Rocky Mountain Analytical
Changing lives, one test at a time

D-Spot 25-hydroxyvitamin D_3

Clinical Information for Professionals

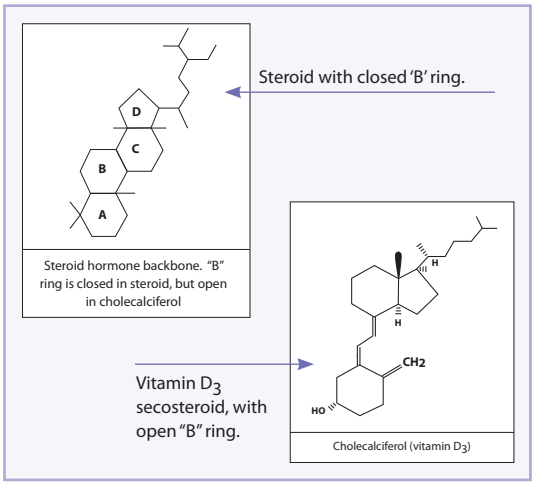
D-Spot

Introduction to Vitamin D

Vitamin D is a group of fat soluble prohormones that play a critical role in regulating calcium and phosphorus levels in the body. Not technically a vitamin since sunlight triggers its synthesis in skin, vitamin D is a secosteroid molecule, and exerts its effect by activating vitamin D receptors (VDRs). [1]

Secosteroids are very similar in structure to steroid hormones. The major difference is that the B-ring carbon atoms are open in secosteroids, but closed in steroid molecules. (see diagram at right)

Vitamin D is considered the most important secosteroid in the body because it directly regulates genes. Therefore, deficiencies or insufficiencies can have a significant deleterious impact on health.



The Vitamin D Family

The vitamin D family includes the following important molecules:

cholecalciferol or vitamin D_3

- Ultraviolet B (UVB) radiation penetrates the skin and converts 7-dehydrocholesterol into previtamin D_3, which is isomerized to vitamin D_3.
- Just 30 min of UVB irradiation achieves maximum daily production of vitamin D_3. Beyond 30 minutes exposure, excess vitamin D_3 is destroyed by sunlight, thereby preventing vitamin D toxicity.
- Vitamin D_3 is the form of vitamin D used in most over-the-counter supplements
- Vitamin D_3 is used exclusively to fortify dairy foods in Canada.
- Vitamin D_3 has a very short half-life in serum, making direct measurement impracticable.

ergocalciferol or vitamin D_2

- Ergocalciferol occurs naturally in mushrooms (portabella, shiitake, button).
- Ergocalciferol is available by prescription in Canada as Ostoforte®. It is also commonly referred to as synthetic vitamin D.
- Vitamin D_2 binds less tightly to the vitamin D receptor and is metabolized slightly differently than vitamin D_3.
- Levels of vitamin D_2 are naturally low in serum unless it is being supplemented.

25-hydroxyvitamin D or 25-OHD

- A long half life (2 to 3 weeks) and larger quantities in serum (nmol/L)make 25-OHD the most suitable molecule to assess for deficiency or sufficiency of vitamin D.
- It is present in both D_3 and D_2 forms.

1,25 dihydroxyvitamin D or 1,25-(OH)2D

- 1,25-(OH)2D is the molecule that activates vitamin D receptors.
- Is also present in D_2 and D_3 forms.
- 1,25-(OH)2D is difficult to measure due to its low serum levels (pmol/L), which are a thousand-fold less than 25-OHD.
- 1,25-(OH)2D levels are highly influenced by parathyroid hormone and calcium/ phosphorus balance.

D-Spot
25-hydroxyvitamin D_3

The Importance of Vitamin D

What is an Optimal Level?

