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REVIEW
MANAGEMENT OF ENDOCRINE DISEASE

Value and limitations of assessing vitamin D nutritional status and advised levels of vitamin D supplementation

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Abstract

The growing attention to the role of vitamin D in skeletal and extra-skeletal diseases over the last decade induced an increased demand for vitamin D determination as well as a dramatic rise of sales of vitamin D supplement. However, several critical points in this field remain to be clarified. We lack a clear consensus about the definition of vitamin D deficiency, insufficiency, and sufficiency. The identification of different thresholds defining vitamin D status has relevant implications in clinical practice. In fact, the worldwide prevalence of low vitamin D status is highly varying according to the level of 25(OH)D utilized to define sufficiency. Therefore, the assessment of 25-hydroxyvitamin D levels may have a critical role, but a number of different technical problems associated with its determination may interfere in interpreting the results. The hydrophobic nature of vitamin D and the tight binding to its carrier (vitamin D binding protein), the different forms circulating in blood, and the issue of standardization are among the most important factors influencing the measurement of this metabolite. Another controversial point relies on the conflicting guidance on prevention and treatment of vitamin D deficiency endorsed by different medical and scientific communities. In particular, uncertainty exists about how to replete vitamin D stores, how to maintain normal 25(OH)D levels after repletion, which form of vitamin D is preferable for supplementation, and which route of administration and dosing regimens are advisable. Finally, concerns have been raised regarding vitamin D toxicity and its adverse effects.

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Introduction

There has been a growing interest in vitamin D during the last decades, which has boosted an increasing number of scientific papers on this topic. This interest, also shared by the lay community, mainly derives from the recognized effect of vitamin D on mineral metabolism and neuromuscular function (1, 2) and the purported effect on other aspects of health: cardiovascular (3, 4, 5), endocrine (6, 7), metabolic (8), neurological (9, 10), neoplastic (11), articular (12), immunological (13, 14), etc. Furthermore, vitamin D has also been linked to mortality (15, 16). The logical consequence of this surge of attention has been an increased demand for the determination of serum 25(OH)D levels (the best available index of vitamin D nutritional status) with substantial associated costs, in order to prove that insufficiency or deficiency of vitamin D was the causative factor of that particular disease and, *vice versa*, when the subject was repleted with vitamin D, he/she was protected or could be considered at lower risk.

Vitamin D is mainly derived from sun light exposure of the skin (17), only one-fifth being introduced by dietary sources from animal (cholecalciferol-D_3) or plant (ergocalciferol-D_2) origin. In order to be fully active, both ergocalciferol and cholecalciferol undergo 25-hydroxylation in the liver generating 25(OH)D_2 and 25(OH)D_3. This is the major rate-limiting step primarily dependent on the parent compound and therefore explaining the well-known seasonal variation of 25(OH)D (18). In normal subjects, the kidney adds an hydroxyl group in position 1 giving rise to the final metabolites 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$. A reduction in serum calcium, phosphorus, or fibroblast growth factor 23 (FGF23) and an increase in parathyroid hormone (PTH) stimulate the activity of CYP27B1 hydroxylase. In this context, it is important to note that opposite changes (i.e. an increase in serum calcium, phosphorus, and FGF23 and a reduction in PTH) determine a conversion of 25(OH)D toward the production of 24,25$(OH)_2$D. The possibility of producing another metabolite by inducing hydroxylation in position 26 (25,26$(OH)_2$D) exists. The physiological role of these last two metabolites is still an object of debate (19).

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Vitamin D status is defined by the measurement of 25(OH)D; this term refers to both circulating forms ($25(OH)D_2$ and $25(OH)D_3$) of the vitamin. There are a number of reasons why the concentration of total $1,25(OH)_2D$ cannot be utilized as a marker of vitamin D status; this is because of its short half-life (4–15 h vs 21–30 days of 25(OH)D), low concentrations of the final metabolite (picomole vs nanomole), and owing to the fact that a very small amount of 25(OH)D can be converted to $1,25(OH)_2D$, thus giving the false idea of sufficiency. Only when 25(OH)D falls below 4 ng/ml (corresponding to 10 nmol/l, being 1 ng/ml = 2.5 nmol/l), there is a concomitant decrease in $1,25(OH)_2D$ (19).

