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Vitamin D, myth or miracle?

J.P.M. WIELDERS

In the last decennium, the medical laboratories have witnessed a large increase of requests for vitamin D analyses. This is attributed to the fast growing pile of publications on this interesting prohormone and the awareness of a moiety of doctors of its multiple effects. On the other hand, a large group of doctors is waiting for more evidence and considers vitamin D a hype and its effect a myth. The clinical presentation of rickets is known for many centuries. One century ago, it was recognized that rickets could be cured by a certain compound in cod liver oil. Since that day this substance was called vitamin D, although it was shown only a few years later that rickets could be cured with sunshine or UV radiation as well.

The essential role of vitamin D in calcium homeostasis and healthy bones is well known. Vitamin D is available in two forms, cholecalciferol (D3) that is generated in our skin after sun exposure or artificially prepared from wool-fat and ergocalciferol (D2) prepared from plant-sterols. Vitamin D2 is not available in the Netherlands. Most of our own vitamin D storage has been endogenously produced from April until October (in the Netherlands). As such vitamin D is not a vitamin but a (pre) prohormone with many effects outside calcium homeostasis. Only at wintertime we need a supplement and a few decades ago the use of vitamin D suppletion was very common in the Netherlands. The policy of suppletion to children up to 4 year still exists, but at present about 40 % of children at that age do not get the prescribed 400 iU/day.

Meander Medisch Centrum, Amersfoort

E-mail: jpm.wielders@meandermc.nl

One decade ago, a local general practitioner consulted our laboratory concerning a women with severe muscle weakness, which turned out to be a presentation of vitamin D deficiency. This initiated our interest and we started investigating the prevalence and clinical presentation of vitamin D deficiency in the general population and in risk groups. Further more, we have been involved in method development and validation. Finally we felt a moral obligation to provide medicinal professionals with information about clinical aspects vitamin D by means of lectures, publications, a website and the organization of two vitamin D symposia.

Prevalence of vitamin D deficiency

At the end of last century, most publications about vitamin D were either a description of cases and rather small cohort studies, or more academic research in endocrinology journals. Inspired by a publication of Glerup in Denmark and our experiences with several patients, we started with two small pilot studies. Our first study was performed in March 2001 about the prevalence and presentation of hypovitaminosis D in veiled women in the Netherlands (1) of rickets within a group. In a group of 51 Turkish women aged 14-63 years, we found 82% to be severely deficient (25(OH) D3 < 20 nmol/L). All but one woman in this group had levels below 50 nmol/L. At that time, the Dutch Health Council considered 30 nmol/L as sufficient for men and women at all ages. About half of the deficient Turkish women complained of muscle pain, muscle weakness or fatigue. In the Dutch control group, we found 6% below 20 nmol/L and 56% below 50 nmol/L. These results confirmed the presence of a possible serious public health problem in the Netherlands.

Next, we studied the prevalence of vitamin D deficiency in 34 institutionalized patients in a psycho - geriatric ward (2) and found 47 % below 20 nmol/L 25(OH) D3 and 91% below 50 nmol/L. We treated the deficient psychogeriatric patients with 800 iE vitamin D3 and 1000 mg calcium per day and found their vitamin D levels after 6 month to be 66 - 112 nmol/L.
In a group of 197 elderly home-living patients from general practitioners with complaints of malaise, we found about 12 % below 20 nmol/L and about 25 % below 30 nmol/L (Wielders, unpublished). We showed by our small studies that myopathy and weakness are correlated or possible causal related to hypo-vitaminosis D without overt osteomalacy.
Our laboratory participated in a cross-sectional study about prevalence of deficiency and its determinants in a representative multi-etnic multisite general practitioner patient group of 613 adults (3).
The prevalence of 25(OH)D3 deficiency defined as < 25 nmol/L was higher in Turkish (41.3%), Moroccan (36.5%), Surinam South Asian (51.4%), Surinam Creole (45.3%) and sub-Saharan African (19.3%) adults, compared to the Dutch group (5.9%). Major modifiable determinants were consumption of fatty fish, use of vitamin D supplements and the area of uncovered skin.