Although there is no agreed upon definition for what constitutes an optimal level of 25-OHD, various sources have pegged the **minimum** for optimal health somewhere in the range of 75 to 100 nmol/L:

- A Harvard School of Public Health review found that 75 nmol/L was the minimum 'most advantageous' 25-OHD level.[2]
- The Endocrine Society considers a 25-OHD_3 level of 100 nmol/L as the minimum 'ideal' level for non-skeletal health benefits.[3]

For the D-Spot, Rocky Mountain Analytical uses 80 nmol/L as a minimum and 200 nmol/L as an upper limit for the optimal range. This range is consistent with serum 25-OHD ranges for other accredited laboratories in Alberta. Table 1 on page 3 lists all the ranges for the D-Spot.

As more than 200 genes are influenced or regulated by the vitamin D secosteroid family, deficiencies or insufficiencies of vitamin D can have wide-ranging effects. The current Canadian average 25-OHD level is 67.7 nmol/L[4], whereas the minimum considered optimal for health lies somewhere between 75 and 100 nmol/L. This means more than 70% of Canadians do not have levels considered optimal for health. A 2010 study by Grant estimated the health benefits that could be gained by achieving an average 25-OHD level of 105 nmol/L in Canada as follows:

- reduce the health-related economic burden by $14.4 billion.
- reduce deaths by 37,000 (16.1% decrease) per year.[5]

The estimated reduction in deaths from improved vitamin D status is based on the numerous pathologies and diseases associated with vitamin D deficiency or insufficiency:

Bone

- Calcium and phosphorus absorption are markedly decreased in the presence of vitamin D deficiency.
- Vitamin D plays a critical role in controlling calcium and phosphate levels. If these levels are not adequately controlled, bone conditions such as rickets in children or osteoporosis in adults may occur. [6]

Endocrinology/Autoimmune

- Polymorphisms in the vitamin D receptor are associated with development of Type I diabetes. [7]
- Women who consume the recommended daily amount of vitamin D or more were 30% less likely to develop rheumatoid arthritis.[8]
- The lowest levels of 25-OHD_3 were associated with highest prevalence of Type II diabetes.[6]

Cancers

- Levels of 25-OHD below 50 nmol/L are associated with a 30 to 50% increased risk of incident colon, prostate and breast cancers, in conjunction with higher mortality from these cancers. It is hypothesized that 1,25-(OH)2D induces apoptosis and prevents angiogenesis in malignant cells, thus reducing their survivability.[9]

Heart

- Vitamin D deficiency has been associated with congestive heart failure and hypertension.[10]
- Peripheral vascular disease may be reduced by maintaining adequate 25-OHD levels.[11]

Neurology/Psychiatry

- Women who consume at least the recommended daily amount of vitamin D, were 40% less likely to develop multiple sclerosis (MS) than those on lower doses.[12]
- Supplementation with vitamin D is associated with a 22% decrease in risk of falls. [7]
- Vitamin D deficiency has been linked to an increased incidence of schizophrenia and depression.[10]
- People with Alzheimer's dementia have lower 25-OHD_3 levels than matched controls without dementia.[7]

Note: Despite the plethora of population studies demonstrating associations between vitamin D deficiencies and various disease states, few outcome studies of the effect of vitamin D supplementation on disease have been published.

Vitamin D Supplements

Cholecalciferol - Vitamin D_3

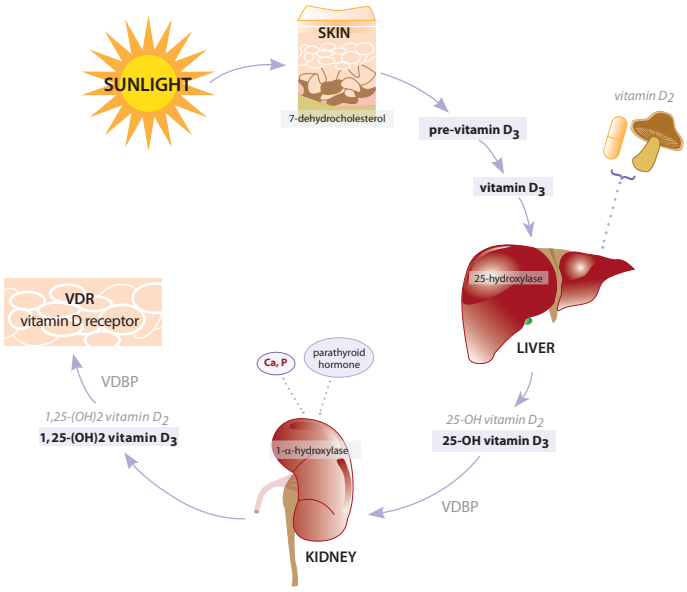
- Cholecalciferol metabolites have superior affinity for vitamin D-binding proteins compared to ergocalciferol (D_2) [13]
- Cholecalciferol is manufactured primarily by ultraviolet irradiation of 7-dehydrocholesterol from lanolin. As an animal product, lanolin-derived vitamin D is unsuitable for vegans. Yeast and lichen sources may be available in some markets.
- Each1000 IU of supplemented vitamin D_3 raises 25-OHD by approximately 15 to 25 nmol/L. [2,14]
- D_3 is the form found in most supplements.

