



Vitamin D

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Formation and activation of vitamin D

World-wide, most humans depend on sun exposure to satisfy their requirement for vitamin D. Solar ultraviolet B photons are absorbed by 7-dehydrocholesterol in the skin, where their photolytic action transforms the 7-dehydrocholesterol into pre-vitamin D₃ and vitamin D₃ (cholecalciferol). It is estimated that approximately one billion people world wide have some degree of vitamin D deficiency [1]. In the U.K. and the U.S.A. this deficiency is especially prevalent among the elderly population, and one of the reservations that have been expressed about excessive cholesterol lowering therapy is that this might further reduce the production of vitamin D from cholesterol.

The production of active vitamin D in the human is a multi-stage process, of which the first step is the photolysis of 7-dehydrocholesterol in the skin. Season, latitude, time of day, skin pigmentation and ageing all influence cutaneous production of vitamin D₃. Sensible sun exposure, which would entail the exposure of arms and legs twice a week for 5 to 30 minutes (depending on season, latitude, and skin pigmentation) between the hours of 10 am and 3 pm, is considered a safe and effective way to obtain vitamin D₃. Excess vitamin D₃ so produced is, in turn, destroyed by sunlight and therefore excessive exposure to the sun does not cause vitamin D intoxication. However, use of high Sun Protection Factor (SPF) cream has been estimated to reduce vitamin D production by 99%, since these creams block the exact wavelengths of UVB radiation required for the photolysis of 7-dehydrocholesterol [1]. Studies also show that humans living above a latitude of 35 degrees North (e.g. Crete) or below 35 degrees South (e.g. Sydney) receive insufficient UVB radiation in the winter for adequate vitamin D synthesis.

Once formed in the skin, vitamin D₃ is transported to the liver and metabolized to 25-hydroxyvitamin D₃ (also known as calcidiol) by the action of hepatic cytochrome P450. 25-hydroxyvitamin D₃ is stored in the liver until required metabolically, at which time it is transported to the kidney and converted into its biologically active form, 1,25-dihydroxy vitamin D₃ (1,25-dihydroxycholecalciferol, 1,25 DHCC or calcitrol) by the action of a renal tubular mitochondrial 1-alpha-hydroxylase. Extra-renal conversion to 1,25-dihydroxy vitamin D₃ is also possible to a limited extent. Physiological vitamin D production in the human thus requires exposure to the sun, functional liver cells and functional renal cells.

Vitamin D₃, which is a 27-carbon derivative of cholesterol, can also be obtained nutritionally by the consumption of animal products. Vitamin D₂, in contrast, is a 28-carbon derivative of the plant sterol ergosterol; as well as containing one more carbon atom than vitamin D₃, D₂ has an extra unsaturated carbon-carbon bond and an extra methyl group in its chemical structure. Both vitamin D₂ and D₃ are inactive and require the same hydroxylation reactions in the liver and kidney to achieve biological activity; this process is itself regulated by parathyroid hormone (PTH).

Vitamin D measurement

The term vitamin D covers a group of fat-soluble secosteroids and their metabolites. Biolab uses a chromatographic method to measure vitamin D in serum; this technique separates and quantifies vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol), the vitamers of most interest in the assessment of vitamin D status in humans [2]. As well as giving individual concentration values for D₂ and D₃, this method provides a visual check of the chromatographic profile, which in turn informs the analyst as to the integrity

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of the clinical specimen. While many commercial, antibody-based vitamin D assays do not distinguish between these two forms of the vitamin, variations in the cross-reactivity of the antibodies used (which may also cross-react with other vitamin D metabolites) has led to some confusion in the results, especially in subjects receiving either vitamin D₃ or vitamin D₂ supplementation. The majority of NHS laboratories still use non-chromatographic, antibody-based methods for vitamin D measurement, although some of the larger laboratories have now introduced chromatographic systems for this purpose.

