Review Article

The resurgence of the importance of vitamin D in bone health

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Dietary reference intakes (DRIs) for Vitamin D (VitD) have been traditionally established based on plasma 25-OHD concentration sufficient to prevent rickets and osteomalacia. While nutritional rickets is still prevalent in developing countries, hypovitaminosis D is becoming widespread around the world regardless the latitude. Emerging evidence has unravelled the physiological roles of VitD beyond calcium homeostasis. Hypovitaminosis D has been linked to cancers, diabetes, CVD, periodontal diseases and influenza. Hypovitaminosis D is multifactorial and is related to VitD scarcity in foods, latitude, solar-irradiation, atmospheric-pollution, skin-pigmentation, clothing, sunscreen-use and indoor activities, etc. Plasma 25-OHD concentration range from 25-138 nmol/L. A higher plasma 25-OHD concentration is linked to higher bone-mass in adolescents, pre- and post-menopausal women. Plasma 25-OHD ≥75 nmol/L has been shown to enhance calcium absorption, suppress PTH elevation, reduce the risks of bone loss and fractures, and certain extra-skeletal diseases. VitD supplementation with 10ug/d is insufficient to lower fracture risks. Combined VitD and calcium supplementation in higher doses has been found superior to VitD alone to increase bone-mass in adolescents and to reduce non-vertebral fractures in postmenopausal women. In future, DRIs for VitD are likely to be established beyond its skeletal roles to include multiple health outcomes. However, the desirable level of VitD has yet to be defined. Furthermore, redefining the upper-tolerable-level of VitD intake is necessary to prevent hypercalcemia and toxicity. There is also a urgent need to harmonize laboratory methods in VitD assay in different laboratories.

Key Words: vitamin D, hypovitaminosis D, plasma 25-OHD, dietary reference intake, bone mass, fracture

INTRODUCTION

The discovery of steroid hormone vitamin D (VitD) that cures rickets and osteomalacia[1] has led to the establishment of dietary reference intakes (DRIs) of VitD based on plasma 25-hydroxyvitaminD_3 (25-OHD) concentration sufficient to prevent rickets and osteomalacia.[2-3] VitD acts in concert with parathyroid hormone (PTH) to modulate calcium homeostasis and bone metabolism. Reduction in plasma ionized calcium triggers PTH secretion to stimulate the production of 1,25-dihydroyvitamin D_3 in the kidney, an active metabolite of VitD targeting the gut to enhance calcium absorption. PTH and VitD also act to increase calcium resorption and phosphorus excretion from the renal distal tubules. In bone, both hormones stimulate osteoblasts to produce RANKL which promotes osteoclastogensis and activates resting osteoclasts to increase bone resorption. As a result, calcium is mobilized from the gut, kidney and bone to restore plasma calcium concentration to normal.[4] Normal range of plasma 25OHD spans from 25-138 nmol/L.[2] The lower limit of plasma 25OHD varies ~20-37.5 nmol/L, plasma 25-OH concentration below 37.5 nmol/L is defined as hypovitaminosis D.[2] In the UK, plasma 25-OHD concentration at 25 nmol/L is used as the lower cutoff for adequate VitD status.[3] Food sources of VitD is limited in cod liver oil, fatty fish, livers, egg yolks, and fortified foods such as margarine. Therefore, cutaneous photosynthesis of VitD under the sun remains the principal source of VitD for human. Factors that potentially affect VitD status are genetics, adiposity, skin pigmentation, age, latitudes, seasons, atmospheric pollution, diets, clothing and use of sun-screen, etc.[5]

Nutritional rickets and hypovitaminosis D

Nutritional rickets under 5-y old attributable to calcium and/or VitD deficiencies is still a public health problem in developing countries at both temperate and tropical latitudes, as well as among the at risk groups in Western developed countries.[6-7] Since 1900's, despite interventional strategies by using food fortification, public education on regular sunlight exposure, and VitD supplementation targeting vulnerable groups to combat against rickets, cases of rickets have been reported in 60 countries over the last 20 years.[6] Nutritional rickets is more subtle in stunted children coexisted with chronic malnutrition.[7]

Furthermore, there is mounting evidence to suggest that VitD insufficiency is widespread beyond childhood in every continent regardless the latitude.[8-16]

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There is an increasing concern of a widespread of hypovitaminosis D around the world.[17-18]