Measurement of 25(OH)D

The diagnosis of hypovitaminosis D (either deficiency or insufficiency) is therefore based on the current concentration and measurement of total 25(OH)D. However, there are a number of technical problems that should be born in mind in order not to misinterpret the results.

There are at least three major reasons impeding the achievement of a robust result; these are represented by the hydrophobic nature of the compound with the tight binding to its carrier (vitamin D binding protein (DBP)), the different forms circulating in blood, and the issue of standardization (Fig. 1).

As 25(OH)D is a lipophilic substance tightly linked to DBP, this generates some technical problems. Furthermore, endogenous lipids may affect binding and chromatographic separation, as they co-extract from plasma and serum. An important preventive measure to be adopted is avoiding sunlight exposure of the sample because this may induce degradation of the vitamin; the latter also applies to the standard employed in some assays. In contrast, the 25(OH)D is a very stable metabolite; multiple freeze and thaw cycles have no significant effect on determination of 25(OH)D in serum (20, 21). Indeed, in one of the most recent papers addressing the problem of the optimal threshold for defining vitamin D status, the authors performed the measurement of 25(OH)D in a blood sample taken at autopsy; they stated that, unlike PTH and calcium, 25(OH)D was found to be stable in various experiments for at least 10 days postmortem (22).

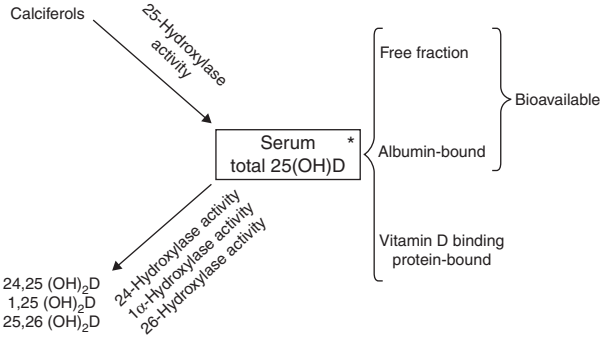

Figure 1 Factors influencing serum levels of total 25(OH)D and its partition in blood. *$24,25(OH)_2D$, 3-epimer-25(OH)D, isobars are recognized by some assays.

As previously stated, total circulating 25(OH)D is the sum of two metabolites, $25(OH)D_2$ and $25(OH)D_3$. However, not all the immunoassays employed in clinical practice are able to detect $25(OH)D_2$. Cavalier *et al.* (23) were one of first to enlighten this problem; indeed, they demonstrated that after vitamin D_2 administration, contrary to what would have been expected, there was no increase in total serum 25(OH)D with one of the methods employed. This finding has obvious clinical implications in subjects treated with vitamin D_2 or in countries (i.e. USA) in which vitamin D_2 is the only FDA-approved product (24). This methodological problem, possibly related to a stronger affinity of the DBP for $25(OH)D_2$ (25), poses an individual treated with D_2 at risk of vitamin D intoxication, because with some assays he/she will unlikely reach the 'laboratory' sufficiency. Therefore, according to the authorities in the field (19, 26), the ideal method of measurement should equally detect both metabolites. Isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS) is currently considered the referent method for 25(OH)D assay because it measures $25(OH)D_2$ and $25(OH)D_3$. However, HPLC may also be utilized, and according to Cavaliers' (23) and our own data (27, 28), the Diasorin RIA is endowed with these characteristics.

