Vitamin D in the clinical environment- an overview

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Clinical interest in vitamin D has increased substantially in recent years, a result of its association with a diverse range of diseases and conditions. Parallel to this has been a significant increase in requests for laboratory measurement of vitamin D status with reports of 100 % increases year-on-year. Worldwide interest in vitamin D is reflected in the number of studies being conducted on all aspects of its physiology, pathology, therapeutic, and health-associated advantages. Over 500 interventional or observational trials are registered with ClinicalTrials.gov and the years between 2000 and 2009 have seen a 300 % increase in the number of publications relating to vitamin D. Currently over 200 papers a month are being published on the topic.

History

No one knows when vitamin D appeared in the evolutionary process. Several years ago Holick demonstrated that a phytoplankton (*Emiliania huxleyi, calcareous algae*), with a lineage dating back 750 million years, was capable of synthesising ergosterol with subsequent conversion to vitamin D_2 after exposure to UV light [1] and suggested that the vitamin may have been acting to protect the cells against UV radiation damage. Also vitamin D_2 may have facilitated influx of calcium and other ions from the exterior milieu into the cells. The appearance of terrestrial vertebrates 350 million years ago necessitated a mechanism for maintaining calcium homeostasis in a setting where calcium was not readily available. Although nothing is known of how this occurred from an evolutionary perspective vitamin D and sunlight became critical to the process.

Source and metabolism

There are two forms of vitamin D: D_3 and D_2. The latter is produced by plants, fungi, and plankton and is not synthesised endogenously in humans, whereas vitamin D_3 is produced in the skin. Both of these vitamins are produced under the influence of ultraviolet B (UVB) radiation, a portion of the sun's radiation energy with wavelengths in the region 290–315 nm. In animals and humans UVB passes through skin and is absorbed by 7-dehydrocholesterol. A complex series of reactions results in previtamin D_3 formation, which is rapidly thermoisomerised to vitamin D_3 [2].

Very few foods contain sufficient vitamin D to satisfy daily needs. Examples include oily fish and cod liver oil. Some foods are fortified with vitamin D, e. g. milk, margarine, and cereals. In conditions where adequate sunlight exists UVB induced cutaneous production provides greater than 90 % of our vitamin D requirements. Vitamin D (D meaning D_3 or D_2) is bound to vitamin D binding protein and transported to the liver where it is 25-hydroxylated. 25-hydroxyvitamin D (25OHD) is the major circulating form of the vitamin and is regarded as an index of vitamin D status. Further hydroxylation at the 1-position occurs in the kidney to produce the biologically active form of the vitamin, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D).

Vitamin D assessment

Immunoassays (both radioimmunoassay and chemiluminescence assays) have been the mainstay for the laboratory determination of 25OHD, and these assays have been used in many epidemiological studies. However, concern has been expressed about the lack of comparability between assays and laboratories even when the same assay is employed. In addition, immunoassays have shown significant variability in their ability to equally detect D_3 and D_2. All assays demonstrate 100 % cross-reactivity with 25OHD_3, but some give a more variable response towards 25OHD_2 which may be a confounding factor in areas where vitamin D_2 is used as a supplement. More recently, liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been used to detect and quantify 25OHD. This technology is capable of measuring both 25OHD_3 and 25OHD_2 with good sensitivity and precision. Methods for analysing vitamin D using mass spectrometry have become increasingly 'user-friendly' making the technique suitable for high-throughput clinical chemistry laboratories. In 2009 Chromsystems GmbH introduced the first commercially available reagent kit (**MassChrom®**) for the precise analysis of 25OHD_3 and 25OHD_2 by LC-MS/MS with minimal pre-chromatography sample preparation, on-line extraction, and short assay time.

The lack of comparability and agreement between assays has led to calls for a global effort on method standardisation [3]. Recently the National Institutes of Standards and Technology in the US developed a standard reference material (NIST 972) for circulating vitamin D_3 and D_2 analysis as an aid in method validation [4].

Vitamin D status: global perspective and what is a 'normal' vitamin D status

Vitamin D deficiency/insufficiency is a major public health issue worldwide [5, 6]. No demographic group is spared including institutionalised and community living elderly, adolescents, children, different ethnic groups and degrees of skin pigmentation across all continents. There is currently no consensus on what constitutes a normal vitamin D status. Indeed, the concept of a global ideal vitamin D concentration for optimal health has not been validated. Different thresholds may exist for different medical conditions or different population groups and will depend on what end-points are used to evaluate vitamin D status.
However, in order to define target values for vitamin D three thresholds are commonly accepted [7]:

Deficiency:
< 25 nmol/l; defined as high risk for developing bone disease.

Insufficiency:
25–80 nmol/l; defined as the vitamin D concentration which normalises parathyroid hormone concentration.

Sufficiency:
80–125 nmol/l or higher; defined as optimal concentration for maximal health benefit.

Toxicity:
> 325 nmol/l are regarded as toxic.

It should be stressed that these cut-points are not universally accepted.

Determinants of vitamin D production in skin

Several factors will determine efficiency of cutaneous vitamin D_3 synthesis. A person's position on the planet plays an important role. Distance from the equator determines how much UVB radiation strikes the earth's surface and is a function of the solar zenith angle and how much ozone the solar radiation travels through the atmosphere. This depends on latitude, season, and time of day. Above latitude 37°, during the months October to March, very little UV gets through with little or no cutaneous vitamin D_3 production. Other factors influencing UVB penetration of skin are weather conditions and pollution, skin pigmentation and age, type of clothes worn when outdoors, time spent outdoors (UV light cannot penetrate glass), and sunscreen use (correctly applied spf 15 can filter out > 90 % UVB). Non-UV factors influencing vitamin D status are diets poor in vitamin D content, genetic differences in various population groups (e. g. polymorphisms in vitamin D receptors and vitamin D-binding protein), and reduced renal function (alterations in the homeostatic mechanisms involving vitamin D).

Consequences of vitamin D deficiency

Classically, severe or chronic vitamin D deficiency is associated with rickets in children and osteomalacia in adults. It is also a factor in the pathogenesis of osteoporosis and is associated with increased risk of hip fracture. More recent research has implicated vitamin D deficiency in a long list of conditions including several types of cancer, heart disease, vascular calcification, stroke, hypertension, autoimmune diseases, multiple sclerosis, diabetes, depression, cognitive function, chronic pain, osteoporosis, muscle weakness, and periodontal disease (overviews in references 1 and 6).

Conclusion

Although there is still a great deal of debate on how to correct vitamin D deficiency/insufficiency, it is estimated that a daily supplemental intake of 800–1000 IU is needed to maintain an average circulating concentration of 75 nmol/l [8]. This is well above the recommended allowance of 400 IU for adults 51–70 years old and 600 IU for adults older than 70 years of age. Exposure of skin to sunlight as a means of generating vitamin D has engendered a lot of discussion and disagreement. The dermatology community is opposed to deliberate exposure of skin to sun citing long-term risk of skin cancer while others advocate that judicious sun exposure (10–15 minutes to the arms and legs, for example) can generate significant cutaneous vitamin D synthesis.

Interest in vitamin D continues to grow exponentially. Crucial to these research efforts are reliable and reproducible 25OHD assays. The use of proficiency testing, the introduction of standard reference material, and the introduction of advanced methodologies such as mass spectrometry should bring this goal closer.

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