures. The extra-skeletal consequences can be muscle weakness, falls, and acute respiratory infection, and are the subject of large ongoing clinical trials. ECTS advise improving vitamin D status by fortifying food and using vitamin D supplements in risk groups [2].

Fortification of foods by adding vitamin D to dairy products, bread, and cereals can improve the vitamin D status of the entire population, but quality assurance needs to be monitored to prevent poisoning. Specific risk groups such as babies and children up to 3 years old, pregnant women, the elderly, and non-Western immigrants should routinely receive vitamin D supplements. Future research should include genetic studies to better define individual vulnerability to vitamin D deficiency and Mendelian randomization studies for the effect of vitamin D deficiency on long-term non-skeletal outcomes, such as cancer [3].

The correct diagnosis of this condition and the identification of factors that improve or worsen may contribute to the development of more effective strategies for the treatment of populations at risk, such as the elderly and postmenopausal women [5]. Thus, the efforts of the Bone Metabolism Department of the Brazilian Society of Endocrinology and Metabology (SBEM) are increasing in the development of recommendations based on the evidence available in the scientific literature for the diagnosis and treatment of this condition [6].

In this context, parathyroid cells express the enzyme 1-alpha-hydroxylase and can synthesize the active form, 1.25(OH)2D intracellularly, from the serum pool of 25(OH)D [7]. In situations of hypovitaminosis D, the lower intracellular synthesis leads to secondary hyperparathyroidism that is associated with increased bone resorption. There is an inverse correlation between PTH and 25 (OH) D, described in children and the elderly. Several cutoff values of 25(OH)D for normalization of PTH have been published, and most are concentrated between 28 and 40 ng/mL (70 to 100 nmol/L) [8].

The absorption of calcium by the intestine is dependent on the action of active vitamin D in the duodenum, through a saturable transcellular process, whose stimulus leads to the synthesis of proteins such as calbindin-D9k (CaBP-9k) and the epithelial apical channel TRPV6 [8]. However, there is evidence that the unsaturated transport that occurs with part of the absorption of calcium in the human ileum also influences vitamin D [9-12].

Therefore, the present study aimed to make a broad analysis of the world literature to compose the State of the Art on serum vitamin D levels and its adequate supplementation, to prevent and mitigate various diseases, based on randomized clinical studies, analysis, and latest international conferences and consensus.

Methods
Study Design

The present study followed a literary review of randomized clinical studies, meta-analysis, and the latest international consensus, following the rules of the PRISMA 2009. Available in: http://www.prisma-statement.org/

Table 1 shows the main variables of the present study that have addressed according to the classification of the acronym PICOS (P = Patients; I = Intervention; C = Control (Control); O = Outcomes; S = Study Design (Type of studies).

Study eligibility criteria

Only randomized clinical studies, systematic reviews, meta-analysis, and the latest international conferences and consensus included.

Selection of studies and risk of bias in each study

The Cochrane instrument was adopted to assess the quality of the included studies [13].

Data sources and research strategy

The search strategies for this systematic review have based on the keywords (MeSH Terms) "Vitamin D; (25(OH)D); Hypovitaminosis; Immunity; Inflammatory Process; Disease trigger; Acute respiratory syndrome; COVID-19; SARS-CoV-2 ", with publications from 2015 to 2020, to analyze the most recent scientific publications. The SCOPUS (Elsevier and non-Elsevier database), COCHRANE Library, PubMed (MEDLINE biomedical literature, life science magazines, and online books) and SCIENCE DIRECT (Elsevier database) databases were used, including the National Institutes of Health RePORTER Grant database and clinical trial records. Also, a combination of keywords with the Booleans OR, AND, and the operator NOT have used to target scientific articles of interest.

Table 1. Table of PICOS (Patients; Intervention; Control; Outcomes; Study Design).

PATIENTS	Patients with vitamin D deficiency or insufficiency
INTERVENTION	Vitamin D supplementation from 50 nmol/L to 180 nmol/L.
CONTROL	Dosage less than or equal to 20 ng/mL.
OUTCOMES	Resolution or reduction of symptoms and complications. Improvement in the quality of life.
STUDY DESIGN	Randomized clinical studies, systematic reviews, meta-analysis and latest international consensus.