Some other metabolites may be the origin of spurious results. Among them, it is worthwhile to mention $24,25(OH)_2D$, which may represent up to 10–15% of the total quantity of 25(OH)D. Antibody-based methods, particularly those involving no chromatographic steps, cannot resolve $24,25(OH)_2D$ and include this metabolite in the estimation of total 25(OH)D. Some commercial kits offer corrections for this metabolite but such correction appears to be inaccurate at high or low values. Recently, there has been new interest in the assay of $24,25(OH)_2D_3$ owing to some findings demonstrating, for example, that the enzyme 24-hydroxylase (CYP24A1) is stimulated by FGF23 (29); that idiopathic infantile hypercalcemia may in part be derived by loss-of-function mutations in CYP24A1, so that the levels of this metabolite are undetectable (30); and that CYP24A1 defects in adults are associated with nephrolithiasis or nephrocalcinosis (31). Furthermore, genome-wide association studies have demonstrated that CYP24A1 variation is one of the four genetic determinants identified so far causing variability of serum 25(OH)D (32); therefore, the levels of 25(OH)D may also reflect fast and slow metabolizers with corresponding high or low serum $24,25(OH)_2D$ levels.

There are two other substances that can be the cause of spurious results; the first one is the 3-epi-25(OH)D

epimer which is a related molecule present in varying concentrations in normal subjects (33) that may interfere with the results obtained by LC–MS/MS. Another possible interference could derive from isobars, even though more detailed investigations are needed concerning these substances (19, 34). Epimers and isobars are compounds with the same molecular weight as that of vitamin D metabolites forming the same mass to charge parent and product ion pairs upon ionization.

One of the most important problems in this field is represented by the great variability in the results obtained among laboratories that utilize different methods, as also recently underscored (35). This is an old problem (36), partly overcome in recent years, mainly derived by the lack of a reference standard; before the adoption of such a standard, there was also a great variability when comparing three different laboratories employing what is now considered the gold standard of measurement, i.e. the LC–MS/MS (37). The absence of certified reference material for 25(OH)D is the most important factor determining the imprecision in identifying individuals with vitamin D levels below the optimal threshold, anyway defined; this often leads to the perception that an individual was classified as sufficient or insufficient based on the laboratory used for the determination. This has obvious important clinical implications, particularly in redefining worldwide vitamin D status (38), as demonstrated in a recent paper by Perna *et al*. (39). The National Institute of Standards and Technology (NIST) has developed a standard reference material (SRM 972) in order to solve this problem. The SRM consists of four pools of serum, each with varying levels of vitamin D metabolites. Chromatographic resolution of the 3-epimer of $25(OH)D_3$ proved to be essential for accurate measurement of the vitamin D metabolites present in these serum samples (40). The importance of the standardization process is demonstrated by the success story of serum total cholesterol (41). Presently, there are several ways for participation in the vitamin D Standardization Program (VDSP). Among them, the NIST-NIH Vitamin D Metabolites Quality Assurance Program (http://www.nist.gov/mml/csd/vitdqap.cfm), the DEQAS program, the VDSP's CDC Standardization-Certification Program, and finally the possibility of collaborating with VDSP to standardize 25(OH)D made sometime in the past as part of studies that have been completed. Standardizing values measured in the past require re-measuring total 25(OH)D concentration in a statistically designed subsample of stored sera (~100 samples) from the study by a laboratory that has been standardized to the NIST reference measurement procedure (41).

Vitamin D binding protein

DBP is the main serum carrier of vitamin D metabolites (albumin is a lower affinity binder), whose published normal reference range is 30–60 mg/dl (42). In physiological conditions, about 83% of total 25(OH)D in the circulation is bound to DBP (42, 43). The term bioavailable 25(OH)D refers to the readily available form of circulating vitamin D, that is free 25(OH)D combined with albumin-bound 25(OH)D.