Our interest was raised in a possible correlation of vitamin D with fatigue and vague complaints in pregnancy (4). To determine the prevalence of vitamin-D deficiency among pregnant women and their newborns, we performed a descriptive study on pregnant women visiting our ward for obstetrics, over a period of one year (5). For 545 women of Dutch/West-European origin and 131 pregnant women of non-Western immigrant origin (mainly Turkish and Moroccan), we measured 25(OH)D3 and calcium in their 10th and/or 30th week of pregnancy. A severe deficiency was found (calcidiol < 20 nmol/L) in 55% of non-European women compared to 5% of Dutch/West-European women (figure 1A). From cord blood samples, a severe vitamin-D deficiency (calcidiol < 13 nmol/L) was found in 54% of the newborns of non-European origin compared to 6% of the Dutch/West-European newborns (figure 1B). Our conclusion that more than half of the non-European pregnant women and their newborns were severe vitamin-D deficient was hot news in all Dutch newspapers, it was cited on radio and television, and discussed in interviews. We proposed screening for vitamin D deficiency and adequate suppletion for pregnant women from this risk group and pointed at causes and possible consequences of vitamin-D deficiency for mother and child. In a recent retrospective study we found 400 iU per day given to vitamin D deficient pregnant women, being the Dutch Health Council advice, too low for reaching at least 50 nmol/L (Borst, Duk and Wielders, submitted).
The growth and development of the most severe deficient newborns was a matter of serious concern to us. So, we performed a clinical and biochemical follow-up at the age of about one and a half year. No clinical signs of rickets were detected, but 43% of 86 toddlers had continued in vitamin D deficiency (25(OH)D < 50 nmol/L). The most severe deficiency was found in non-Western immigrant children (Hogeman & Wielders, submitted). This points at a possible shortage of suppletion or low compliance regarding the Dutch advice of 400 iE per day under 4 years.
At present, five years after the publication of our perinatal study, our recommendation is still not shared by the majority of midwifes and gynaecologists but vitamin D has become a serious item in the discussion of new guidelines for pregnancy and new-born care.

Method development, validation studies and analytical aspects

Around the millennium turn, the vitamin D status was often assessed by measuring both 25(OH)D3 (calcidiol) and 1,25(OH)2 D3 (calcitriol). We postulated that measurement of calcitriol is obsolete for patients with hypovitaminosis D without osteomalacy. In a small study with 51 Turkish women and 20 deficient elderly Dutch adults, we found that calcitriol is kept within normal range even in severe 25(OH) vitamin D 3 deficiency (6). This implies that measurement of calcitriol for assessment of the vitamin D status in the general population is useless, which was confirmed by prof. R. Vieth and prof. M. Holick (oral communications).
In vitamin D kit inserts and medical laboratory sampling handling guides it is often stated that samples should be stored frozen and protected from light. On the other hand, food industries report excellent heat stability of vitamin D in natural matrices like milk or fat. Preanalytical stability data for vitamin D in human blood are scarce and incomplete. Therefore, we tested the stability of 25(OH)D in human blood samples under routine laboratory conditions like storage at room temperature and in the refrigerator, under artificial light or after several freeze-thawing cycles (7).