Currently there are few agreed indications for 1,25-dihydroxy vitamin D (calcitriol) measurement; levels of this biologically active form of vitamin D reflect demand for ionised calcium rather than body vitamin D status (i.e. when calcium levels fall, levels of 1,25-dihydroxy vitamin D rise). 1,25-dihydroxy vitamin D is relatively unstable *ex-vivo* and more variable in concentration than 25-hydroxy vitamin D. The plasma half life of 1,25-dihydroxy vitamin D is 8 hours, as compared to approximately one month for 25-hydroxy vitamin D₃. In contrast the plasma half life of 25-hydroxy vitamin D₂ is 8 days, with one tenth the potency of 25-hydroxy vitamin D₃ [3]. This explains why vitamin D₃ is currently the predominant form now used in supplementation, even though vitamin D₂ was formerly more commonly used.

Indications

Until the renewed upsurge of interest in vitamin D over the past few years [4], the criteria for vitamin D measurement were based on its action in the formation and maintenance of bone, i.e.:

1. In the elderly, vitamin D deficiency causes osteomalacia with subsequent osteoporotic fractures [5], while children with vitamin D deficiency may develop infantile rickets [6].
2. Osteoporosis has also been associated with failure to adequately convert 25-hydroxy vitamin D to 1,25-dihydroxy vitamin D [7].

Subjects with malabsorption, individuals consuming a high phytate diet (which promotes malabsorption of certain nutrients) and patients on anti-convulsants (which speed up catabolism of vitamin D) have also long been recognised as being at risk from vitamin D deficiency. There is now good evidence for vitamin D having important non-skeletal roles in human physiology [1], in fact being an essential nutrient for every cell in the body, which greatly expands both the indications for its measurement and the public health concerns as to the effects of its deficiency.

The diseases in which there is a newly-appreciated association with low vitamin D levels include glucose intolerance, diabetes mellitus, metabolic syndrome, cardiovascular disease, myocardial infarction, hypertension, obesity, heart failure, myopathy, inflammatory bowel disease, multiple sclerosis, psoriasis, tuberculosis, upper respiratory tract infections, polycystic ovarian syndrome, and several types of cancer. The association between vitamin D deficiency and increased cancer risk is particularly strong. There is also good evidence that vitamin D deficiency is a significant cause of cardiovascular disease and of impaired resistance to various types of infection.

Vitamin D and cardiovascular disease

Since the hydroxylation and activation of vitamin D is regulated by parathyroid hormone (PTH), vitamin D deficiency has the effect of producing sustained elevation in PTH. The relationship between raised PTH and the risk of cardiovascular disease has been investigated in a recent cross-sectional study which evaluated 654 adults between the ages of 55 and 96 years without a history of coronary heart disease, revascularization, or stroke. Results revealed that those with higher concentrations of 25(OH)D had a significant dose-dependent decrease of the intima-media wall thickness of the carotid artery ($P = .036$), linking vitamin D deficiency to development of subclinical atherosclerosis [8]. This finding was supported by research showing that vitamin D deficiency increases systemic inflammation, as confirmed by elevated levels of C-reactive protein and interleukin-10 [9,10,11]. In addition, the administration of vitamin D analogues has been shown to down-regulate the inflammatory markers and reduce plaque production and instability [11].

Vitamin D deficiency has also been shown to up-regulate the renin-angiotensin-aldosterone system, resulting in hypertension [12], an important risk factor for cardiovascular disease. In an animal study of vitamin D deficiency, mice with vitamin D receptor knockout showed increased blood pressure, increased serum angiotensin-converting enzyme levels and increased tissue renin levels [13,14]. In humans it has been shown that 1,25(OH)₂D has an inhibitory effect on renin synthesis, decreasing blood pressure [15]. It has also been demonstrated that exposure to UVB, but not UVA, radiation on a regular basis both elevates serum 25(OH)D levels above 100 nmol/L and decreases blood pressure by 6 mm Hg in hypertensive patients [16]. In support of this observation, the NHANES III study recently found that those individuals with higher serum 25(OH)D concentrations had a self-reported mean systolic blood pressure approximately 3 mmHg below that of individuals with lower serum 25(OH)D concentrations [17].

