Vitamin D

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The fat-soluble secosteroid vitamin D₃ is synthesised in UV (ultraviolet)-B exposed human skin. It regulates calcium and phosphorus homeostasis and thus plays an important role in bone mineralisation. Moreover, vitamin D₃ contributes to the control of cellular growth and differentiation as well as to the responsiveness of the innate and adaptive immune system. At insufficient sun exposure, the molecule is a true vitamin and needs to be taken up via diet or by direct supplementation with pills. During the past 50 000 years, the need of sufficient vitamin D₃ synthesis acted an evolutionary driver for skin lightening of anatomically modern humans migrating out of Africa. The vitamin D₃ metabolite 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, also called calcitriol), which is produced primarily in the kidneys, is the exclusive high-affinity ligand of the transcription factor vitamin D receptor (VDR), which was finally cloned in 1988 (Baker et al., 1988). The link to rickets let initial vitamin D research focus on its role in calcium and phosphorus homeostasis and bone formation. However, since some 35 years it is also known that 1,25(OH)₂D₃ can control the growth of cancer cells (Colston et al., 1982) suggesting that it has a potent anticancer action. At the same time, a role of 1,25(OH)₂D₃ in the differentiation of cells of the immune system was discovered (Abe et al., 1981). Later on, many evidences accumulated demonstrating that vitamin D serves as an important regulator of the response of both the innate and the adaptive immune system (Chun et al., 2014). This finally provided a cellular and molecular explanation for the cure of tuberculosis by sunlight exposure.

For obtaining full benefits of the disease-preventive potential of vitamin D₃, the molecule or its metabolites should circulate in sufficiently high concentrations in the bloodstream. In contrast, today’s widespread vitamin D deficiency contributes to a number of serious disorders including musculoskeletal diseases, which are, in addition to rickets, osteomalacia, fracture risk, risk of falls and sarcopenia, and nonmusculoskeletal disorders, such as increased risks for cancers, cardiovascular disease, hypertension, all-cause mortality, infectious and autoimmune diseases (Holick, 2007).

Synthesis of Vitamin D

Under UV-B (290–315 nm) exposure, the cholesterol precursor 7-dehydrocholesterol converts to pre-vitamin D₃ (most efficient are 295–297 nm) and then isomerises in a nonenzymatic reaction into vitamin D₃ (Figure 1). Cholesterol biosynthesis is an evolutionary very old pathway and, for example, phytoplankton produces vitamin D₃ since more than 500 million years (Tremезегус et al., 2006). The same reaction takes place in human skin, when it is exposed to sufficient doses of UV-B, that is humans...
Figure 1  **Vitamin D synthesis pathway.** Vitamin D₃ is synthesised endogenously in the skin under essential catalysis by UV-B or taken up by diet. In the liver, vitamin D₃ is converted by the enzyme CYP2R1 into the circulating form 25(OH)D₃ and then in the kidneys (and in many additional tissues and cell types) by the enzyme CYP27B1 into the high-affinity VDR ligand 1,25(OH)₂D₃.

This diagram illustrates the synthesis pathway of vitamin D. Vitamin D₃ is produced in the skin under the influence of UV-B light or is acquired through dietary intake. In the liver, it is converted by CYP2R1 into 25(OH)D₃, the most abundant form in the human body. Further metabolism by CYP27B1 generates 1,25(OH)₂D₃, the active hormone.

Vitamin D is synthesized in the skin under the influence of UV-B radiation. However, at latitudes below 37°N, UV-B radiation is insufficient for vitamin D₃ synthesis throughout the year in European winter months. Hence, only at latitudes above this range can human skin produce vitamin D₃ on its own when staying in the sun. People living at lower latitudes must rely on dietary sources or vitamin D supplementation.

Lifestyle changes over the past 50,000 years, such as increased indoor activities and reduced exposure to sunlight, have significantly reduced endogenous vitamin D₃ production. This reduction has led to a dependency on external vitamin D₃ supply, making vitamin D₃ a vitamin in its original sense. The average human diet is not an efficient source of vitamin D, containing only small amounts of vitamin D₃ in oily fish, egg yolk, and some mushrooms, and vitamin D₂ (ergocalciferol) in foods like green leafy vegetables and fortified foods.

