Controversies in Vitamin D: Summary Statement From an International Conference

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Context: Vitamin D is classically recognized as a regulator of calcium and phosphorus metabolism. Recent advances in the measurement of vitamin D metabolites, diagnosis of vitamin D deficiency, and clinical observations have led to an appreciation that along with its role in skeletal metabolism, vitamin D may well have an important role in nonclassical settings. Measurement of the circulating form of vitamin D that best describes total body stores, namely 25-hydroxyvitamin D, can be unreliable despite many sophisticated methodologies that have been proposed and implemented. Likewise, evidence from clinical studies showing a beneficial role of vitamin D in different disease states has been controversial and at times speculative. Moreover, the target concentrations of 25-hydroxyvitamin D to address a number of putative links between vitamin D inadequacy and nonskeletal diseases are further areas of uncertainty.

Setting: To address these issues, an international conference on "Controversies in Vitamin D" was held in Pisa, Italy, in June 2017. Three main topics were addressed: (i) vitamin D assays and the definition of hypovitaminosis D; (ii) skeletal and extraskeletal effects of vitamin D; (iii) therapeutics of vitamin D.

Results: This report provides a summary of the deliberations of the expert panels of the conference.

Conclusions: Despite great advances in our appreciation of vitamin D metabolism, measurements, biological actions on classical and nonclassical tissues, and therapeutics, all of which this report summarizes, much more work remains to be done so that our knowledge base can become even more secure. **(*J Clin Endocrinol Metab* 104: 234–240, 2019)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 28 June 2018. Accepted 26 October 2018.
First Published Online 31 October 2018

Abbreviations: DBP, vitamin D-binding protein; LC, liquid chromatography; vitamin D_2, ergocalciferol; vitamin D_3, cholecalciferol.

Although recognition of rickets and related musculoskeletal diseases date back to three or four centuries ago, their underlying causes were largely unknown until the identification in 1922 of the secosteroid that we know as vitamin D (1). Interest in this hormone slowly increased over the years but literally exploded with the discovery of the activation pathway of vitamin D to 25-hydroxyvitamin in the liver and to the active product itself, 1,25-dihydroxyvitamin D, in the kidney. Advances in biochemical assessment of vitamin D metabolites, appreciation that such levels bear importantly on therapeutic goals in a variety of metabolic bone diseases, and linkage to extraskeletal aspects of vitamin D action all have contributed to the current understanding of activated vitamin D as a pleiotropic hormone that appears to have a potential physiological role in all human tissues.

Vitamin D is a fat-soluble vitamin consisting of the plant form known as ergocalciferol (vitamin D_2) and the animal form known as cholecalciferol (vitamin D_3). The source of vitamin D_2 in the human is dietary intake and supplements, whereas the source of vitamin D_3 in the human can be endogenous synthesis as well as from the diet and supplements. Vitamin D_3 is produced by the skin upon UV-B irradiation of 7-dehydrocholesterol (2). Subsequently, vitamins D_2 and D_3 are hydroxylated at Position 25 in the liver to form 25-hydroxyvitamin D, the major circulating form of vitamin Da, and ultimately to the fully active moiety by hydroxylation at Position 1 in the kidney to form 1,25-dihydroxyvitamin D (3). The same enzyme responsible for the formation of 1,25-dihydroxyvitamin D in the kidney, namely CYP27B1, is also found in many extrarenal tissues and is able to produce 1,25-dihydroxyvitamin D in a para- or autocrine fashion. Measurement of 25-hydroxyvitamin D is currently accepted as the best index of vitamin D stores. In the circulation, 25-hydroxyvitamin D is bound primarily to vitamin Da, binding protein (DBP). The prevalence of vitamin D deficiency, as assessed by circulating concentrations of 25-hydroxyvitamin D, ranges from 27% to 91%% in North America, a representative observation that illustrates that vitamin D deficiency is widespread and a global health problem (4). Speaking to the difficulty of defining deficiency prevalence, over a 16-year period in the United States, from 1988 to 2004, the national percentage of adults achieving serum 25-hydroxyvitamin D levels of at least 30 ng/mL was thought to have precipitously declined from 60% to 30% (5); however, after retrospective standardization, no decline was evident (6). This point raises the confounding issue of accurate measurement of circulating 25-hydroxyvitamin D. Numerous factors account in the aggregate for such measurement difficulties, the key ones of which are methodological interference and lack of assay standardization (7).