Ergocalciferol - Vitamin D_2

- Ergocalciferol is less than 1/3 the potency of cholecalciferol and has a shorter half-life. [12]
- A head-to-head comparison of 25-OHD levels after supplementation with D_2 or D_3 showed both forms raised 25-OHD for the first three days. From day 4 on, 25-OHD levels steadily increased with D_3 supplementation, but decreased to baseline by day 14 in patients taking D_2.[12]

Vitamin D Metabolism

After vitamin D_3 has been synthesized or supplemented, it is transported to the liver and converted to 25-hydroxyvitamin D_3 (25-OHD_3), the molecule measured to assess vitamin D status. 25-OHD_3 is converted in the kidneys to its active form, 1,25-dihydroxyvitamin D_3 (1,25-(OH)$2D_3$). Binding of 1,25-(OH)$2D_3$ to vitamin D receptors (VDR) influences many cellular processes. In fact, over 200 genes are known to be regulated by interaction of (1,25-OH)2D with vitamin D receptors. The diagram at right illustrates the synthesis and metabolism of the vitamin D family.



Assessing Vitamin D Status

Measure the Right Molecule

The physiologically active metabolite of vitamin D is 1,25-(OH)2D. However, its relatively short half-life and low concentrations (pmol/L) render 1,25-(OH)2D of limited use as an indicator of vitamin D status. In addition, its levels are highly influenced by concentrations of both parathyroid hormone and calcium.

Vitamin D_3 is also a poor marker of vitamin D status because it is rapidly hydroxylated, giving it a very short half-life in serum. In contrast, 25-OHD has a half-life of 2 to 3 weeks and is present in serum at concentrations a thousand-fold higher than 1,25-(OH)2D. Consequently, measurement of 25-OHD has become the preferred indicator of vitamin D nutritional status.

Table 1: D-Spot Reference Ranges

Status	25OHD_3 Level
Severe Deficiency	<25 nmol/L
Mild to Moderate Deficiency	25 to 80 nmol/L
Optimal	80 to 200 nmol/L
High	200 to 250 nmol/L
Toxicity possible	>250 nmol/L

Note: Standard International (SI) units are used in laboratories in Canada and Worldwide, but not in the United States. The U.S. reports 25-OHD in ng/mL rather than nmol/L. For reference purposes, the conversion factor from ng/mL to SI units is 2.5 (Example: 30 ng/mL = 75 nmol/L).

Use the Right Method

Although 25-OHD can be measured via radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISA) and other methods, measurement by LC-MSMS is considered the gold-standard. LC-MSMS measurement of 25-OHD has the highest degree of sensitivity and specificity of all the methods.

Measure What's Measurable.

While 25-OHD can be measured as either 25-OHD_2 or 25-OHD_3, Rocky Mountain Analytical only reports the 25-OHD_3 form. We have opted out of analyzing 25-OHD_2 for the following reasons:

- The currently available standard certified reference materials (NIST- National Institute of Standards and Technology) for 25-OHD do not contain appreciable levels of vitamin D_2, which means laboratories cannot independently verify the accuracy of their D_2 numbers.[15]
- Canada uses vitamin D_3 exclusively to fortify dairy foods, therefore Vitamin D_2 levels are naturally low unless D_2 is being supplemented.