Recently, the interest in DBP has considerably increased. DBP circulates in three major polymorphic forms, thus producing six allelic combinations occurring at different frequencies among ethnic populations (44). The different allele forms of DBP circulate at varying concentrations and possess different binding affinities for 25(OH)D and $1,25(OH)_2D$; therefore, both these variables have the potential to influence bioavailability of vitamin D (44). These data are in accordance with the recent genome-wide association studies showing that lower affinity forms of DBP are associated with lower circulating levels of 25(OH)D so that the affinity of the binding may regulate both the total and free 25(OH)D levels (33, 45) (Fig. 2). In this context, it is important to note that a recent study has demonstrated the association between bone mineral density (BMD) and levels of free 25(OH)D but not total circulating values of the vitamin (46). Along these lines, a recent longitudinal study showed that the known associations of low 25(OH)D concentrations with clinical outcome are related to common genetic differences in the vitamin D receptor (47).

Vitamin D and the search for a threshold

The definition of vitamin D deficiency, insufficiency, and sufficiency is currently challenging as an overall

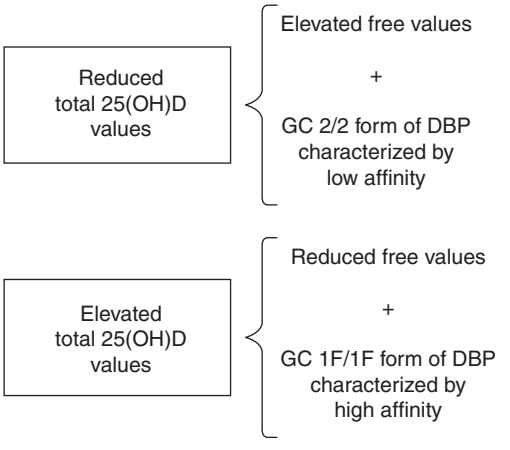

Figure 2 A theoretical approach to the interrelationships among total, free vitamin D and binding affinity of vitamin D binding protein (DBP). Depending on the isoform present in serum, the active 25(OH)D fraction (that is, the free fraction) may be elevated or reduced despite corresponding reduced or elevated total values of the vitamin. Different DBP turnover rates for the genetic variants may also have a role.

consensus is still lacking (48, 49, 50). It represents a crucial issue, as the identification of different thresholds defining vitamin D status has varying implications in clinical practice. First, the worldwide prevalence of low vitamin D status is highly varying according to the level of 25(OH)D utilized to define sufficiency. Consequently, the choice to initiate vitamin D supplementation may change, as well as the goals of therapy, the dosing strategy, and the decision about who should be screened, if necessary, and how often (51).

In recent years, a number of position statements and clinical practice guidelines have been published to define the optimal vitamin D status and the health outcomes associated with its alteration (52, 53). Many different recommendations on dietary intakes needed to reach and maintain sufficient 25(OH)D levels have been proposed as well (54, 55, 56, 57, 58, 59).

In this context, the publication of the two most authoritative reports on these issues (one released from the Institute of Medicine (IOM) committee's 2011 report on dietary reference intakes for calcium and vitamin D and the other from the Endocrine Society clinical practice guideline for the evaluation, treatment, and prevention of vitamin D deficiency) (58, 59) has lead to confusion among clinicians, researchers, and the public because of the disagreement in data interpretation. The conclusions of the two reports indeed differ considerably. The US Endocrine Society (ES) reported a 25(OH)D level <20 ng/ml (50 nmol/l) as the 'cut off' to define vitamin D deficiency, a 25(OH)D level between 21 and 29 ng/ml (52.5 and 72.5 nmol/l) to define vitamin D insufficiency, and a 25(OH)D level more than 30 ng/ml (75 nmol/l) as the optimal level. In contrast, the IOM concluded that 25(OH)D levels above 20 ng/ml are needed for good bone health for almost all the individuals (97.5% of the population), while a level of 16 ng/ml (40 nmol/l) meets the need of approximately half the population. According to the IOM, higher levels of 25(OH)D have not been consistently shown to confer greater benefits, in turn challenging the concept that 'more is better'. The controversy has been fuelled by several factors that should be taken into account in interpreting the results of the current literature. These include: the difficulty to distinguish the sole effect of vitamin D as the majority of intervention trials co-administered calcium; the difficulty to exactly measure the relative contribution of sunlight exposure, food fortification, and multivitamins intake; the lack of randomized controlled trials assessing the effect of vitamin D supplementation on health outcomes other than bone; and the complexity to compare studies utilizing different 25(OH)D assays (24, 60, 61, 62, 63). On the other hand, it should be emphasized that the two reports are intended for different populations (the general population for the IOM report and the population at risk for deficiency for the ES), and this could partly explain the controversy surrounding their respective recommendations.