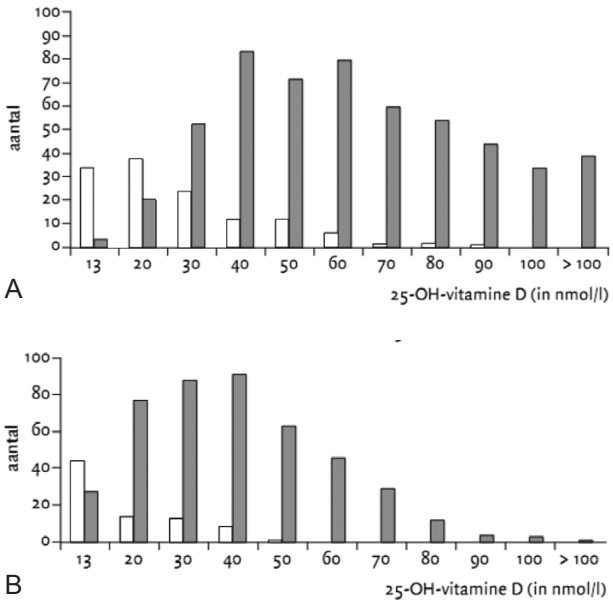

Figure 1. (A) Frequency distribution of 25 OH vitamin D3 in pregnant women. West-European women in grey bars, non western immigrants in white. The concentration given in the graph is the upper level of the interval. (B) Frequency distribution of 25 OH vitamin D3 in cord blood of newborns. West-European baby's in grey bars, non western baby's in white.

We found 25(OH)D3 in its natural state - bound to vitamin D binding protein - to be very stable at room temperature, even for unprocessed whole blood. For either serum or unprocessed samples, the decreases noted after 3 days room temperature were less than the analytical interassay imprecision. There appears to be no need for frozen serum transport and storage of serum at 4 °C for a week, and up to 4 freeze-thawing cycles are permissible. This preanalytical stability is a prerequisite for reliable analysis. Testing it is often lacking in method development or validation studies. Our laboratory participated in several method validation studies from commercial firms, publications are in preparation or submitted.

Clinical studies and optimal vitamin D levels

A main item in the performance of clinical studies of new drugs is the need for randomized clinical trials. For vitamin D this is difficult to perform since its main source is sun exposure (life style) and food like fatty fish. Hence for vitamin D the majority of the studies is descriptive or cohort based. Another difficulty is the absence of international consensus about the levels for deficiency and insufficiency. International vitamin D peers have set the minimal level at 75 nmol/L based on multiple health outcomes. This level is close to the observed lower end of the summer level for Western Europeans. The Dutch Health Council 2008 Advice was based on bone health criteria: a minimal level of 30 nmol/L, except for women > 50 years and men > 70 years (then 50 nmol/L is the minimum). We pointed at evidence for non-calcemic effects of vitamin D and inconsistencies in the Dutch Health Council use of the 'no observed adverse effect level' of 200 nmol/L and a maximum intake of 2000 iU vitamin D per day (8,9). We made a plea for 75 of 80 nmol/L as the lower end of an optimal vitamin D status. Recently the American Institute of Medicine set the minimum level at 50 nmol/L and the maximum daily intake at 4000 iU per day. In 2011 the Dutch CBO guideline for osteoporosis acknowledged the positive effect of vitamin D levels > 50 nmol/L for bone health, fracture risk reduction and the prevention of falls. It is expected that the Dutch Health Council will follow the new American guideline next year.

Vitamin D deficiency is associated with susceptibility to active tuberculosis (TB) in many settings.
In vitro studies and studies on human volunteers showed that two first-line anti-tuberculosis drugs, isoniazid and rifampicin, reduce 25(OH)D concentrations but results in different publications were controversial. We measured the vitamin D status during treatment with isoniazid and rifampicin of 81 Tanzanian hospitalised patients with pulmonary TB (10). Starting from 91 nmol/L as an average, we found an average increase of 10 nmol/L 25(OH)D during the first 2 months of treatment, with no effect on PTH, calcium, phosphate or magnesium. Improved dietary intake and increased sunlight exposure may have contributed to this increase. The optimal vitamin D status of patients treated for TB is still unknown, but our data do not support the presence or development of poor vitamin D status during treatment.
Patients with inflammatory bowel disease (IBD) are at risk of osteoporosis. Our study evaluated seasonal vitamin D status, determinants of vitamin D deficiency and effects of vitamin D supplementation in adult IBD patients (11). A standardized questionnaire was used for demographic and lifestyle data i.e. IBD activity, health behaviour and vitamin D intake through diet and ultraviolet light. At late-summer we found 39% of the included 316 patients were vitamin D deficient (25(OH)D < 50 nmol/L). At late-winter, 57% of the follow-up patients were deficient. Independent protective determinants of vitamin D deficiency were oral vitamin D supplementation, recent sun holiday and regular solarium visits. We concluded that vitamin D deficiency is common in IBD patients, but prevalence might be comparable with the general population. Determinants for low vitamin D levels were IBD activity and elevated inflammatory markers, suggesting that increased risk of osteoporosis in IBD might be more related to the inflammation than to vitamin D deficiency.
Other studies about prevalence, confounders and clinical presentation of vitamin D deficiency for patients with gynaecological cancer, mental retardation (institutionalized patients) and psychiatric patients with aggressive behaviour are running.
Our recent vitamin D review in the leading Dutch medical journal (12) was their most downloaded publication last year, proving the interest of the Dutch doctors for this theme. We presented a balance of causal effects (bone health, muscle health, proper functioning of the immune system) and associated effects that need more evidence: increased risk for colon, prostate, breast cancer, increased risk for autoimmune diseases like DM and MS. We hold 75 nmol/L or higher as optimal level and consider 50 nmol/L as a minimum level. In our review we advocated the use of a loading dose like 50.000 iE or 100.000 iE in cases of severe deficiency and clinical complaints. Vitamin D will most likely become an important item in preventive medicine.

