Vitamin D Deficiency and Toxicity

Laboratory Support of Diagnosis and Management

CLINICAL BACKGROUND

Vitamin D-associated Disorders

Vitamin D deficiency, as defined by the Institute of Medicine (IOM) and professional organizations such as the Endocrine Society, is common in the general population. The most severe deficiencies are associated with rickets in children and osteomalacia and osteoporosis in adults; vitamin D deficiency is also associated with muscle weakness and secondary hyperparathyroidism, both of which can exacerbate these bone disorders and/or their clinical impact.[1,2] In many cases, however, significant vitamin D deficiency can exist without immediate clinical symptoms, presenting a future risk to bone health if the condition is not diagnosed for many years.

Evidence, mostly from observational studies, suggests a link between vitamin D deficiency and non-bone diseases as well. These diseases include cancer (especially colorectal and breast cancer), cardiovascular disease, autoimmune diseases, diabetes, depression, and neurocognitive dysfunction.[1,3,4]

In rare cases, ingestion of large doses of vitamin D results in vitamin D toxicity, which can manifest as hypercalcemia, hyperphosphatemia, suppressed parathyroid hormone level, hypercalciuria, and soft tissue calcification, including nephrocalcinosis and kidney stones.[1,5] The risk of vitamin D toxicity may be increased in individuals who have[5]:

- granulomatous disorders such as sarcoidosis and tuberculosis
- some lymphomas that produce 1,25-dihydroxyvitamin D in activated macrophages
- a genetic defect causing a 25-hydroxyvitamin D-24-hydroxylase deficiency

Physiology

Vitamin D is a fat-soluble vitamin that promotes bone health by enhancing intestinal absorption of calcium and phosphorus[1,6]; without sufficient absorption of calcium and phosphorous parathyroid hormone (PTH) levels can increase and result in bone resorption.[6] More recent discoveries suggest that vitamin D may have additional biological functions. For example, vitamin D receptors have been found in a number of tissues including colon, breast, prostate, and brain. The active form of vitamin D (1,25-dihydroxyvitamin D) is involved in the control of more than 200 genes, including those involved in immune function[7] and regulating cell growth and apoptosis.[1]

The term "vitamin D" typically refers to 2 molecular forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is the main form and is produced in the skin in response to sunlight.[6] However, both forms can be obtained in relatively small amounts through normal diet. Both are also available as over-the-counter supplements and as high-dose prescription formulations in the United States.[3] Vitamin D_3 is also available as a high-dose supplement.

Vitamins D_2 and D_3 are metabolized in the liver to their respective 25-hydroxyvitamin D (25[OH]D) metabolites, 25(OH)D_2 and 25(OH)D_3, which are converted in the kidneys to their corresponding active forms **(Table 1)**.[4] Experts disagree about the relative potencies of vitamins D_2 and D_3; some experts claim that the 2 forms are equally effective in raising 25(OH)D levels, whereas other experts assert that vitamin D_2 is less effective than vitamin D_3 in elevating 25(OH)D.[8,9]

Table 1. Important Vitamin D Forms

Name	Alternate Name	Abbreviation	Description
Ergocalciferol	Vitamin D_2	NA	Plant-based form
Cholecalciferol	Vitamin D_3	NA	Animal-based form; form produced in human skin
Ercalcidiol	25-hydroxyvitamin D_2	25(OH)D_2	Main circulating form of vitamin D_2
Calcidiol	25-hydroxyvitamin D_3	25(OH)D_3	Main circulating form of vitamin D_3
Ercalcitriol	1,25-dihydroxyvitamin D_2	$1,25(OH)_2D_2$	Active form of vitamin D_2; has a short half-life in the blood
Calcitriol	1,25-dihydroxyvitamin D_3	$1,25(OH)_2D_3$	Active form of vitamin D_3; has a short half-life in the blood
3-epimer	3-epi-25-hydroxyvitamin D	3-epi-25(OH)D	Low-activity form; significant levels present in infants

Recommended Vitamin D Intake

The American Association of Clinical Endocrinologists (AACE) recommends vitamin D and calcium supplementation to prevent osteoporosis in postmenopausal women.[2] In conjunction with calcium, vitamin D supplementation increases bone mineral density and reduces the risk of fractures and falls in postmenopausal women.[2] The American Academy of Pediatrics (AAP) also recommends supplementation with vitamin D to prevent rickets in children, especially for breastfed infants.[10] Indeed, vitamin D supplementation/therapy is now the standard of care for preventing and treating rickets.[6,10]

Optimal intake levels, however, remain a subject of debate. In 2011, the IOM increased the recommended daily vitamin D intake from 200-600 IU/d to 400-800 IU/d, depending on age.[11] Other experts believe that these increases were not sufficient; they recommend as much as 1,000-2,000 IU/d **(Table 2)**.[6] These same experts believe that an intake up to 10,000 IU/d is safe.