Seasonal variation in VitD status has been observed in populations living in the temperate zones of the world. For instance, UK is located between the latitudes between 50-59^{0}N. Plasma 25OHD of adults is highest in Jul-Sep and lowest in Jan–Mar.[19] Atmospheric UV exposure varies with latitude, suitable Ultraviolet B light wavelengths (295-315 nm) for cutaneous synthesis of VitD is not available in UK during winter at latitude ≥52°N. Hence, the UK populations rely on their body stores and diets to maintain VitD status in winter. A recent survey[14] in 7434 British adults aged 45y found an alarmingly high prevalence of hypovitaminosis D among the UK populations in winter and spring (serum 25OHD concentrations <25, <40, < 75 nmol/L were 16%, 47% and 87% respectively; whereas in summer, the proportions were 3.2%, 15% and 61% respectively). In fact, significantly more residents in Scotland had serum 25OHD <40 nmol/L than those living in England & Wales (p<0.0001). In Boston, USA (40°N), postmenopausal women were found to have increased spinal bone loss in winter but bone gain in summer.[20]

VitD and bone health

The beneficial effects of VitD on bone mass have been evaluated in childhood and adolescence,[21-25] premenopausal years,[26] and postmenopausal women with respect to bone mass maintenance[26-27] and fractures prevention.[27] There is an inverse association between plasma levels of PTH and VitD in adolescents within a wide range of calcium intakes.[11, 28-29] So far there has not been an appropriate health outcome to suggest if an increase in PTH is beneficial or deleterious to the long-term bone health in adolescents. In adults, optimal VitD status has been shown to suppress plasma PTH. An increased PTH level up-regulates bone turnover leading to low bone mass and increased fracture risks.[30-32] Plasma 25OHD concentration at ~ 80 nmol/L (range 75-110 nmol/L) has been found desirable because plasma PTH stops rising and plateaus off even if 25OHD level continues to increase beyond 80 nmol/L.[31,33] Furthermore, meta-analyses evaluating VitD supplementation on BMD, fracture risks[34] and risks of falls[26] have found that VitD doses (17.5-20 μg/d) which are greater than the current DRIs, i.e., 5-10 ug/d[2-3] are required to raise plasma 25OHD concentration to ≥ 75 nmol/L in order to see its beneficial effects on bone. Hence, recent findings suggest that the Current VitD DRI values are not sufficient to raise plasma 25OHD level to optimize bone mass status and to prevent fractures.

Synergistic effect of VitD and calcium on bone integrity

A 2-y calcium-fortified milk and calcium-VitD-fortified milk trial[8] in 757 10-y old girls from Beijing showed that girls receiving extra VitD from calcium-fortified milk compared to those receiving calcium-fortified milk only had significantly greater gains in total body BMC (2.4 *v.* 1.2 %) and BMD (5.5 *v.* 3.2 %). A recent meta-analysis showed that Vitamin D alone showed no significant effect on hip fractures among institutionized frail elderly but VitD combined with calcium supplementation reduced hip fractures and non-vertebral fractures.[35]

Functional outcomes for optimal vitamin D status

Functional outcomes such as plasma VitD level sufficient to suppress a rise in plasma PTH, optimize bone gain, reduce bone loss and fracture risks, maximize calcium absorption and neuromuscular strength of the lower extremities could be potentially used to define optimal VitD status.[17,36] Take serum 25OHD level and calcium absorption as an example, fractional calcium absorption is proportional to serum 25OHD concentration in the range of 20-80 nmol/L among healthy US men and women. Fractional calcium absorption maximizes at 80 nmol/L and then levels off even if serum 25OHD level goes up[37]. However, an inverse correlation between fractional calcium absorption and serum 25OHD level has been observed in children and adolescents in China[38] and USA[39], who have lower serum 25OHD level that may reflect nutritional adaptation by increasing the conversion of serum 25OHD to 1,25OHD or by-passing the VitD dependent route for calcium absorption in these children. Hence, more research is needed to evaluate the relation among calcium, VitD, PTH and bone mass status in populations habituate to lower VitD and calcium intakes.

Physiological functions of VitD beyond calcium and bone metabolism

Vitamin D receptor (VRD) responsible for the regulation of calcium and bone metabolism is not only found in cells of parathyroid gland, bone, gut and renal tubules but also found in liver, heart, skeletal muscle, skin, lymphocytes, colon, ovary, etc. This has aroused research interest to study the relation between VitD and physiological functions beyond calcium homeostasis and bone metabolism.[4] Plasma 25OHD level has been linked to cancers[40] especially colorectal cancer,[41] Type I and Type 2 diabetes,[42-44] cardiovascular diseases,[45] periodontal disease[46-47] and influenza.[48] Hence, there is an increasing evidence to demonstrate the diversified roles of VitD in health and disease prevention beyond its calcemic functions.