Note: The D-Spot does not include 25-OHD_2, which means ergocalciferol (vitamin D_2) supplementation will not be reflected in the report.

When to Test

Testing at the end of winter (March or April) typically reflects 25-OHD at its lowest, while testing at the end of summer (August or September) generally reflects 25-OHD at its highest. Patients who are taking vitamin D supplements can test any time. When a dosage change has been made, it is best to wait 4 to 6 weeks before testing.

Causes of Vitamin D Deficiency [16]

Reduced Synthesis

- **Season, latitude, and time of day** – *fewer UVB photons reach the earth during winter months. In Canada, little or no vitamin D_3 is produced from October to April.*
- **Sunscreen use** – *SPF8 sunscreen reduces vitamin D3 synthesis by 92.5%, and SPF15 by 99%.*
- **Skin pigment** – *absorption of UVB radiation by melanin in dark skin reduces vitamin D_3 synthesis by as much as 99%.*
- **Aging** – *7-dehydrocholesterol in the skin decreases with age. Vitamin D_3 synthesis is decreased by about 75% in a 70 year old.*
- **Skin conditions** *that decrease 7-dehydrocholesterol levels (e.g. skin grafts for burns) may reduce vitamin D_3 synthesis.*

Reduced Absorption

- **Malabsorption** – *diseases that affect fat absorption (e.g. cystic fibrosis, celiac disease, Whipples' disease, Crohn's disease, or bypass surgery) impair vitamin D absorption and storage.*
- **Cholesterol lowering medication** - *abnormally low cholesterol impairs vitamin D synthesis (insufficient 7-dehydrocholesterol).*

Increased Sequestration

- **Obesity** – *excess fat cells store vitamin D thereby reducing its availability to serum. As the body-mass index increases, 25-OHD levels decrease.*

Breastfeeding

- *increasesd infant risk of vitamin D deficiency when breast milk is deficient in vitamin D and is sole source of infant nutrition.*

Disease

- **Liver failure** – *results in decreased 25-OHD production.*
- **Nephrotic syndrome** – *causes 25-OHD to spill into urine.*
- **Chronic kidney disease** – *decreases serum levels of 1,25-(OH)2D.*
- **Primary hyperparathyroidism** – *elevated parathyroid hormone levels result in increased metabolism of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D.*
- **Hyperthyroidism** – *enhances metabolism of 25-OHD thereby reducing serum 25-OHD levels.*
- **Tumor-induced osteomalacia** – *tumor secretions decrease renal 25-hydroxyvitamin D-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-dihydroxyvitamin D.*
- **Granulomatous disorders** - *macrophages inside granulomas convert 25-OHD to 1,25-(OH)2D, thus decreasing 25-OHD and increasing 1,25-(OH)2D.*

Increased Catabolism

- *medications (anticonvulsants, glucocorticoids, anti-retroviral and antirejection medications) promote destruction of 25-OHD and 1,25(OH)2D to inactive calcitroic acid.*

Genetic mutations

- **Pseudovitamin D deficiency rickets** – *mutation of the renal 25-hydroxyvitamin D-1α-hydroxylase gene (CYP27B1) leads to reduced or no renal synthesis of 1,25-dihydroxyvitamin D.*
- **Vitamin D-resistant rickets** – *mutation in vitamin D receptor gene causes partial or complete resistance to 1,25-dihydroxyvitamin D.*
- **Vitamin D-dependent rickets type 3** – *overproduction of hormone-responsive-element binding proteins causes partial or complete resistance to 1,25-dihydroxyvitamin D.*
- **Autosomal dominant hypophosphatemic rickets** – *gene mutation causes decreased renal 25-hydroxyvitamin D-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-dihydroxyvitamin D.*
- **X-linked hypophosphatemic rickets** – *gene mutation causes decreased renal 25-hydroxyvitamin D-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-(OH)2D.*

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