The primary health outcomes of vitamin D nutrition utilized to define vitamin D sufficiency are those related to skeletal health. Actually, maximal intestinal calcium absorption, serum PTH suppression (52, 53), reduced risk of falling, prevention of fractures, increase in BMD, and reduced histomorphometric findings of osteomalacia from bone biopsy are the most important parameters considered in both reports to identify the optimal vitamin D status (58, 59, 64, 65, 66).

Table 1 briefly summarizes the different conclusions about these skeletal outcomes reached by the two professional organizations. Concerning the other possible benefits of vitamin D, both reports concluded that existing data are not sufficient to support the recommendation of vitamin D supplementation to reduce the risk of extra-skeletal acute and/or chronic diseases (58, 59).

Vitamin D and supplementation: general considerations

The controversy between the IOM and the ES on the definition of 'sufficiency' and the different goals of supplementation and treatment generated very different recommendations about vitamin D intakes. The IOM concluded that children aged 0–1 year require 400 IU/daily vitamin D (corresponding to 10 μg/daily vitamin D, being 1 μg vitamin D = 40 IU), all other children and adults up to the age of 70 years require 600 IU/day (15 μg/day) and adults over the age of 70 years need 800 IU/day (20 μg/day). On the contrary, the ES recommended a dose of vitamin D ranging from 400 to 1000 IU/day (10–25 μg/day) for children aged 0–1 year, 600–1000 IU/day (15–25 μg/day) for children aged more than 1 year, and 1500–2000 IU/day (37.5–50 μg/day) for adults aged 18 years or more (9, 10). Moreover, the ES also recognized that obese children and adults may require as much as two to three times the recommended dose due to the influence of body fat on vitamin D storage and metabolism (67).

The tolerable upper intake for those aged 9 years and older was set at 4000 IU/day (100 μg/day) by both the reports; however, the ES stated that larger doses may be needed to correct vitamin D deficiency in certain clinical situations (for example, 10 000 IU/day (250 μg/day) for adults aged ≥ 19 years). Also, the IOM recognized that such an intake is not associated with intoxication. Finally, both the IOM and the ES recommend that either vitamin D2 or vitamin D3 could be used as they have the same efficacy to raise and maintain circulating 25(OH)D levels (58, 59).

Vitamin D and the supplementation: the discussion

Since the publication of these two differing recommendations, a lively debate ensued among clinicians and

Table 1 The position of the IOM and the US Endocrine Society (ES) in the definition of vitamin D sufficiency based on different skeletal outcomes.

Skeletal outcome	IOM	US ES
Intestinal calcium absorption	A threshold for normal calcium absorption occurs at a serum level of 25(OH)D of 5–10 ng/ml (102, 103)	Calcium absorption is optimized at serum 25(OH)D level of 30 ng/ml (104)
Evidence of osteomalacia by histomorphometry from bone biopsy	Only 1% of patients with 25(OH)D levels above 20 ng/ml have osteomalacia (23)	No evidence of osteomalacia in all patients with 25(OH)D more than 30 ng/ml (8.5% of patients with 25(OH)D levels above 20 ng/ml have osteomalacia) (23)
Relationship between serum 25(OH)D and serum PTH levels	No evidence for a plateau of serum PTH levels or a plateau for 25(OH)D levels between 10 and 50 ng/ml; however, PTH levels can vary significantly by sex, age, time of day, kidney function, and dietary calcium (105, 106)	PTH begins to plateau in adults who have 25(OH)D levels between 30 and 40 ng/ml (1, 107)
Fracture risk reduction	Vitamin D at 800 UI/day provides some benefit in fracture risk reduction in elderly but only when calcium supplementation was added to the treatment (108, 109, 110)	Antifracture efficacy of vitamin D started at 25(OH)D levels of at least 30 ng/ml. This level is reached only in trials that gave at least 800 UI/day vitamin D_3 (111, 112)
Fall risk reduction	Vitamin D supplementation does not prevent falls. The reanalysis of data presented by the US ES indicates that there is no significant dose–response relationship between the risk of fall and the achieved 25(OH)D levels (63, 113)	Vitamin D supplementation at doses at least 800 UI/day reduces the risk of falls, particularly in elderly with vitamin deficiency at baseline. A gain in lower extremity strength or function and an improvement in body sway are also observed (115, 117)
Increase in BMD	Intervention studies showed little increase in BMD in vitamin-D-replete participants. Moreover, little or no additional benefit in BMD is observed with serum 25(OH)D levels above 20 ng/ml (116, 117, 118)	Higher hip BMD is associated with high serum 25(OH)D levels throughout the reference range of 9–37 ng/ml (119, 120)