Obtaining Sufficient Amounts of Vitamin D

As much as 68% to 77% of the population is estimated to have suboptimal (<30 ng/mL) levels of vitamin D.[12-14] Sufficient amounts can be obtained through adequate sunlight exposure and/or a diet containing enough vitamin D-rich foods such as oily fish. Other animal-based foods provide small amounts of vitamin D_3, and some plant-based foods provide small amounts of vitamin D_2.[6] For individuals who do not get enough vitamin D by these means, supplementation is available. Vitamins D_2 and D_3 are both available in fortified foods and in over-the-counter supplements that range in dose from 400 IU to 50,000 IU. Vitamin D_2 is also available by prescription in liquid (8,000 IU) or high-potency (50,000 IU) capsule formulations. For vitamin D deficiency, the recommended treatment dosage, length of treatment, and maintenance therapy dosage differ based on whether the patient is an adult, child, or infant.[9] Higher vitamin D doses may be required for people who are obese, have a malabsorption syndrome (eg, cystic fibrosis, celiac disease, Crohn's disease),[1] have undergone bariatric surgery, or are taking medications that affect vitamin D metabolism.[6]

Table 2. IOM and Endocrine Society Recommendations for Vitamin D Intake[6,11]

Life-stage Group[a]	IOM Recommendations (IU/d)		Endocrine Society Recommendations (IU/d)[b]	
	Intake	Upper Limit[c]	Intake	Upper Limit[c]
0-6 months	400[d]	1,000	400-1,000	2,000
6-12 months	400[d]	1,500	400-1,000	2,000
1-3 years	600	2,500	600-1,000	4,000
4-8 years	600	3,000	600-1,000	4,000
9-18 years	600	4,000	600-1,000	4,000
19-30 years	600	4,000	1,500-2,000	10,000
31-50 years	600	4,000	1,500-2,000	10,000
51-70 years	600	4,000	1,500-2,000	10,000
71+ years	800	4,000	1,500-2,000	10,000
Pregnant or lactating women (14-18 years)	600	4,000	600-1,000	4,000
Pregnant or lactating women (19-50 years)	600	4,000	1,500-2,000	10,000

[a] Includes normal, healthy individuals of both genders unless otherwise specified.
[b] Estimated intake needed to maintain blood 25(OH)D levels above 30 ng/mL.
[c] Maximum level that is expected to have no risk of adverse effects to healthy individuals. 1 µg of vitamin D is equivalent to 400 IU.
[d] Refers to adequate intake (intake estimated to maintain protective 25(OH)D levels in a group of healthy individuals with limited sun exposure and vitamin D stores) instead of recommended intake, which could not be established because of insufficient evidence.

Determining Vitamin D Status

Laboratory measurement of 25(OH)D in blood is the accepted means for determining vitamin D status.[6] 25(OH)D levels can be used in: 1) diagnosing vitamin D insufficiency or deficiency, thus identifying individuals who may benefit from supplementation; 2) monitoring response to vitamin D supplements; and 3) diagnosing vitamin D toxicity. There is no consensus about the optimal 25(OH)D level, but many experts accept a minimum of 30 ng/mL with a range 40 ng/mL to 60 ng/mL as optimal.[1,2,6] Suboptimal levels are broken into 2 groups: insufficiency (21 ng/mL to 29 ng/mL) and deficiency (≤20 ng/mL).