The extraskeletal disorders that have been associated with low vitamin D status include dermatologic and cardiovascular diseases, malignancies, and immune and metabolic disorders (8). Understandable interest in benefits that could potentially be obtained by vitamin D supplementation has grown, resulting in many clinical studies across a range of experimental settings. Despite this intense interest, vitamin D supplementation has failed to provide unequivocal documentation of benefits with respect to most therapeutic areas (7, 9–11). In this report, we summarize recent advances in our knowledge of vitamin D, with the goal to provide insights and a blueprint for further studies.

This international conference on "Controversies in Vitamin D" that was held in Pisa, Italy, in June 2017, reviewed and analyzed the scientific literature regarding the role of vitamin D in the maintenance of human health. A panel of international experts approved several statements that were focused on three topics: (i) vitamin D assays and definition of hypovitaminosis D; (ii) skeletal and extraskeletal effects of vitamin D; (iii) therapeutic use of vitamin D. This report is a summary of the deliberations of the expert panels, each of which has a full-length publication (12–14). As this report is a summary of those findings, further details can be found in those published articles.

Methods

The conference consisted of thematically based presentations by invited participants chosen by members of the Steering Committee who are represented by the authorship of this report.

Selection of participants was made with regard to the thematic content of the conference, recognizing that it was not possible to invite all experts because of constraints of time and other circumstances. Systematic reviews of the entire literature relevant to the individuals' presentations were not required but in many cases, were conducted. Controversial topics included the Institute of Medicine's recommendation of adequate levels of vitamin D in a healthy population without osteoporosis or other metabolic bone disease. Other controversial topics included the following: target levels of 25-hydroxyvitamin D for those with osteoporosis or other metabolic bone diseases, ethnic differences, assay methodologies for 25-hydroxyvitamin D (including DBP) and pitfalls associated with them, skeletal and extra skeletal actions of vitamin D, and therapeutic uses.

Vitamin D assays and the definition of hypovitaminosis D

Vitamin D adequacy is usually determined by the measurement of serum total 25-hydroxyvitamin D concentration, defined as the sum of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 levels (15, 16). The serum concentrations of other vitamin D metabolites and components of vitamin D metabolism, such as the

three-epimer of 25-hydroxyvitamin D, $1,25(OH)_2D$, $24,25(OH)_2D_3$, DBP, and free 25-dihydroxyvitamin D, are not generally used clinically to assess an individual vitamin D status but rather in research studies or in special situations (17, 18). For example, the $1,25(OH)_2$ active form of vitamin D is measured if there is a defect in the conversion of 25(OH)D to $1,25(OH)_2D$, as found in renal failure, hypoparathyroidism, some genetic forms of rickets, and oncogenic osteomalacia. $1,25(OH)_2D$ is also measured if hypercalcemia is suspected as a result of toxic ingestion of calcitriol or because of overproduction of $1,25(OH)_2D$ in granulomatous diseases or in some lymphomas. The $24,25(OH)_2D$ form is measured in relationship to 25-hydroxyvitamin D if hypercalcemia is thought to be a result of a deficiency in the CYP24A1 enzyme that is responsible for an inactivation pathway of vitamin D. Measurement of these other forms of vitamin D are hampered by lack of standardized assays (19).

Serum total 25-hydroxyvitamin D is a difficult analyte to measure because it is relatively hydrophobic and binds tightly to DBP (20). Immunoassays and liquid chromatography (LC)-tandem mass spectrometry techniques can be used to assess serum 25-hydroxyvitamin D concentrations, but they all can be problematic. With immunoassays, antibody sensitivity and specificity are critically important. Low-affinity antibodies for 25-hydroxyvitamin D_2 could underestimate the total 25-hydroxyvitamin D level (21). Crossreactivity of the antibody with $24,25(OH)_2D_3$ could also affect the accuracy of the 25-hydroxyvitamin D measurement (22, 23). The 25-hydroxyvitamin D measurement can also be compromised by its tight binding to DBP or by the presence of other serum proteins in the sample (matrix interference) (24–26). Methods based on LC can also be problematic if they do not properly resolve the 3-epi-25-OHD_3 metabolite. Thus, inclusion of this metabolite will overestimate the 25-hydroxyvitamin D level (23). This epimer is generally low in adults but can be high in young children (27, 28). Recognition of these potential pitfalls in 25-hydroxyvitamin D measurement led to the international Vitamin D Standardization Program that was founded in 2010 (29). It spearheaded development of reference measurement procedures and standard reference materials and approaches allowing the reporting of standardized 25-hydroxyvitamin D data. LC-tandem mass spectrometry is currently considered to be the most accurate and precise method for measuring 25-hydroxyvitamin D and vitamin D metabolites, not only for research but also in clinical situations. Despite this recommendation, automated immunoassays that measure only total serum 25-hydroxyvitamin D are still routinely used in many clinical laboratories.