Serum levels of 25-hydroxyvitamin D₃ (25(OH)D₃) are used as a biomarker for vitamin D status. The Institute of Medicine (IOM) recommends serum levels of 25(OH)D₃ of ≥50nM for both bone and overall health. Vitamin D sufficiency should be reached by daily supplementation with vitamin D₃ doses of 10–15μg (400–600 IU) for children and 15–20μg (600–800 IU) for adults. However, the US Endocrine Society suggests serum concentrations of at least 75nM and daily supplementations with 25μg (1000 IU) vitamin D₃ for optimal health.

Prolonged sun exposure or high oral vitamin D₃ intakes can have toxic effects, such as hypercalcemia. Elevated serum calcium levels are caused by increased intestinal absorption and mobilization of calcium from bone, which can result in soft tissue calcification. Therefore, the upper limit for daily vitamin D₃ supplementation was set to 100μg (4000 IU). However, it is not likely that symptoms of toxicity will occur at daily vitamin D₃ intakes below 250μg (10,000 IU).

Biologically, vitamin D₃ is an inert molecule and needs to be enzymatically converted in a two-step reaction to the nuclear hormone 1,25(OH)₂D₃. The cytochrome P450 (CYP) enzyme CYP2R1, which is primarily expressed in the liver, adds a hydroxyl group at carbon 25 of vitamin D₃ creating 25(OH)D₃ (Henry, 2011). This molecule has the rather long half-life of 2–3 weeks in human serum and is the most abundant form of vitamin D in the human body. Therefore, 25(OH)D₃ serum levels are used as biomarker for the vitamin D status (Holnis, 2005).
The mitochondrial enzyme CYB27B1 hydroxylates 25(OH)D3 at carbon 1 producing 1,25(OH)2D3 (Figure 1). Signal transduction pathways stimulated by parathyroid hormone (PTH) and low levels in calcium and phosphate induce CYP27B1 gene expression in the kidneys. In contrast, 1,25(OH)2D3, as well as fibroblast growth factor 23 (FGF23), which is secreted by bone osteoblasts and osteocytes in response to increasing serum phosphate levels, downregulate the CYP27B1 gene (Henry, 2011).

Circulating endocrine levels of 1,25(OH)2D3 primarily derive from proximal tubule cells of the kidneys, while a number of human tissues, such as macrophages of the innate immune system, keratinocytes of the skin and osteoblasts within bones, can produce the molecule in a paracrine and autocrine manner. Vitamin D3 and its metabolites are transported in the bloodstream by the α-globulin carrier protein vitamin D-binding protein (DBP), so that there is only a small fraction of free vitamin D in serum. Moreover, the enzyme CYP24A1 hydroxylates 25(OH)D3 and 1,25(OH)2D3 at carbon 24 and initiates in this way the degradation of the molecules. Importantly, the CYP24A1 gene is one of the most responsive primary vitamin D targets. It is expressed primarily in the kidneys, but is found also in a number of cancer cells. Thus, because of tight control of its synthesis and degradation, serum levels of 1,25(OH)2D3 are some 800 times lower than that of 25(OH)D3.

Evolutionary View on Vitamin D

The historically first role of vitamin D3 was to act as a chemical sunscreen. Phyto- and zooplankton use the photochemical reaction of vitamin D3 production as a protection mechanism against sun exposure. Phyto- and zooplankton use the photochemical reaction of vitamin D3 production as a protection mechanism against sun exposure. Furthermore, the enzyme CYP24A1 hydroxylates 25(OH)D3 and 1,25(OH)2D3 at carbon 24 and initiates in this way the degradation of the molecules. Importantly, the CYP24A1 gene is one of the most responsive primary vitamin D targets. It is expressed primarily in the kidneys, but is found also in a number of cancer cells. Thus, because of tight control of its synthesis and degradation, serum levels of 1,25(OH)2D3 are some 800 times lower than that of 25(OH)D3.

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The historically first role of vitamin D3 was to act as a chemical sunscreen. Phyto- and zooplankton use the photochemical reaction of vitamin D3 production as a protection mechanism against UV-B-induced DNA (deoxyribonucleic acid) damage. Since the marine food chain starts with plankton, vitamin D3 accumulates in the liver of many deep-water fish, such as cod. This explains why cod liver oil is rich in vitamin D3.