Conclusion

Vitamin D is neither a myth nor a miracle. It is a prohormone, not a vitamin and its effects are well beyond calcium homeostasis. A high prevalence of deficiency exists, especially in the elderly, institutionalized people, and non-Western immigrants, which needs to be taken care of.
Our publications, symposia and lectures increased the awareness for this health problem and provided part of the data on which new Dutch guidelines are (going to be) build.

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Universitair Medisch Centrum
Utrecht

Advanced methods in diagnosis and translational research of red blood cell disorders

R. van WIJK and W. van SOLINGE

Diagnostics and research on benign disorders of the red blood cell have always been an important focus of the Department of Clinical Chemistry and Haematology of the University Medical Center Utrecht (UMCU). Both diagnostic and research activities are performed in close collaboration with the Department of (pediatric) Hematology/Oncology of the UMCU and other (inter)national hospitals. In this brief review, an update will be provided with regard to the current status of diagnostic developments, as well as to approaches aimed at a better understanding of the pathophysiology of red blood cell disorders, in particular those related to hereditary hemolytic anemia.

The red blood cell and hereditary hemolytic anemia

The moment the mature red blood cell (RBC) leaves the bone marrow, it is optimally adapted to perform the binding and transport of oxygen, and its delivery to all tissues. This is the most important task of the erythrocyte during its estimated 120-day journey in the blood stream. The membrane, hemoglobin, and proteins involved in metabolic pathways of the RBC interact to modulate oxygen transport, protect hemoglobin from oxidant-induced damage, and maintain the osmotic environment of the cell. The biconcave shape of the RBC provides an optimal area for respiratory exchange. The latter requires passage through microcapillaries, which is achieved by a drastic modification of its biconcave shape, made possible only by the loss of the nucleus and cytoplasmic organelles and, consequently, the ability to synthesize proteins. Hence, disturbances of proteins that constitute the RBC membrane, the red cell's major protein (hemoglobin), or of proteins involved in red cell metabolism may ultimately result in decreased RBC survival: hemolysis.

Advanced diagnostics for hereditary hemolytic anemia – enzymopathies and membrane disorders

RBC enzyme and membrane disorders constitute 2 of the major causes of hereditary hemolytic anemia. Nevertheless, they are rare diseases. The diagnosis of a RBC enzymopathy due to disturbed metabolism basically relies on enzyme activity measurements of a variety of enzymes, deficiencies of which are known to have a clear causal relationship with a hematological phenotype (1). We are currently equiped to perform extensive biochemical analysis of these clinically relevant enzymes in adults and, notably, in children (as children's RBCs are quite different from adult). In most cases of deficient enzymatic function we perform DNA sequence analysis of the respective gene. In this way, the diagnosis of a red blood cell enzymopathy is confirmed on both the biochemical and molecular level, thereby strenghtening the correct diagnosis of

Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

E-mail: r.vanwijk@umcutrecht.nl