Overall, the literature supports the premise that a low total 25(OH)D concentration is associated with an increased risk of cardiovascular disease. Several mechanisms explain the protective effects of vitamin D on the cardiovascular system: a) involvement of the vitamin D-PTH axis, b) the regulation of inflammation with its link to atherosclerosis, c) the regulation of the renin-angiotensin system, d) the effect on insulin secretion and insulin sensitivity, and e) metabolic syndrome [18].

Vitamin D and cancer

The first article demonstrating a relationship between solar radiation and cancer mortality in North America was actually published in 1941 [19]. In 1980 an article in the International Journal of Epidemiology proposed that vitamin D status may be directly related to cancer risk [20]. Since that time, many studies have been published that demonstrate an inverse relationship with solar radiation and cancer mortality for various types of cancers including breast, rectum, ovary, prostate, stomach, bladder, oesophagus, kidney, lung, pancreas, uterus, non-Hodgkin lymphoma, and multiple myeloma [21].

One study, published in 2007, was a prospective study, designed as a 4-year, population-based, double-blind, randomized placebo-controlled trial, looking at the effect of vitamin D intake on the incidence of all cancers [21]. Its purpose was to determine if calcium alone or calcium plus vitamin D had an effect on reducing the incidence of all types of cancer. The study participants included 1024 community-dwelling women who were randomly selected from a population of healthy postmenopausal women from nine rural counties in Nebraska. The participants' mean baseline serum 25-hydroxyvitamin D level was 72 nmol/L. The results showed that both the calcium-only and the calcium plus vitamin D groups had lower rates for all cancers compared with the placebo group ($P < .03$). The relative risk for the development of cancer at the study's end was 0.402 for the calcium plus vitamin D group ($P = .013$) and 0.532 for the calcium-only group ($P = .063$). The 12-month serum 25-hydroxyvitamin D level in the calcium plus vitamin D group increased to 96 nmol/L which was associated with a 35% reduction risk of cancer. The authors concluded that improving vitamin D nutritional status substantially reduced all-cancer risk in postmenopausal women and that baseline and treatment-induced serum 25-hydroxyvitamin D concentrations were strong predictors of cancer risk.

Other studies relating cancer to vitamin D have shown that people living at higher latitudes are at increased risk for Hodgkin's lymphoma as well as colon, pancreatic, prostate, ovarian, breast, and other cancers. In addition, people living at higher latitudes are more likely to die from these cancers as compared to those living at lower latitudes [1]. Epidemiological studies, both prospective and retrospective, have shown that individuals who have serum 25-hydroxyvitamin D levels less than 50 nmol/L have an associated 30% to 50% greater risk of colon, prostate, and breast cancer as well as a higher mortality rate from these cancers [1]. In addition, analysis of the Women's Health Initiative showed that women who had a serum 25-hydroxyvitamin D level less than 30 nmol/L had a 253% increase in the risk of colorectal cancer over an 8-year follow-up period [22].

Vitamin D and infectious disease

The link between vitamin D deficiency and susceptibility to infection has been suggested for many years. The observation that children with nutritional rickets were more likely to experience infections of the respiratory system led to the coining of the phrase "rachitic lung" [23]. Vitamin D has been shown to induce antimicrobial gene expression, which results in the synthesis of cathelicidins and defensins in human immune cells and epithelial cells. This partly explains the antibiotic effect of vitamin D [24].

The use of vitamin D₃ from cod liver oil was the cornerstone of tuberculosis treatment until the introduction of anti-infective chemotherapy in the 1950s [25]. The discovery that vitamin D receptor (VDR) and 1 α -hydroxylase, the enzyme necessary for conversion of vitamin D into its active form, are present in cells of the immune system, including circulating mononuclear cells [26,27], revolutionized the field of vitamin D immunology. Recent studies have demonstrated that vitamin D regulates the expression of specific endogenous antimicrobial peptides in immune cells [28]; this action suggests a role for vitamin D in modulating the immune response to various infectious diseases.