Table 2. An example of the search structure in PubMed, the same search strategy was used in the other databases.

PubMed	Vitamin D **OR** (25 (OH) D) **OR** Hypovitaminosis
AND	
PubMed	Immunity **OR** Inflammatory Process **OR** Disease trigger
PubMed	Acute respiratory syndrome **OR** COVID-19 **OR** SARS-CoV-2
NOT	
PubMed	Case reports **OR** Active vitamin D analogues

The title and abstracts were examined under all conditions. The research structure used in the databases was shown in Table 2.

Results and Discussion
Main current outcomes

The 2nd International Conference on Vitamin D Controversies was held in Monteriggioni (Siena), Italy, from 11 to 11 September 14, 2018 [2]. The purpose of this meeting was to address controversies and timely topics in vitamin D research, to review the available data related to these topics and controversies, to promote discussions to help resolve remaining problems and ultimately to suggest a research agenda to clarify areas of uncertainty. Several questions from the first conference (2017) have revised, such as assays used to determine the serum concentration of 25-hydroxyvitamin D (25(OH)D), which remains a critical and controversial issue to define the status of vitamin D. State definitions nutritional status (ie sufficiency, insufficiency, and deficiency) have also reviewed. New areas have been revised, including vitamin D threshold values and how they should be defined in the context of specific diseases, sources of vitamin D, and risk factors associated with vitamin D deficiency. Also discussed were non-skeletal aspects related to vitamin D, including to reproductive system, neurology, chronic kidney disease, and falls (Tables 3 and 4). The

therapeutic role of vitamin D and the findings of recent
clinical trials have also been addressed [2].

Table 3. Declarations of the Second International Conference [2].

1.	Existing data is insufficient to define "low" or "high" thresholds for vitamin D status with any degree of certainty due to the lack of standardized 25 (OH) D measurements in vitamin D research.
2.	The current approach to defining vitamin D status using a concentration of 25 (OH) D.
3.	Due to the variability of the test, the circulation of "25 (OH) D", measured by the multiplicity of existing tests, cannot be simply blindly grouped in meta-analysis.
4.	For research and publication of data, 25 (OH) D assays should demonstrate standardization or alignment with reference methodology.
5.	Laboratories must participate in a proficiency testing program based on the accuracy of 25 (OH) D, p. DEQAS, or College of American Pathologists.
6.	Some documentation that the 25 (OH) D test methodology works correctly in the study environment (for example, pregnancy, hemodialysis). Given the deficiencies in the tests, manufacturers should develop tests with a comparable capacity to accurately measure 25 (OH) D2 and 25 (OH) D3 in various clinical circumstances.
7.	It seems reasonable to recommend cholecalciferol instead of ergocalciferol.
8.	The risk of developing rickets/osteomalacia increases at a concentration of 25 (OH) D <30 nmol/L. This threshold may vary depending on other conditions, such as calcium and phosphate nutrition, parathyroid hormone (PTH) levels, and season.
9.	The concentration of 25 (OH) D varies between normal individuals between approximately 50-125 nmol/L. With admitted uncertainty, an upper 25 (OH) D threshold of 125 nmol/L is advisable.
10.	The most recent food content analysis of vitamin D confirms low vitamin D content in non-fortified food supplies.
11.	There appear to be differences in bioavailability and availability, perhaps biological effects between 25 (OH) D3 and 25 (OH) D2 under certain circumstances.
12.	Vitamin D or 25 (OH) D3 can be added to the food supply by feeding animals enriched food.
13.	The latest data on exposure to very high natural levels exposure to sunlight has led to uncertainty about how much vitamin D3 can be produced after exposure to the sun.
14.	Individuals with dark skin can produce vitamin D3 to a greater extent than previously thought.
15.	The use of sunscreen does not significantly affect the production of vitamin D3 in the skin.
16.	Glucocorticoids are associated with vitamin D deficiency and/or resistance.
17.	Pathophysiology appears to be multifactorial.
18.	Vitamin D supplementation improves skeletal health in glucocorticoid-induced osteoporosis when combined with calcium.
19.	The precise effects of active vitamin D compounds in the treatment of some very common

complications of chronic kidney disease are far from fully established.