When animal species evolved and some moved from calcium-rich water to the calcium-poor terrestrial environment, the control of calcium homeostasis became a speciality of the developing vitamin D endocrinology (Bouillon and Suda, 2014). This also explains why only vertebrates have a full endocrine system, formed by plasma transport proteins, metabolising enzymes and a high-affinity receptor. Later on, endocrinology of vitamin D got also involved in modulating the response of the immune system as well as cellular growth and differentiation.

The evolutionary precursors of the nuclear receptor VDR were ligand-independent transcription factors being primarily involved in the control of cellular metabolism (Escriva et al., 2004). In a multistep process, these ancestral nuclear receptors learned to bind and to be activated by metabolic compounds, such as bile acids in the case of the VDR precursor (Makishima et al., 2002). Further evolution of the ligand-binding domain (LBD) of this receptor molecule resulted in a ligand-binding pocket (LBP) that accommodates with high specificity and affinity 1,25(OH)2D3 (Carlberg and Molnár, 2012).

Anatomically modern humans, which developed some 200–300 000 years ago in East Africa, had dark skin, in order to prevent UV-mediated degradation of the circulating methyl-group donor folate. Despite dark skin, the intensive sun exposure at the equator allowed sufficient vitamin D3 synthesis, as witnessed by average 25(OH)D3 serum levels of 119 nM in traditionally living Maasai people in Tanzania and Kenya (Luxwolda et al., 2012). Since humans had more than 150 000 years about the same lifestyle as Maasai, their physiology and biochemistry adapted to this rather high vitamin D status.

After the migration of modern humans to less sunny regions in Asia and Europe, which started some 50–60 000 years ago, the need of sufficient endogenous vitamin D3 production caused an evolutionary pressure for genetic adaptation in form of gradual skin lightening (Hochberg and Templeton, 2010). This process took some 10–30 000 years, which in an evolutionary scale is very fast. In contrast, the immigration of light skin Europeans to the Americas and Australia and the involuntary transfer of dark skin Africans to the Americas within the last 500 years were too quick for an efficient genetic adaptation. For example, dark skin persons living in the United Kingdom or in Canada do not have sufficient endogenous vitamin D3 production at these latitudes. In general, many humans live nowadays at latitudes to which their skin colour is not adapted. Moreover, because of colder climate and cultural traditions, most humans cover nearly their entire body with textile. This is an additional important contribution to the low endogenous vitamin D3 production and causes a rather high rate in vitamin D deficiency even in sunny geographic regions, such as on the Arabic peninsula.

Individuals differ in their genomes primarily via single nucleotide variations (SNVs) resulting in phenotypic and physiological differences. Some of these SNVs relate to the vitamin D status, that is to the individual’s serum 25(OH)D3 levels, such as rs2282679 (in the GC gene encoding for DBP), rs12785878 (near the DHCR7 (7-dehydrocholesterol reductase) gene) and rs10741657 (close to the CYP2R1 gene) (Wang et al., 2010). The rather low rate of endogenous vitamin D3 synthesis of contemporary humans parallels with many disease-promoting lifestyle changes, such as reduced physical activity and increased body mass index. However, evolution selects for benefits that result in a higher number of offspring reaching a reproductive age, but it does not protect from aging-related diseases. Thus, the higher vitamin D status in the past protected humans against infectious diseases, such as tuberculosis, rather than against disorders that normally occur at higher age, such as cancer and cardiovascular disease.

Genomic Actions of Vitamin D

The nuclear receptor VDR is the only protein encoded by the human genome that binds 1,25(OH)2D3 at subnanomolar concentrations (Haussler et al., 1997), that is basically all physiological effects of vitamin D are mediated by this receptor. VDR is one of the some 1600 human transcription factors and shows highest expression in metabolic tissues, such as kidneys, intestine and bone (www.proteinatlas.org/ENSG00000111424-VDR/tissue). However, low to moderate VDR expression levels are also found in more than half of the 400 tissues and cell types that form the human body, that is all of these are responsive to vitamin D.
VDR is a member of the nuclear receptor superfamily; other well-known members of which are the receptors for the steroid hormones cortisol and oestriadiol, glucocorticoid receptor (GR) and oestrogen receptor (ER) (Evans, 2005). The molecular mechanisms of nuclear receptors are very similar. For example, VDR, ER and GR have a structurally conserved LBDs, each of which contains in its lower part an LBP that is perfectly adapted in its size and shape for accommodating the respective specific ligand (Nagy and Schwabe, 2004) (Figure 2).