The work of Rook et al [29] and Crowle et al [30] in the 1980s demonstrated that vitamin D enhanced bactericidal activity of human macrophages against *Mycobacterium tuberculosis*, the causative agent of TB. This discovery led to new interest in the role of vitamin D in the immune response to bacterial pathogens. Liu et al [28] provided the mechanism as to how vitamin D might enhance innate immunity. This group demonstrated that stimulation of macrophage-bound Toll-like receptor 2/1 complex by *M tuberculosis*-derived antigens up-regulates the expression of both the vitamin D receptor and CYP27B1, the enzyme that converts 25-hydroxyvitamin D (25-OHD) to its active 1,25-dihydroxyvitamin D [1,25-(OH)₂D] form. Intracellular 1,25-(OH)₂D then interacts with the receptor and leads to induction of the antimicrobial peptide cathelicidin and killing of intracellular *M tuberculosis*. In vitamin D deficiency, the infected macrophage is unable to produce sufficient 1,25-(OH)₂D to upregulate production of cathelicidin. Cathelicidin itself has a broad-spectrum action against a variety of other pathogens, but also has activity in the immune system beyond microbial killing. Vitamin D is also known to regulate the expression of β -defensin, another antimicrobial peptide with multiple effector functions within the immune system [31]. Endoscopic studies in humans have demonstrated that β -defensin is secreted in the gastric mucosa after infection by *Helicobacter pylori* [32] and may therefore constitute a major aspect of immune defense against this bacterial pathogen at the mucosal surface.

The seasonality of viral respiratory tract infections such as those caused by influenza and rhinovirus ("the common cold") is understood to be a major contributor to seasonal variations in human mortality [33]. It has been argued that vitamin D status may be a contributor in determining population susceptibility to seasonal epidemics as well as to the degree of associated morbidity and mortality [34]. It is well known that often the exuberance of the host immune response, rather than the viral pathogen itself, determines the clinical severity and mortality risk associated with viral diseases such as influenza [35]. Vitamin D modulates cytokine profiles in animal models of autoimmune disease through limiting excessive production of pro-inflammatory cytokines, such as tumor necrosis factor α and interleukin-12, thus leading to suppression of inflammation [36]. These studies and those mentioned above support the hypothesis that optimal vitamin D status in the host may have a key immunoregulatory function in viral respiratory infections, down-regulating toxic cytokine responses, while promoting removal of infectious organisms [34].

Vitamin D conversion factors

In line with current best practice, Biolab vitamin D reports are in molar units (nanomoles per litre, or nmol/L) for serum vitamin D. However, much of the scientific literature and also current US laboratory reports use mass units for expressing results (nanograms per millilitre, or ng/mL, which is the same value as micrograms per litre of $\mu\text{g/L}$).

The factor used to convert $\mu\text{g/L}$ (or ng/mL) to nmol/L is 2.5, i.e. if the reference interval for serum vitamin D is 30 – 80 $\mu\text{g/L}$, this converts to 75 – 200 nmol/L.

This conversion factor is actually based on the chemical formula of 25-OH vitamin D₃, which is present in serum at a higher concentration than 25-OH vitamin D₂ (which may not be present at all). To be precise:

25-OH vitamin D₃ - $\mu\text{g/L}$ to nmol/L = $\times 2.496$, or nmol/L to $\mu\text{g/L}$ = $\times 0.4007$

25-OH vitamin D₂ - $\mu\text{g/L}$ to nmol/L = $\times 2.423$, or nmol/L to $\mu\text{g/L}$ = $\times 0.4127$

In terms of vitamin D dosage, 1 μg of vitamin D is equivalent to 40 IU (international units), i.e. 25 μg of vitamin D is equivalent to 1000 IU.

Recommended vitamin D intake

The current recommended daily allowance for vitamin D has been made obsolete by the emerging evidence of its non-skeletal actions; most nutritional practitioners in the UK now recommend an increased daily vitamin D intake of 1000 IU, or more. It is preferable that such recommendations should be based on serum measurements to assess the patient's vitamin D status. If the lower level of serum 25-hydroxyvitamin D considered to be sufficient is 75 nmol/L, most individuals will need to obtain about 4000 IU/d or more vitamin D₃ from all sources [<http://www.youtube.com/watch?v=emjCzaHtSrg>. Heaney RP. What is a vitamin D deficiency?]. Because they do not get enough vitamin D₃ through their diet or live under conditions that prohibit adequate sun exposure, most healthy adults will need an extra 2000 IU per day of vitamin D₃ to achieve a serum concentration of 75 nmol/L or greater. Steady state concentrations are not reached until after about 90 days, so 3 – 4 months on a regime of modified diet or supplementation should be allowed before re-testing.