20. Pending more solid data, it can be assumed that oral vitamin D, such as cholecalciferol, can provide benefits in various clinical settings related to chronic kidney disease.

21. Vitamin D is essential to prevent rickets and osteomalacia.

22. Vitamin D metabolites, except the active metabolite.

23. May play a role in fracture repair.

24. Vitamin D supplementation with adequate calcium intake.

25. It can decrease the incidence of fractures in the elderly.

26. The elimination of nutritional rickets remains a health priority.

Table 4. Vitamin D as a risk factor for non-skeletal health [2].

1. The relationship between vitamin D status and cancer is based on plausible in vitro data, data from animals, and clinicians.

2. Randomized clinical studies indicate that vitamin D supplementation did not significantly reduce the risk of cancer, but significantly improved cancer survival. However, deficiencies in the test designs provide a cautionary note.

3. Appropriate selection of subjects (perhaps starting with a high-risk population) and other variables should be considered as components of the ideal project.

4. Studies to determine the effect of vitamin D on cancer risk should be carried out for more than 3-5 years, given the time course of oncogenesis.

5. Vitamin D may be involved in the pathogenesis of Chron's disease in line with its potential role in the immune response, as it occurs in other autoimmune diseases.

6. Due to the heterogeneity in the study design, different serum concentrations, baseline levels of vitamin D and different doses of vitamin D administered in studies, the harmonization of these variables is a prerequisite for the interpretation of the potential role of vitamin D in autoimmune diseases.

7. It is known that obesity and diabetes are associated with vitamin D deficiency, but the mechanisms involved have not yet been elucidated.

8. Vitamin D supplementation has not been shown to improve outcome measures for diabetes and obesity.

9. Bariatric surgery has several important results related to vitamin D metabolism, including reduced absorption.

10. Severe vitamin D deficiency is common in patients after bariatric surgery and correction require much more doses compared to obese people, which requires higher doses than non-obese individuals.

11. Calcium malabsorption may persist after correction of vitamin D deficiency due to reduced intestinal activity that activates calcium absorption.

12. These patients have an increased fracture rate compared to obese people of the same age.

13. **The adequacy of vitamin D for mothers and babies is important.**

14. **The implication of vitamin D deficiency during pregnancy is not fully understood. Results from meta-analysis suggest that improving maternal vitamin D status may decrease the risk of pregnancy outcome and may have long-term effects as well as beneficial effects on the child, such as asthma.**

15. **Chronic vitamin D deficiency in animals can predispose to the development of chronic degenerative neurological diseases.**

16. **Chronic degenerative neurological diseases in humans have been linked to vitamin D deficiency, but a potential causative role remains elusive.**

17. **Vitamin D deficiency is associated with muscle dysfunction and decreases in the elderly.**

18. **Vitamin D supplementation may reduce or increase the risk of falls in individuals moderately deficient in vitamin D.**

19. **The ability to "see" or "not to see" an effect on falls and perhaps other results of vitamin D actions may depend on whether the studied population is vitamin D or not.**

Table 5. Important recommendations from 25 (OH) D [2,3].

Diseases	Relevance (Deficiency or insufficiency)	Dose Recommended 25(OH)D (ng/mL)
In chronic kidney disease	✓ Higher risk for vitamin D deficiency;	>40
Osteoporosis	✓ Secondary hyperparathyroidism; ✓ Increase in bone resorption; ✓ Increase in the number of falls;	30<x<50
Obesity and Bariatric Surgery	✓ Obese people have lower vitamin D than non-obese people; ✓ Bar Bariatric surgery as an aggravating factor; ✓ Secundário Secondary hyperparathyroidism;	30<x<70
Gestation*	✓ The low weight of the newborn; ✓ Low bone mass; ✓ Cardiovascular risk markers in children; ✓ Gestational diabetes, pre-eclampsia; ✓ Bacterial vaginosis;	30<x<70
Cardiovascular disease	✓ Regulation of genes involving the production of renin; ✓ the proliferation of cardiac and vascular muscle cells; ✓ Negativa negative regulation of C-reactive protein and other pro-inflammatory factors; ✓ 40% increase in the risk of death from coronary artery disease (CAD); ✓ Grave Severe vitamin D deficiency in patients with stabilized CVD is related to 50% more death from stroke and three to five times more sudden death; ✓ Reduction in systolic blood pressure levels;	30<x<70
	✓ Reduces insulin resistance and increases its secretion, through the modulation of the immune and inflammatory process;	30<x<70