INDIVIDUALS SUITABLE FOR TESTING

- Individuals with suspected vitamin D deficiency (eg, those with persistent, nonspecific musculoskeletal pain)
- Individuals at increased risk for vitamin D deficiency (eg, pregnant, lactating, dark-skinned, elderly, obese, or house-bound individuals; those dwelling in latitudes higher than 33° north or 33° south; and infants who are breastfeeding without vitamin D supplementation or from vitamin D-deficient mothers)
- Individuals being treated with vitamin D_2 or vitamin D_3 supplementation
- Individuals with disorders associated with reduced 25(OH)D levels **(Table 3)**[1,6,7,15-29]
- Individuals taking certain medications associated with reduced 25(OH)D levels **(Table 3)**
- Individuals with suspected toxicity (eg, those with hypercalcemia of obscure origin)

Table 3. Effect of Various Disorders and Medications on 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Concentrations[1,6,7,15-29]

Disorder	25(OH)D Concentration	$1,25(OH)_2D$ Concentration
Chronic kidney disease[1,15]	↓ or N	↓
Fat malabsorption disorders, short bowel syndrome, inflammatory bowel disease, Crohn's disease[6,16]	↓	N or ↑
Hypercalcemia of cancer[a,17,18]	↓ or N or ↑	N or ↓
Hypophosphatemic rickets[b,1,19]	N	N or ↓
Lymphoma, granulomatous disorders[1,6]	↓	↑
Medications that increase vitamin D metabolism (eg, anticonvulsants, antiretrovirals, and glucocorticoids) [c,1,6,28]	↓	N
Nephrotic syndrome[c,7,20]	↓	N
Nutritional rickets or osteomalacia[21]	↓	↓ or N or ↑
Obesity[c, 6,22,29]	↓	N
Osteoporosis[1,19]	↓ or N	↓ or N or ↑
Primary hyperparathyroidism[1,6,23]	↓ or N	N or ↑
Secondary hyperparathyroidism[24]	↓	N or ↑
Severe parenchymal liver disease (impaired 25-hydroxylation of vitamin D)[c,7,25]	↓	N
Tumor-induced osteomalacia (oncogenic osteomalacia)[1,26]	N	↓
Vitamin D-dependent rickets, type I[d,27]	N	↓
Vitamin D-dependent rickets, type II[e,27]	N	↑
Vitamin D (25[OH]D) toxicity (uncommon)[7]	↑	↓ or N

25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; N, normal; ↑, elevated; ↓, reduced.

[a] PTHrP (parathyroid hormone–related peptide)-mediated.

[b] Autosomal-dominant or X-linked hypophosphatemic rickets.

[c] $1,25(OH)_2D$ levels are usually normal for patients taking the medications listed, and those with nephrotic syndrome, hepatic disease, or obesity. However, $1,25(OH)_2D$ levels can be high normal or elevated due to secondary hyperparathyroidism associated with lower levels of 25(OH)D. They can also be low if there is inadequate substrate for the kidneys to produce $1,25(OH)_2D$.

[d] Also called pseudo-vitamin D-deficiency rickets.

[e] Also called hereditary vitamin D-resistant rickets.

TEST AVAILABILITY

Tests offered by Quest Diagnostics to assist in the diagnosis of vitamin D deficiency or toxicity are presented in **Table 4**. This table is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

TEST SELECTION

25(OH)D

Measurement of 25(OH)D, the main form of circulating vitamin D, is used to determine vitamin D status and to monitor most patients receiving vitamin D therapy.[6] Immunoassays measure total 25(OH)D concentrations and not its separate components. On the other hand, the liquid chromatography–tandem mass spectrometry (LC/MS/MS) method measures 25(OH)D_2, 25(OH)D_3, and total 25(OH)D concentrations. LC/MS/MS is the reference method endorsed by experts at the National Institute of Standards and Technology (NIST), the Centers for Disease Control and Prevention (CDC), and the National Institutes of Health (NIH).[30-32]

Unlike some immunoassays,[33-36] the immunochemiluminometric assay used by Quest Diagnostics accurately and precisely measures total 25(OH)D levels. It is suitable for diagnosing vitamin D insufficiency or deficiency in most patients. The method is calibrated to the NIST standard reference material (SRM) 972, ensuring its accuracy. Furthermore, it has been certified by the CDC Vitamin D Standardization Certification Program, demonstrating precision and lack of bias in the method as compared to the CDC's LC/MS/MS reference method.