researchers on several controversial points. In particular, uncertainty was raised about the following: i) how to replete vitamin D stores; ii) how to maintain normal 25(OH)D levels after repletion; iii) which form of vitamin D is preferable for supplementation; iv) which route of administration and which dosing regimens are advisable; and v) vitamin D toxicity and adverse effects.

Achieving and maintaining vitamin D sufficiency

The optimal dosage to reach sufficiency remains poorly defined. In general, according to a rule of thumb, for every 100 IU (2.5 μg) vitamin D taken, 25(OH)D levels increase to about 1 ng/ml, but with a huge inter-individual variability.

Several factors may account for such a variability: the initial 25(OH)D concentration, patient's weight, adequacy of the dose according to compliance, the type of vitamin D administered (D_2 or D_3), renal function, and genetic factors. The variability in absorption, the inaccuracy of 25(OH)D assessment, as well as unknown factors also probably contribute to the variability of the dose–response relationship (68, 69, 70).

Controversy also exists on whether supplementation should be given daily or intermittently (e.g. weekly, monthly, quarterly, or once a year). It has been shown that circulating levels of 25(OH)D increase similarly when oral vitamin D is given daily, weekly, or monthly, provided that the total amount is identical. However, it must be recognized that a universal supplementation guideline does not exist, most likely the result of great disparity among countries in the availability of vitamin D supplements (71).

Another crucial point is that the immediate aim of treatment should be quick normalization of 25(OH)D levels, as well as vitamin D stores. This quick 'correction' can be accomplished with an initial period of high-dose vitamin D. An intermittent high-dose therapy (the so-called 'Stoss' therapy) is an interesting option to avoid non-adherence to treatment, although a regimen of regular low dose is a reasonable alternative. Studies comparing these two different regimens actually reported inconsistent results, and both high dose (dosing interval <2 months) and more regular low dose seem to offer similar efficacy (72, 73, 74). The maximum safe bolus of vitamin D remains uncertain. A number of papers reported that a single oral dose of 300 000–600 000 IU D_2 or D_3 rapidly enhances serum 25(OH)D and reduces PTH levels in patients with deficiency (27, 28, 75). However, the study by Sanders *et al.* (76) showing that 500 000 IU oral dose of cholecalciferol increased the risk of falls and fractures

among older women deserves attention. Another trial reported that 300 000 IU ergocalciferol given i.m. for 3 years to elderly people during fall season did increase fracture risk (77). No plausible biological explanation has been given for these results, whose interpretation remains merely speculative. However, these papers raise the possibility that infrequent high doses of vitamin D may be unsafe, probably because they induce large and rapid fluctuation in vitamin D status, thus counteracting any possible beneficial outcome. The debate is still open: the rate and magnitude of the increase in serum 25(OH)D levels may be critical, as well as at which time points 25(OH)D concentration should be measured after dosing. On the other hand, it is undeniable that, on a population basis, the utilization of intermittent large doses could aim to overcome the problem of compliance (78, 79, 80, 81).