The origin of anatomically modern humans at the equator also implies that the human genome is evolutionary rather adapted to a constant vitamin D status than to level changes between summer and winter. Thus, while the levels of cortisol and oestriadiol change on a daily (in all humans) and monthly basis (in premenopausal females), respectively, the endocrinology of vitamin D aims on a constant hormone level.

Another highly conserved domain of nuclear receptors is their DNA-binding domain (DBD). The VDR DBD recognises the hexameric sequence RGKTSAS (R = A or G, K = G or T, S = C or G) in the major groove of genomic DNA. In vitro experiments indicated that VDR preferentially forms heterodimers with the nuclear receptor retinoid X receptor (RXR) on a direct repeat of two hexameric motifs spaced by three nucleotides (DR3) (Carlberg et al., 1993; Sone et al., 1991). This was confirmed by genome-wide analysis of VDR binding sites in six human cell types, but only for approximately 15% of all 23,000 detected sites (Tuoresmäki et al., 2014). Accordingly, there must be additional mechanisms how VDR associates with genomic DNA (Carlberg and Campbell, 2013; Carlberg, 2017). Interestingly, the genome-wide VDR binding pattern is rather cell-specific, which has an impact on the panel of vitamin D target genes in VDR expressing tissues and cell types (Carlberg, 2014; Campbell, 2014).

In the past, vitamin D and its metabolite 1,25(OH)2D3 were best characterised for their physiological role in calcium homeostasis and bone formation. However, to date most genome-wide data are available for the actions vitamin D in cells of the haematopoietic system, such as peripheral blood mononuclear cells (PBMCs), monocytes and macrophages (Carlberg, 2014). This emphasises the role of VDR and vitamin D in the control of innate and adaptive immunity.

Genomic DNA is always wrapped around nucleosomes forming chromatin. The default stage of chromatin is densely packed heterochromatin preventing the access of transcription factors and most other nuclear proteins to genomic DNA (Beisel and Paro, 2011). Thus, chromatin has an intrinsic repressive potential conserving the epigenome of a differentiated cell. Per cell type this leaves only some 100–200,000 chromatin sites, being primarily located at promoter and enhancer regions, accessible to transcription factors (ENCODE-Project-Consortium et al., 2012). The epigenome can be modulated on the level of DNA methylation, histone acetylation and methylation and three-dimensional chromatin organisation and is controlled by chromatin modifying and remodelling enzymes that can read, write or erase posttranslational chromatin marks and reposition nucleosomes, respectively.

VDR colocalises with a number of nuclear proteins, such as the pioneer transcription factor PU.1 and the chromatin organiser CTCF (Carlberg, 2017). Moreover, VDR is a component of a large protein complex containing chromatin modifying and remodelling enzymes, corepressor, coactivator and mediator proteins (Molnár, 2014). In this way, VDR and its ligand 1,25(OH)2D3 can modulate many aspects of the epigenome, such as patterns of posttranslational histone modifications and accessible chromatin (Seuter et al., 2016). This means that vitamin D3 has a direct effect on the epigenome. Vitamin D-triggered changes in the epigenome can serve as a first step in modulating the transcriptome of a cell (Carlberg, 2014). A high vitamin D status correlates with the availability of 1,25(OH)2D3 in the nuclei of VDR expressing tissues and cell types. Therefore, the vitamin D status of an individual should have an impact on his/her epigenome and subsequently on the transcriptome.

Interestingly, VDR is also located in the cytoplasm and can interact with membrane invaginations (caveolae). In this way, vitamin D3 can stimulate signal transduction pathways that are mediated by mitogen-activated protein kinase and cyclic adenosine monophosphate. These rapid, nongenomic actions of vitamin D3 were found in the intestine, vascular smooth muscle and pancreatic β-cells.