As a rule of thumb, the serum 25-OH vitamin D concentration will increase by 2.5 nmol/L (equivalent to 1 ng/mL) for every additional 100 IU vitamin D₃ that is ingested daily [1,37]. Therefore, if a patient has a baseline concentration of 25 nmol/L (which indicates vitamin D deficiency), an additional 3000 IU per day would be required to achieve a level of 100 nmol/L, which would put this patient in the sufficiency category.

A diet high in oily fish such as salmon, sardines, mackerel, and tuna can supply additional vitamin D₃ from 250 to 1000 IU per serving. For example, 3.5 oz of fresh, wild salmon can provide 600 to 1000 IU of vitamin D₃, whereas 3.5 oz of fresh, farmed salmon can supply only 100 to 250 IU of vitamin D₃. Likewise, 3.6 oz of canned tuna contains about 230 IU of vitamin D₃. In addition, 8 oz of fortified milk, orange juice, or yogurt contains about 100 IU of vitamin D₃ [1]. Our serum measurements confirm that most patients do not consistently take in enough vitamin D through their diet each day to maintain adequate serum concentrations, and therefore supplementation is most often recommended. Both vitamin D₂ and D₃ have been demonstrated to allow serum 25-hydroxyvitamin D concentrations to reach adequate levels; however, vitamin D₃ has been shown to maintain these concentrations for a longer period of time. As a result, supplementation with vitamin D₃ is generally recommended over vitamin D₂.

This is in spite of the fact that the risk of toxicity is greater with vitamin D₃ than it is with vitamin D₂ (due to its longer half life). Biolab has recorded serum vitamin D values in excess of 1500 nmol/L in subjects who have mistakenly consumed excessive amounts of vitamin D over a period of months. Side effects can occur when blood concentrations reach 300 nmol/L. Symptoms include nausea, vomiting, constipation, headache, sleepiness, and weakness, with raised plasma calcium concentrations and hypercalciuria. However, doses of vitamin D₃ of 10 000 IU per day have been shown to be taken without side effects and this is considered to be the safe upper limit of daily intake.

Synonyms: Vitamin D, 25-hydroxy vitamin D₃, 25-hydroxy cholecalciferol, calcidiol.

Patient preparation

No special preparation is required and the patient can continue to take other nutritional supplements and medication before the collection of the sample, but should avoid taking vitamin D containing supplements for 24-48 hours.

Specimen requirements

Serum separator tubes (plain gel tubes - available from Biolab on request). If posted, samples must reach Biolab within 24 hours.

Methodology

High pressure liquid chromatography (HPLC), quantitating both vitamin D₃ and vitamin D₂ (if present).

Turn around time: 5 working days.

Interpretation

The serum concentration of 25-hydroxy vitamin D is the most sensitive and useful index of vitamin D status. There is a seasonal variation in 25-hydroxy vitamin D in temperate regions of the globe, with lower levels being prevalent in the winter.

Biolab vitamin D results are reported using NIST (National Institute of Standards and Technology, USA) aligned calibration standards. For healthy subjects, with no medical condition and normal sun exposure, the serum reference interval for 25-hydroxy vitamin D is 75 – 200 nmol/L (30 – 80 µg/L).

The treatment target for subjects with medical conditions that may be associated with vitamin D deficiency is a serum range of 125 – 150 nmol/L (50 – 60 µg/L).

The level of vitamin D₂, which is of plant origin and is the form contained in certain supplements, is reported separately from the level of vitamin D₃. Total 25-hydroxy vitamin D can be taken as the sum of 25-hydroxy D₃ and 25-hydroxy D₂. Most subjects have very low levels of vitamin D₂ in comparison to D₃.

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Presentations and lectures on vitamin D

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2. Heaney RP What is a vitamin D deficiency. <http://www.ucsd.tv/search-details.aspx?showID=15751>.