Multiple functional outcomes for defining optimal vitamin D status

The discovery of the diversified physiological roles of VitD in health maintenance and disease prevention has led to the belief that the definition of healthy VitD status cannot be solely based on plasma 25OHD concentration sufficient to prevent rickets, osteomalacia, fractures or to optimize calcium absorption. It has been suggested that multiple functional outcomes involving various tissues or organs should be used to derive the optimal plasma 25OHD level which in turn can better define optimal VitD status. In order to maximize bone mass, to reduce the risks of fractures, colon cancer, periodontal diseases and maintenance of neuromuscular strength[26] and to maximize calcium absorption,[37] the minimum plasma 25OHD concentration and its desirable range of concentrations have been proposed to be 75 nmol/L and 90-100 nmol/L respectively.[17,26]

Upper safety level of VitD intake

The process of converting cutaneous 7-dehydrocholesterol to previtamin D_3 under the sun, which then isomerizes to Vitamin D_3 in the body is under metabolic

regulation. Hence, prolonged sunlight exposure will not lead to excess production of Vitamin D_3. Both previtamin D_3 and Vitamin D_3 can be degraded and excreted in the urine. However, excess doses of oral VitD supplements can be toxic.[2] The European Union set the tolerable upper level of VitD intake for infants and children (0-10-y old) and children and adults (aged ≥11-y old) at 25 ug/d and 50 μg/d respectively.[49] In the UK, the tolerable upper level of VitD intake was set at 25 ug/d across the general population.[50] In the USA, the tolerable upper levels of VitD intake for infants aged 0-12-mo, children and adolescents aged 1-18-y are 25 μg/d and 50 μg/d respectively, and for adults (50 μg/d)[2]. There are emerging views that the current safety tolerable upper level of VitD intake for adults established in Europe and USA are 5 folds lower than the proposed 250 μg/d[51]. Health hazard evaluation in both short- and long-term use of VitD supplements, with dosage exceeding the current upper tolerable level, is mandatory to determine hypercalcemia and toxicity, if any. However, a recent risk assessment of VitD supplementation studies using high doses of VitD supplements were based on studies conducted for a few weeks, or with high doses but were given intermittently over months, which did not permit enough time to observe the steady state of equilibrium and to observe any long-term effects of hypercalcemia or toxicity.[51]

Inter-laboratory variation in determining VitD

Different laboratory techniques have been used to measure plasma 25OHD concentration including enzyme-linked immunoassay (ELISA), radioimmunoassay (RIA), high-performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectrometry (LC–MS). Plasma 25OHD analysed by RIA in an automated format providing a high sample throughput has been widely used in clinical and research laboratories. However, some RIA methods have been found unable to detect $25OHD_2$ with the same efficiency as $25OHD_3$ which may underestimate VitD status of patients when using Vitamin D_2 as supplement.[52-53] HPLC is an accurate method offering an advantage of separating and detecting $25(OH)D_2$ and $25(OH)D_3$ in plasma. The samples go through HPLC and followed by UV detection is highly reproducible and has been considered the gold standard method.[54] LC-MS has also been found an accurate method of choice for assaying 25OHD. However, both HPLC and LC-MS methods require expensive equipment and highly experienced staff, and the sample throughput is slow.[54] The International Vitamin D Quality Assessment Scheme (DEQAS) formed in 1989 aims to monitor the performance of 25OHD analysis. Currently, there are over 100 registered laboratories from 18 countries. DEQAS in 2004 reported that different laboratories using different methods produce different results based on the same samples. Most 25OHD commercial assay methods were not accurate enough to measure the true 25OHD value, and the results were highly dependent on the laboratory and operator.[53] Hence, Global efforts in standardization and calibration of analytical methods is necessary to harmonize VitD assay among laboratories so that plasma 25OHD concentrations are comparable between laboratories across the world.

DISCUSSION AND CONCLUSION

Emerging evidence has shown a widespread of hypovitaminosis D among populations at different latitudes of the world. There is an association between hypovitaminosis D and the prevalence of skeletal and extra-skeletal diseases. The current DRI of VitD (5-10 μg/d) has been reported to be insufficient to raise plasma 25OHD concentration to a level sufficient to optimize health and prevent diseases beyond rickets and osteomalacia. However, the desirable level of VitD is still debatable in the scientific community and has yet to be redefined. Public health interventions on hypovitaminosis D, namely food fortification, public education on diets and life-style in particular with regular sunshine exposure, and VitD supplementation to vulnerable groups are necessary. Further research is indicated to study the pathophysiology of VitD insufficiency on bone integrity across the life-span, and to delineate the cause and effect of VitD insufficiency and diseases beyond the skeletal system. Most research linking hypovitaminosis D and its related diseases were conducted in the Western developed countries, more research is clearly indicated in the developing countries to evaluate the relation between hypovitaminosis D and diseases by taking into account of different cultures, life-styles and adaptation in the people habituate to lower intakes of calcium. Furthermore, redefining the upper tolerable level of VitD intake is necessary to prevent hypercalcemia and toxicity. There is also a urgent need to harmonize laboratory methods in VitD assay for standardizing VitD assays in different laboratories across the world.

AUTHOR DISCLOSURES

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