The quest for a standardized approach to the accurate determination of 25-hydroxyvitamin D concentration is a way toward a clearer definition of vitamin D status, whether one is concerned about deficiency, sufficiency, or excess (15). With this caveat in mind, current guidelines suggest that 25-hydroxyvitamin D values <12 ng/mL (30 nM) are associated with an increased risk of rickets/osteomalacia, whereas 25-hydroxyvitamin D concentrations between 20 and 50 ng/mL (50 to 125 nM) appear to be safe and sufficient for skeletal health in the healthy general population (30). It is not clear how or whether these guidelines should be considered with regard to individuals who have metabolic bone diseases, such as osteoporosis or primary hyperparathyroidism.

Skeletal and extraskeletal actions of vitamin D: current evidence and outstanding questions

A causal role of vitamin D deficiency in the pathogenesis of skeletal disorders, such as rickets and osteomalacia, has been clearly established (31). In the context of vitamin D deficiency based on nutritional grounds or via inactivation of vitamin D receptor or 1α-hydroxylase (CYP27B1), humans and mice have abnormal epiphyseal growth plates. Interestingly, however, clinical, radiological and histological hallmarks of rickets and osteomalacia can be rescued by dietary or genetic interventions aimed at restoring sufficient calcium absorption (32, 33). These data clearly indicate the importance of normal vitamin D signaling for adequate intestinal calcium absorption and in turn, bone homeostasis (34).

Numerous clinical trials and meta-analyses have investigated the effect of vitamin D alone or with calcium on fracture incidence, showing that vitamin D supplementation can be effective in certain populations at the modest daily dose of 800 IU. These populations include older subjects, $\geq$70 years of age, vitamin D-deficient populations, and in subjects living in residential care settings (35, 36). In contrast, other meta analyses have not been consistent with this view, illustrating that differing results could well be a function of the specified parameters of the analyses that vary from study to study in the ages of the populations, chosen cut points of vitamin D, ethnicity, the duration of the study, whether calcium was included in the trials, and other factors (37).

Muscle weakness is classically described as a common clinical feature in severe vitamin D-deficient states, and in these cases, improvement in muscle strength can be expected after vitamin D replacement (38). In observational studies, that association between lower levels of 25-hydroxyvitamin D and higher fall risk and fractures could be related to muscle dysfunction (39, 40). Several meta-analyses have suggested that adequate vitamin D supplementation can prevent falls and improve muscle function, especially in elderly and deficient populations (41–43).

Vitamin D may play a role in the pathogenesis of several extraskeletal disorders involving the dermatological, cardiovascular, immune, or metabolic system. The skin represents a well-established example of a nonskeletal action of vitamin D signaling. Despite the fact that no clear association between vitamin D and psoriasis incidence has been found (44), topical application of vitamin D analogs is the most common treatment of psoriasis (45). The beneficial effect of vitamin D in psoriasis has been attributed to a vitamin D-induced reduction of epidermal cell proliferation and to its anti-inflammatory properties.

A role for vitamin D in the regulation of the immune system was proposed several years ago when the vitamin D receptor was shown to be expressed in T lymphocytes (46). More recently, vitamin D was found to regulate both arms of the immune system, by inhibiting the adaptive immune response and by promoting the innate immune response. As a result of these observations, it has been proposed that vitamin D deficiency could enhance both the risk of infections (47) and autoimmune diseases, such as multiple sclerosis and type 1 diabetes (48, 49). However, these observations have not been substantiated by larger randomized clinical trials that are needed to elucidate the impact of vitamin D deficiency and/or supplementation in immune system disorders. On the other hand, one of the most consistent associations in this regard has been found between poor vitamin D status and obesity or type 2 diabetes (50, 51). In

contrast, intervention studies have not shown a consistent or clear effect of vitamin D supplementation on prevention of type 2 diabetes (52), nor on some aspects of the metabolic syndrome, such as glucose tolerance, blood pressure, or serum lipids levels (53, 54). Finally, numerous preclinical studies suggest a potential role of vitamin D on cancer development and progression (55). Vitamin D receptors are expressed in many different cancer cell lines (56), and animal models in which vitamin D receptors are knocked out are more prone to develop different types of cancers, such as breast, colon, and skin tumors when provoked by carcinogens (57). Additionally, the association between low serum 25-hydroxyvitamin D level and colorectal cancer risk (58) has not been validated with beneficial effects of vitamin D supplementation (59).

Therapeutic use of vitamin D

Cutaneous exposure to UV-B irradiation is historically a major source of vitamin D in humans (34). With public health, environmental, and cultural issues that have led to a reduced role for the cutaneous production of vitamin D_3 in humans, other sources have become more important. Vitamin D-fortified foods and vitamin D supplementation have supplanted, to a large extent, the prominent role that the skin used to play in the vitamin D economy of many individuals. Vitamins D_2 and D_3 are the most common forms of dietary supplementation (15).