Diabetes	✓ Epidemiological studies show that children with vitamin D deficiency have a 2.4-fold increased risk of developing DM1. ✓ In the EURODIAB study, there was a 33% reduction in the risk of developing DM1 in supplemented children.	
Cancer	✓ Correlation between levels of heatstroke and mortality from some types of cancer, as well as skin color seems to be related to the increased prevalence of colorectal, breast, and prostate cancer; ✓ A decrease in 1-alpha hydroxylase (CYP27B1) and vitamin D receptor (VDR) is observed as cancer progresses; ✓ *In vitro* and *in vivo* studies show the direct or indirect effect of 1.25 (OH) 2D3 and its analogs on proliferation, differentiation, apoptosis, angiogenesis, invasion, and inflammation of malignant cells; ✓ Microarray studies show that 1.25 (OH) 2D3 influences the transcription of a large number of genes, mainly in the control of apoptosis.	30<x<70
Autoimmune disease	✓ 1.25 (OH) 2D3 inhibits T cell proliferation, suppresses immunoglobulin synthesis and proliferation, prevents the formation of IFN-γ (interferon-γ) and IL-2 (interleukin-2); ✓ Increases the activity of suppressor T cells (TH2). ✓ Interacts with factors inherited from MHC class II. ✓ A high intake of vitamin D presents a 42% lower risk of developing the disease.	30<x<70
Innate immunity	✓ Antimicrobial action, including Mycobacterium tuberculosis, by stimulating the production of cathelicidin (a protein that acts in the destruction of pathological agents); ✓ High doses of vitamin D, 600,000 IU, in patients with tuberculosis, demonstrated greater weight gain and less residual disease in those who received the vitamin in comparison to controls. ✓ A survey of postmenopausal women, who ingested 2,000 IU of vitamin D per day, showed a 90% reduction in upper respiratory tract infections when compared to those who ingested 400 IU per day.	30<x<70
Psoriasis	✓ The active form of vitamin D is a potent inhibitor of keratinocyte proliferation and can be used safely in non-malignant hyperproliferative diseases of the skin, such as psoriasis; ✓ Data from controlled, randomized studies show that the active form is an effective and well-tolerated treatment in patients with mild or moderate chronic psoriasis plaques.	30<x<70
Respiratory diseases	✓ In children with asthma, the level of 25 (OH) D appears to correlate positively with disease control and lung function; ✓ 1,200 IU per day of vitamin D in children was associated with an 83% reduction in the risk of exacerbating the disease. It is assumed that the immunomodulatory effects of vitamin D and the effects on lung function may be useful for the treatment of respiratory diseases.	30<x<50
	✓ Reduced mobility, worsened muscle function, and	

	thus an increased risk of falls.	
Physical and cognitive function in the elderly	✓ Vitamin D receptors have high concentrations in several areas of the central nervous system. Epidemiological studies have shown that low vitamin D intake is associated with cognitive decline, an increased risk of Alzheimer's disease and depression; ✓ Formation and β-amyloid aggregation ✓ Deregulation in the gabaergic system ✓ Increased calcium influx into neurons.	30<x<70

Table 5 below presents the main illnesses related to vitamin D deficiency or insufficiency, as well as the possible recommended doses according to the studies addressed at the Second International Conference and the current vitamin D status in European and Middle Eastern countries and strategies to prevent vitamin D deficiency (European Calcified Tissue Society position statement) [2,3].

Main Clinical Studies, Systematic Reviews and Meta-analysis - Last 3 Years

One study investigated whether the genetic variation associated with the concentration of vitamin D affected changes in bone mineral density (BMD) in response to an intervention in the diet for weight loss.