Once vitamin D stores have been replete, a maintenance dose of 800–2000 IU/day (20–50 μg/day) should be recommended. In particular, long-term supplementation has to be encouraged in special groups that are at high-risk for deficiency. In this regard, many experts have questioned the IOM recommendations as, in the absence of sun exposure and dietary input, a daily dose of 600 IU (15 μg) vitamin D will not maintain blood 25(OH)D levels, even at 20 ng/ml (82, 83). Therefore, higher doses may be necessary to achieve an optimal vitamin D status. Indeed, published data demonstrate that among postmenopausal women, larger doses of between 800 and 2000 IU (20 and 50 μg) vitamin D daily, were not able to achieve sufficiency in all the participants (84, 85). Moreover, in a recent study, Cavalier *et al.* (86) reported that the administration of about 4000 IU/day (100 μg/day) of vitamin D_3 in subjects with baseline serum 25(OH)D levels <10 ng/ml was insufficient to achieve or maintain 30 ng/ml in a significant proportion of subjects. It is noteworthy that the administered dose was very close to the upper safety limit of 4000 IU/day defined by the IOM. We believe that high-risk populations, such as the elderly and institutionalized individuals, should receive a supplementation of higher-than-usually accepted doses to achieve the desired level. Recently, a panel of French experts published specific guidelines for vitamin D supplementation in nursing home residents. The panel agreed that all nursing home residents should be supplemented with a dose of at least 1000–2000 IU/day (25–50 μg/day) vitamin D_3 given intermittently (e.g. weekly, monthly, quarterly, or once a year) to improve compliance and to reduce both daily poly-pharmacy and the burden for the nursing home personnel (87).

Vitamin D supplementation: which type?

Current evidence suggests that ergocalciferol has a considerably lower efficacy than cholecalciferol in raising circulating 25(OH)D levels. This difference between the two calciferols relates to several factors: the different affinity for the DBP and VDR, the different affinity as substrate for hepatic 25-hydroxylase, and a possible difference in the 24-hydroxylation rate. This last point deserves interest. In fact, the metabolism of vitamin D involves 24-hydroxylation in the kidney to form $1,24,25(OH)_3D$. This step is crucial as once $1,24,25(OH)_3D_2$ has been formed, ergocalciferol has been deactivated and, therefore, is irretrievable. On the contrary, the $1,24,25(OH)_3D_3$ still binds to VDR ($\approx$40% more than $1,25(OH)_2D_3$) and must undergo additional side-chain oxidation to be biologically deactivated (88). This additional step gives a vast advantage and potential for cholecalciferol to remain biologically active and, thus, maintains vitamin D status. Available data also document the higher efficacy of cholecalciferol, regardless of the frequency of administration (small daily doses or in larger and more infrequent bolus) (27, 88, 89, 90, 91). The monthly administration of 500 μg oral $25(OH)D_3$ has been proposed as an alternative for vitamin D repletion, without any detrimental effect (92). Moreover, it has been recently demonstrated that 800 IU (20 μg) oral $25(OH)D_3$ per day resulted in a safe, immediate, and sustained increase in serum 25(OH)D levels in all participants compared with vitamin D_3 (1 μg oral $25(OH)D_3$ increases 25(OH)D levels to about 4–5 nmol/l compared with the 1 nmol/l increase with 1 μg vitamin D_3) (13). Taken together, these findings suggest that where available, calcidiol is an option for supplementation, particularly in specific clinical conditions such as advanced liver failure in which the 25-hepatic hydroxylation is impaired.

Vitamin D supplementation: i.m. or oral route of administration?