**Vitamin D Response In Vivo**

Vitamin D intervention studies often focus on the evaluation of the health status of the study participants via questionnaires, medical examination and/or serum biochemistry. In contrast, the

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**Figure 2  Structural view.** The structure of 1,25(OH)2D3 (a) and of VDR’s LBD (b). The LBP is indicated in red. Please note that both structures are in different scale.
5-month vitamin D₃ intervention trial VitDmet (NCT01479933) measured from PBMCs isolated from 71 elderly prediabetic study participants at start and end of the study mRNA (messenger ribonucleic acid) expression of 24 vitamin D target genes (Carlberg et al., 2013). There was a significant correlation between mRNA expression changes and variations in 25(OH)D₃ serum levels (Vukic et al., 2015), that is the vitamin D target genes served as biomarkers for functional consequences of changes in the vitamin D status. Moreover, from more than 100 clinical and biochemical parameters that had been determined in the VitDmet trial only 12, such as PTH serum levels, displayed significant correlations (Saksa et al., 2015). The total 36 vitamin D-dependent parameters allowed to determine how well the study participants respond to vitamin D₃ supplementation. In fact, the tested persons showed an individual response to vitamin D₃, referred to a vitamin D response index (Carlberg, 2016), which allowed to segregate them into high, mid and low responders.

In the VitDbol intervention trial (NCT02063334), healthy human adults were treated once with a vitamin D₃ bolus (2000 μg) and samples were taken already 1 and 2 days after onset of the study (Vukic et al., 2015). Also this experimental approach allowed the determination of the vitamin D response index and segregated the study participants into high, mid and low responders (Carlberg and Haq, 2018). Interestingly, the dynamic response to vitamin D₃, that is a comparison of vitamin D-triggered parameters at two or more time points, does not correlate with the vitamin D status as determined in other vitamin D trials.

The vitamin D response index suggests for each individual a vitamin D₃ supplementation protocol that will direct to a personal optimal vitamin D status. This concept may help to solve the scientific dispute about recommended 25(OH)D₃ serum levels and amounts of daily vitamin D₃ supplementation. Moreover, the inclusion of vitamin D response index measurements in the stratification of study cohorts may be most appropriate, in order to challenge observational studies suggesting that high serum concentrations are recommended to be throughout year above 50 nM (US Institute of Medicine) or 75 nM (US Endocrine Society).

### References


### Glossary

1,25(OH)₂D₃ The biologically most active form of vitamin D, also called calcitriol, and the only high affinity (kₐ 0.1 nM) ligand of the transcription factor VDR (Figure 2).

**Epigenome** The genome-wide analysis of epigenetics and changes in gene functions that are heritable but do not involve changes in the genome. Very dynamic, varies from one cell type to the other and can respond to various signalling pathways.

**Genome** The complete haploid DNA sequence of an organism comprising all coding genes and far larger noncoding regions. With the exception of cancer cells, the genome (3260 Mb) of all of the 400 tissues and cell types that form a human individual is identical and constant over time.

**Transcriptome** The complete set of all transcribed RNA molecules of a tissue or cell type; significantly differs between tissues and depends on extra- and intracellular signals.

**VDR** An endocrine member of the nuclear receptor superfamily and is structurally (Figure 2) and functionally comparable with the nuclear receptors for oestrogen, or cortisol. The transcription factor is the only high affinity target of 1,25(OH)₂D₃, that is basically all biological actions of vitamin D are mediated by the VDR.

**Vitamin D response index** The dynamic molecular response to vitamin D being calculated based on a comparison of vitamin D-triggered parameters, such as mRNA expression of vitamin D target genes in PBMCs or serum levels of parathyroid hormone protein, at two or more time points in relation to changes in 25(OH)D₃ serum levels.

**Vitamin D status** Determined based on the serum concentration of 25(OH)D₃ being the most abundant vitamin D metabolite. For good bone health and overall health, 25(OH)D₃ serum concentrations are recommended to be throughout year above 50 nM (US Institute of Medicine) or 75 nM (US Endocrine Society).
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Further Reading