In the two years of preventing excess weight using new dietary strategies, BMD has measured for 424 participants who have randomly assigned to 1 of 4 diets varying in macronutrient intake. A genetic risk score (GRS) has calculated based on three genetic variants, namely 7-dehydrocholesterol reductase (DHCR7) rs12785878, cytochrome P450 2R1 (CYP2R1) rs10741657 and group-specific component (GC) globulin rs2282679, listed in circulating levels of vitamin D. A significant GRS interaction has found between dietary fat intake and vitamin D at two years in full-body BMD. In the high-fat diet group, participants with the highest GRS showed significantly less reduction in full-body BMD with those with the lowest ERG, while genetic associations were not significant in the low-fat diet group. There was also a significant interaction between dietary fat intake and GRS in the 6-month change in femoral neck BMD [14].

Another study investigated the effect of normalizing vitamin D status and/or reducing levels of parathyroid hormone (PTH) on the activity of cardiovascular health markers.

In a double-blind winter study, we randomized 81 healthy postmenopausal women with secondary hyperparathyroidism (PTH> 6.9 pmol/L) and 25-hydroxy-vitamin D (25 (OH) D) levels <50 nmol/L to 12 weeks of treatment with vitamin D3 70 μg/day (2800 IU/day) or identical placebo. Compared to placebo, treatment with vitamin D3 significantly increased plasma levels of 25 (OH) D and 1.25 (OH) 2D by 230% (95% CI: 189-272%) and 58% (190-271%), respectively. Treatment with vitamin D3 reduced PTH by 17% (11-23%) but did not reduce plasma changes, glycated hemoglobin, lipids and lipoproteins, blood pressure, vascular stiffness, heart rate, and cardiac conductivity. Compared to placebo, treatment with vitamin D3 increased plasma levels of high-density lipoproteins (HDL) by 4.6% (0.12-9.12%) but did not affect other measured indices [15].

A large number of observational studies have reported harmful effects of low levels of 25-hydroxyvitamin D (25OHD) on non-skeletal results. A systematic review of the characteristics of the randomized controlled trials (RCTs) included in the meta-analysis on the non-skeletal effects of vitamin D supplementation carried out. Fifty-four systematic reviews were identified, including data from 210 RCTs. Beneficial effects of vitamin D supplementation have reported in 1 of 4 meta-analysis on depression, 2 of 9 meta-analyzes on blood pressure, 3 of 7 meta-analysis on respiratory tract infections, and 8 of 12 meta-analysis on mortality. Most RCTs performed primarily to determine skeletal outcomes, while non-skeletal effects were assessed as secondary outcomes. Only one-third of the randomized clinical trials had a low level of 25OHD as an inclusion criterion, and an initial mean level of 25OHD below 50 nmol/L was present in only less than half of the analyzes [16].

Also, in a cross-sectional study, 104 healthy postmenopausal women with low levels of 25 (OH) D (<50 nmol/L), who had secondary hyperparathyroidism (SHPT) with high levels of PTH (> 6.9 pmol) were investigated/L, n = 52) or normal PTH

levels (n = 52). The mean PTH value in women with SHPT was 8.5 (interquartile range 7.5-9.7) pmol / L 5.3 (4.4-6.3) pmol/L in women with normal PTH (p < 0.001). Plasma phosphate was significantly lower in women with SHPT than in women with normal PTH (1.01 ± 0.14 vs. 1.09 ± 0.13 mmol/L; p <0.01). In the total cohort, the mean level of 25 (OH) D was 38 (31-45) nmol/L, with no differences between groups. SHPT was associated with impaired muscle strength, assessed by maximum muscle strength and maximum production of strength in knee flexion, with the knee fixed at 60 ° and 90 ° (p<0.05). Postural stability has impaired during the semi-tandem position (p=0.001). However, the two groups did not differ in terms of self-reported physical activity, symptoms related to muscles, quality of life, or lean muscle mass, assessed by dual-energy X-ray absorptiometry [17].