In many countries around the world, both cholecalciferol and ergocalciferol are available as oral or i.m. preparations. In general, oral administration is more physiological and leads to a rapid increase in serum 25(OH)D levels within 3 days (27). With i.m. injection, a gradual increase in serum 25(OH)D levels was observed, thus demonstrating a delayed serum 25(OH)D response (27). This phenomenon is probably due to the sequestration of vitamin D in the muscle and fat, where it is gradually released. It has been hypothesized that this pharmacokinetic profile potentially allows i.m. preparations to overcome the fluctuation of serum 25(OH)D levels following high oral bolus (81). However, this point has not been definitively clarified. On the other hand, i.m. preparations may have specific indications, in particular for intermittent (once- or twice-yearly) high-dose regimens. For example, in patients with short bowel syndrome, such an intermittent i.m. regimen is able to attain vitamin D sufficiency. Moreover, in children or in institutionalized

elderly, the i.m. administration is effective in prevention of deficiency, also improving the long-term adherence to treatment (93).

Vitamin D supplementation and safety

Vitamin D intoxication is rather unusual. After intensive solar ultraviolet B (UBV) irradiation, the skin synthesis of vitamin D is self-regulated since inactive metabolites are produced. This explains why no reports described vitamin D intoxication from excessive sun exposure. Moreover, available data demonstrate that skin synthesis of up to 10 000 IU (250 μg) vitamin D daily is safe. The contribution of dietary intake is usually about 10–20%; hence, intoxication is nearly impossible from this source. Therefore, the potential harm of vitamin D may only come from the excessive ingestion of supplements (94, 95).

A number of studies linked the amount of vitamin D intake with the achieved serum 25(OH)D levels in order to establish a threshold for intoxication. It has been reported that there is no harm with an intake of 10 000 IU/day (250 μg/day) vitamin D, which corresponds to a serum 25(OH)D level of about 88 ng/ml (220 nmol/l) (96). Nevertheless, the IOM recently set the upper safe level of circulating 25(OH)D at about 50 ng/ml (125 nmol/l) on the basis of observational studies showing a U-shaped association between circulating 25(OH)D and some clinical outcomes (frailty, all-cause mortality, cancer, falls, and fractures) (97). Serum 25(OH)D levels beyond this limit are considered potentially harmful. However, this threshold seems to be very conservative, especially if we take into consideration many published studies showing that doses around 4000 IU/day (100 μg/day) vitamin D are safe, even in long-term treatment. The serum 25(OH)D level achieved in these studies was between 30 and 64 ng/ml (75 and 160 nmol/l), and it was not accompanied by any clinical sign of intoxication (95).

Vitamin D supplementation and the real world

Over the last decade, there has been growing attention to the role of vitamin D in various chronic diseases. However, the flourish claims in the media, the increasing scientific publications in peer-reviewed journals and the increasing information available on consumer health web sites had two relevant consequences: a substantial increase in laboratory testing for vitamin D and a dramatic rise of sales of vitamin D supplement. In the USA, many clinical laboratories have experienced increases in vitamin D testing of 100% or more in the last 5 years (98). The amount spent on vitamin D supplements in the USA had risen to tenfold in 10 years, from $40 million in 2001 to $425 million in 2009 (99) and $600 million in 2011 (100). On the other hand, the scenario in Europe seems to be different. In fact, a recent survey carried out in southwest Scotland showed that 69% of patients in whom determination of serum 25(OH)D level was requested had vitamin D levels below 20 ng/ml (50 nmol/l) but only 61% of deficient patients were prescribed any form of vitamin D replacement therapy. Moreover, inadequate doses or inappropriate forms of therapy were frequently suggested (101). These findings highlight that the gap between expert recommendations and clinical practice could be partly explained by the conflicting guidance on definition, prevention, and treatment of vitamin D deficiency endorsed by different medical and scientific communities.

Conclusions and perspectives

Important advances have been made in the understanding of the metabolism, mode of action, and measurement of 25(OH)D. At the same time, the scientific community does not seem to find a consensus on the definition and treatment modalities of hypovitaminosis D. Future investigation should fill these gaps, focusing on accurate measurement of vitamin D without neglecting the possibility of determining the free fraction. The genetic variants regulating circulating 25(OH)D levels and how these traits can influence supplementation and treatment are definite areas of research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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