A recent meta-analysis showed that vitamin D_3 was more efficacious in raising 25-hydroxyvitamin D concentrations than vitamin D_2 (60), but either vitamin D_2 or D_3 appears to be reasonable, first-line approaches for the prevention and treatment of hypovitaminosis D (16). For infants, a daily supplement of 400 IU is advised (61). For adults under 70 years, the daily recommendation is 600 IU, and for those over 70 years, the daily recommendation is 800 IU (61). Not too long ago, single high, loading doses of vitamin D were considered to be attractive to restore normal vitamin D status rapidly and conveniently. More recently, however, studies relating bolus dosing to an increased fall and fracture risk have diminished enthusiasm for this approach (62).

For treatment of nutritional rickets, the minimal recommended dose of vitamin D_2 or D_3 is 2000 IU/day for a minimum of 3 months (30). Numerous hydroxylated metabolites of vitamin D, such as calcidiol, calcitriol, and alfacalcidol, have also been used to achieve vitamin D adequacy in specific clinical settings (63). For example, if hepatic or renal conversion steps of vitamin D activation are impaired, then metabolites that bypass these steps may be helpful. In particular, calcidiol is useful when hepatic hydroxylase activity is impaired, in the setting of fat malabsorption, or following bariatric surgery (63). The 1α-hydroxylated derivatives (calcitriol, alfacalcidol, or eldecalcitol) are useful in renal insufficiency or other settings, such as hypoparathyroidism, when conversion to active vitamin D is impaired (63). In primary hyperparathyroidism, 25-hydroxyvitamin D levels can be low and associated with higher indices of disease activity. Careful supplementation with vitamin D in these individuals can help to control the extent to which the parathyroid hormone is overproduced by abnormal parathyroid glands (64, 65). The lack of the parathyroid hormone in hypoparathyroidism and hyperphosphatemia leads to impairment in the renal activation of 25-hydroxyvitamin D. Conventional therapy includes supplementation with calcium and active vitamin D forms, namely, calcitriol or other analogs (66). However, administration of high doses of 1α-hydroxylated forms can increase the risk of hypercalciuria and hypercalcemia (67, 68). As glucocorticoids exert antivitamin D actions by inhibiting hepatic and renal hydroxylation of vitamin D, supplementation with hydroxylated analogs could also be proposed for patients with glucocorticoid-induced osteoporosis (69, 70).

In general, the extent to which serum 25-hydroxyvitamin D levels will rise following vitamin D supplementation is a function of baseline concentration and body weight. Obesity is associated with lower 25-hydroxyvitamin D concentrations, as the lipophilic pool that forms the reservoir of vitamin D is much greater in obese subjects; they typically need longer exposure to UV-B or higher doses of vitamin D supplementation than normal-sized individuals (71, 72).

Conclusion

This international conference on controversies in vitamin D reviewed the most recent evidence, at the time of the conference, regarding the role of vitamin D in human health. In this report, we have summarized those deliberations that have helped to focus our needs for future research based on our current knowledge. These needs include standardized assays that can be universally adopted to assess vitamin D status in human subjects, clearer insights into the putative nonskeletal actions of vitamin D, and more definitive clinical trials that determine under what conditions and in what disorders vitamin D and its metabolites are useful therapeutically. Ongoing clinical trials, the results of which will be known in the next several years, are awaited.

Acknowledgments

We acknowledge the Steering Committee (Co-chairs John P. Bilezikian and Andrea Giustina) and other members Robert A. Adler, Neil Binkley, Roger Bouillon, Peter R. Ebeling, Marise Lazaretti-Castro, Claudio Marcocci, Rene Rizzoli, and Christopher T. Sempos) and all other workshop participants (Daniel Bikle, Patsy Brannon, Geert Carmeliet, Bess Dawson-Hughes, Hector DeLuca, Ombretta DiMunro, Annemieke Heijboer, Glenville Jones, Uri Liberman, Paul Lips, Gherardo Mazziotti, Salvatore Minisola, Craig Munns, Nicola Napoli, Giovani Passeri, Andreas Pittas, Sylvia Trasciatti, and John White).

Financial Support: The conference was funded by Abiogen (Pisa, Italy). Travel funds were granted to all participants, but no other compensation was received.

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Disclosure Summary: The content, subject matter, participant selection, and manuscript preparation were determined solely by the Steering Committee. The sponsor had no role in the selection of topics, review of any presentations, open or closed

discussions, writing, or review of this or the associated manuscripts.

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