Besides, a placebo-controlled randomized clinical trial with 162 patients evaluated the effect of high doses of vitamin D on insulin sensitivity and risk of progression to diabetes. Of the total number of patients, only 83 completed the 6-month follow-up. After that time, serum 25-hydroxyvitamin D levels were significantly higher in the intervention group (36 ng/mL vs. 16 ng/mL). There was no significant difference between fasting plasma glucose (FPG) or a 2-hour glucose tolerance test (GTT). The score of the homeostatic insulin resistance model (HOMA-IR) was significantly lower in the vitamin D group (2.6 vs. 3.1). The rate of progression to diabetes was significantly lower in the intervention group (28% vs. 3%) [18].

Moreover, another randomized, placebo-controlled study with 50 participants investigated vitamin D supplementation in executive functioning and mental health in a group of Norwegian teenagers during the winter who underwent blood collection and cognitive testing from the Tower of Hanoi (ToH) and London Tower. Participants with a low level of vitamin D had worse scores on the Tower of London tests and the more difficult subtasks on the Tower of Hanoi tests, as well as behavior problems and attention deficit. Before vitamin D supplementation, the general mean status of 25-hydroxyvitamin D was 42 nmol/L, and after supplementation, the average was 62 nmol/L. Also, the group that received vitamin D supplementation improved their performance in the most demanding subtasks of ToH [19].

Another randomized, placebo-controlled clinical study was conducted on 100 overweight breeding women who were allocated to the treatment group, with 50 participants receiving 50,000 IU of vitamin D3

per week. The observed follow-up was 2 months. There was a significant decrease in homocysteine levels, as well as an increase in the levels of 25 (OH) D, calcium, and phosphorus after the first and second months of intervention with vitamin D3 in the treatment group. However, there were no changes in the placebo group [20].

To increase and confirm the scientific evidence, a meta-analysis study of randomized controlled trials included 52 studies with a total of 75,454 participants and performed analyzes on the vitamin D dose (≥ 2,000 and <2,000 IU/day), type of vitamin D (vitamin D2 and vitamin D3), treatment time (daily and intermittent), 25 basal hydroxyvitamin D dosage (≥50 and <50 nmol / L), and mean age (≥70 and <70 years). The results showed that vitamin D supplementation was not associated with all causes of mortality, and significantly reduced the risk of life from cancer by 16%. Also, all causes of mortality were statistically lower in studies with vitamin D3 supplementation than in studies with vitamin D2 supplementation [21].

In this scenario, however, meta-analysis studies of randomized clinical studies have shown that lipid parameters, markers of inflammation, blood pressure, and arterial stiffness are not affected by vitamin D supplementation. Furthermore, it has not yet been shown that vitamin supplementation D in high doses or bolus reduces cardiovascular risks and consequently mortality, even in subgroups with concentrations of 25 (OH) D <50 nmol/L. Therefore, doses of vitamin D in addition to the nutritionally recommended amounts of 600 to 800 IU daily cannot currently be recommended for the prevention of cardiovascular events [22].

In consideration and critical review of four recent meta-analysis on vitamin D and fracture prevention, vitamin D supplementation with or without calcium is supported among adults aged 65 and over who are at risk for vitamin D deficiency and fractures, if administered daily or weekly equivalent monthly doses of 800 to 1000 IU and with good adherence. Vitamin D supplementation may not be effective in primary prevention among adults 50 years of age or older without vitamin D deficiency and osteoporosis. Monthly doses greater than 100,000 IU need further evaluation regarding efficacy and safety [23].

Another study of systematic review and meta-analysis examined the effect of vitamin D supplementation on the serum lipid profile. The average vitamin D supplement per day was 2,795 IU.

Twenty-one trials were conducted in participants with diabetes, 13 studies were conducted in apparently healthy patients, and three trials were conducted in those who were obese or overweight. In 24 (68.6%) trials for which vitamin D deficiency (20 ng/mL) was reported at baseline, participants were sufficient vitamin D at the end of the trial. In 4 (11.4%) studies, no improvement was seen in serum vitamin D after the study. In 7 (20%) clinical trials, participants had sufficient vitamin D (> 20 ng/mL) both at baseline and at the end of the study. In 6 trials, both at baseline and at the end of the test serum vitamin D or serum vitamin D at the end of the test were not reported. A total of 41 randomized controlled trials comprising 3434 participants (n = 1699 in the vitamin D supplementation arm and n = 1735 in the placebo arm) were identified and included in the meta-analysis. Approximately 63.4% of study participants were women, with 14 studies conducted entirely among women. Approximately 24% of the studies had a follow-up duration> 6 months, while the remaining 76% had a follow-up duration <6 months. The improvements in total cholesterol and triglycerides were more pronounced in participants with baseline vitamin D deficiency. Vitamin D supplementation appears to have a beneficial effect in reducing serum levels of total cholesterol, LDL cholesterol, and triglycerides, but not HDL cholesterol levels. Vitamin D supplementation may be useful in patients with hypercholesterolemia with vitamin D insufficiency at high risk for cardiovascular disease [24].

Also, studies have suggested that vitamin D supplementation may increase serum fibroblast growth factor 23 (FGF23) among patients with vitamin D deficiency. Thus, a systematic review and meta-analysis were performed to summarize all available data. Nine studies were eligible for meta-analysis. Seven studies measured serum intact FGF23 and two studies measured serum C-terminal FGF23. Meta-analysis found that intact serum FGF23 increased significantly after oral vitamin D3 supplementation in participants with vitamin D deficiency. Serum C-terminal FGF23 also increased after vitamin D3 supplementation in participants with vitamin D deficiency [25].

Recent studies have shown that vitamin D supplementation decreases oxidative stress (OS) parameters. This systematic review and meta-analysis aimed to investigate the effect of vitamin D supplementation on the parameters of OS. In thirteen clinical trials, vitamin D supplementation increased serum levels of total antioxidant capacity (TAC) and glutathione. In addition, the concentration of malondialdehyde decreased significantly after vitamin D supplementation compared to placebo. There was no difference between the experimental and placebo groups in the subset of studies that administered vitamin D at less than 100,000 IU per month. However, a significant increase in TAC levels was found in the subset of studies that administered vitamin D between 100,000 to 200,000 IU and those that administered vitamin D between more than 200,000 IU monthly [26].

Previous meta-analysis of RCTs on vitamin D supplementation and total incidence of cancer and mortality found inconsistent results, and most included studies that generally administered low doses of vitamin D (≤1100 IU/day). Thus, a recent meta-analysis on RCT looked at higher doses of vitamin D supplements. For the total cancer incidence, 10 studies were included (6537 cases; 3-10 years of follow-up; 54-135 nmol / L of levels reached circulating 25 (OH) vitamin D [25 (OH) D] in the intervention group). The summary RR was 0.98 (95% CI, 0.93-1.03; p = 0.42; I2 = 0%). The results remained null in the tested subgroups, including even when 25 (OH) D levels exceeding 100 nmol / L were reached (RR = 0.95; 95% CI, 0.83-1.09; p = 0.48; I2 = 26%). For total cancer mortality, five trials were included (1591 deaths; 3-10 years of follow-up; 54-135 nmol / L of levels reached 25 (OH) D circulating in the intervention group). Therefore, vitamin D supplementation significantly reduced total cancer mortality but did not reduce the total cancer incidence [27].

Vitamin D and COVID-19

In the COVID-19 scenario, a study analyzed the main functions of vitamin D in reducing the risk of respiratory tract infections. In this sense, the main mechanisms include the induction of cathelicidins and defensins that can reduce the rates of viral replication and reduce the concentrations of pro-inflammatory cytokines that produce inflammation that impairs the lining of the lungs, leading to pneumonia, in addition to increasing the concentrations of anti-inflammatory cytokines. Therefore, this study gathered literary information that vitamin D deficiency can contribute to acute respiratory distress syndrome, lethality rates with increasing age, and chronic disease comorbidity [28]. Thus, it is recommended to use 10,000 IU/day of vitamin D3 for a few weeks, followed by 5,000 IU/day, to increase and maintain 25 (OH) D concentrations between 40-60 ng/mL (100- 150 nmol/L) [28].

Also, a recent literature review study revealed that because older adults are more likely to be diagnosed with Parkinson's disease (PD), advanced age is the biggest risk factor. In addition to its modulating effects on the immune system, it has been suggested that vitamin D supplementation plays a role in slowing the progression of PD and improving the quality of life. Therefore, daily supplementation of 2000-5000 IU/day of vitamin D3 in elderly people with PD has the potential to slow the progression of PD, in addition to offering additional protection against COVID-19 [29].

Also, average vitamin D levels for 20 European countries and the morbidity and mortality caused by COVID-19 were acquired. There is a negative correlation between the average levels of vitamin D (average 56 mmol/L ± 10.61) in each country and the number of cases COVID-19/1 M (average 295.95 and mortality). Vitamin D levels are severely low in the aging population, especially in Spain, Italy and Switzerland, this is also the most vulnerable group of the population to COVID-19. It should be advisable to conduct dedicated studies on vitamin D levels of COVID-19 with varying degrees of disease severity [30].

To assess the general effect of vitamin D supplementation on the risk of acute respiratory tract infection and to identify the factors that modify this effect, a systematic review study and a meta-analysis of the individual data of the participants was carried out randomized clinical trials and was obtained for 10,933 (96.6%) participants. Vitamin D supplementation reduced the risk of acute respiratory tract infection among all participants. In subgroup analysis, protective effects were observed in those who received vitamin D daily or weekly without additional bolus doses, but not in those who received one or more bolus doses. Among those who received vitamin D daily or weekly, the protective effects were stronger in those with baseline levels of 25-hydroxyvitamin D <25 nmol/L than in those with baseline levels of 25-hydroxyvitamin D ≥25 nmol/L. Therefore, vitamin D supplementation is safe and protected against acute respiratory tract infection in general. Patients with vitamin D deficiency and who did not receive bolus doses had the greatest benefit [31].

Limitations and BIAS

The accuracy of vitamin D dosages varies widely between laboratories and between different assays, reaching up to 17 ng/mL. There are still differences in the extraction of vitamin D from its binding protein, a cross measurement of 25(OH)D2, 25(OH)D3 and other metabolites in addition to the lack of standardization and, for this reason, quality control tools such as DEQAS (International Vitamin D External Quality Assessment Scheme) in an attempt to reduce these variations in data analysis.

The most used methods today are competitive assays based on specific antibodies and non-radioactive markers. The aim is to improve comparability between the results obtained with different methodologies. Whichever methods are employed, a precise definition of the normal range is essential. It is also noteworthy that the intra-individual variability can be from 12.1 to 40.3%. Clinical conditions that interfere with serum 25(OH)D levels are highly dependent on environmental and lifestyle factors, particularly exposure to UVB rays.

Conclusion

Dietary sources of vitamin D are scarce, and humans depend mainly on skin synthesis, making hypovitaminosis prevalent in most countries. Laboratory evaluation should be performed by measuring 25(OH)D, and the main groups of individuals at risk for vitamin D deficiency are the elderly, patients with osteoporosis, history of falls and fractures, obese, pregnant women and infants, patients taking medications that interfere with vitamin D metabolism (such as glucocorticoids, anticonvulsants, antifungals), patients with malabsorption syndromes, primary hyperparathyroidism, kidney or liver failure, granulomatous diseases, and lymphomas.

For patients with osteoporosis and increased risk of fractures, recommended that concentrations of 25(OH)D remain above 30 ng/mL for full benefits on the prevention of secondary hyperparathyroidism, decreased risk of falls, and for better impact on the health. For this maintenance, the doses need be between 1,000 and 2,000 IU. Special considerations must have taken to pregnant women and infants, in patients with chronic renal failure, obese patients, and those undergoing bariatric surgery.

There are strong interests in vitamin D supplementation in an attempt to reduce various outcomes such as mortality, cardiovascular complications, diabetes, cancer, autoimmune diseases, cognitive function, among others. Thus, the several clinical studies and current meta-analysis have shown significant results with vitamin D supplementation in

these pathologies, with doses above 30 ng/mL, reaching up to 70 ng/mL and maintaining serum dosage at 50 ng/mL.

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Data sharing statement
No additional data are available

Ethics Approval
Not Applicable.

Informed consent
Informed written consent obtained from the participant

Conflict of interest
The authors declare no conflict of interest.