Quest Diagnostics also offers an LC/MS/MS method. Concentrations of both 25(OH)D forms are independently reported, and the 2 forms are summed to provide the total 25(OH)D concentration. Thus, the QuestAssureD™ 25-Hydroxyvitamin D (D_2, D_3), LC/MS/MS assay differentiates the separate contributions of vitamins D_2 and D_3 to vitamin D status. It detects 25(OH)D_2 and 25(OH)D_3 at an analytical sensitivity of 4 ng/mL, and does not cross-react with the D_2 or D_3 forms of vitamin D, 25-hydroxyvitamin D, or 1,25-dihydroxyvitamin D. This test can be used to help diagnose vitamin D insufficiency or deficiency and to monitor patients receiving vitamin D_2 or D_3 supplementation.

Table 4. Tests Available to Determine Vitamin D Levels

Test Code	Test Name	Method	Description
17306	Vitamin D, 25-Hydroxy, Total, Immunoassay	ICMA	Accurately quantifies and reports total 25(OH)D; useful for diagnosing vitamin D insufficiency or deficiency in most patients[a]; useful for monitoring response to vitamin D supplementation
92888	QuestAssureD™ 25-Hydroxyvitamin D (D_2, D_3), LC/MS/MS	LC/MS/MS	Reference method; accurately quantifies and reports both forms of vitamin D: 25(OH)D_2 and 25(OH)D_3; total 25(OH)D reported as the sum of the 2 forms; useful for diagnosing vitamin D insufficiency or deficiency and for monitoring vitamin D_2 or D_3 therapy[a]
91935	QuestAssureD™ for Infants, 25-Hydroxyvitamin D, LC/MS/MS	LC/MS/MS	Accurately quantifies 25(OH)D_2, 25(OH)D_3, and total 25(OH)D in infants/toddlers <36 months of age; 3-epimer, which is present at relatively high levels in infants, is separated and excluded from measurements in this assay; useful for diagnosing vitamin D insufficiency or deficiency and for monitoring vitamin D_2 or D_3 therapy[a]
16558	Vitamin D, 1,25-Dihydroxy, LC/MS/MS[b]	LC/MS/MS	Accurately quantifies 1,25$(OH)_2$D; useful in differential diagnosis of vitamin D-related diseases and for monitoring vitamin D therapy in patients with chronic kidney disease
16761	QuestAssureD™ 25-Hydroxy and 1,25-Dihydroxyvitamin D, LC/MS/MS[b]	LC/MS/MS	See individual analytes

ICMA, immunochemiluminescent assay; LC/MS/MS, liquid chromatography– tandem mass spectrometry.

[a] May also be used to confirm vitamin D toxicity, which occurs rarely.

[b] This test was developed and its performance characteristics have been determined by Quest Diagnostics. Performance characteristics refer to the analytical performance of the test.

Infants, unlike adults, may have significant levels of the 25(OH)D_3 3-epimer (also known as C3-epimer), a form with reduced activity.[37] In one study, about 75% of infants under 4 months of age and 25% to 40% of infants 4 to 12 months of age had measurable levels (≥3 ng/mL) of the 3-epimer. Although these data indicate that accounting for 3-epimer is most appropriate for infants younger than 12 months of age, a notable proportion (18%) of toddlers between 12 and 36 months old also had measurable levels.[38] LC/MS/MS tests that do not remove the 3-epimer component can overestimate total vitamin D levels, which could lead to undertreatment. One group found that including the 3-epimer in total vitamin D quantitation led to misclassification of 9% of infants as vitamin D sufficient.[37] The QuestAssureD for Infants 25-Hydroxyvitamin D, LC/MS/MS assay differs from the standard LC/MS/MS assay in that it chromatographically separates the 3-epimer, so that the epimer can be excluded from the reported 25(OH)D_3 and total 25(OH)D concentrations.

1,25(OH)$_2$D

Measurement of 1,25(OH)$_2$D is not used to diagnose vitamin D deficiency because most individuals have normal levels due to tight regulation.[6] Furthermore, 1,25(OH)$_2$D may even be elevated in people with secondary hyperparathyroidism because PTH enhances the conversion of 25(OH)D to 1,25(OH)$_2$D.[1] 1,25(OH)$_2$D measurement is reserved for distinguishing some cases of primary hyperparathyroidism from hypercalcemia of cancer and for the differential diagnosis of vitamin D-dependent rickets (type I vs type II) **(Table 3)**. 1,25(OH)$_2$D quantitation is also useful for monitoring vitamin D therapy in patients with chronic kidney disease, who may have normal 25(OH)D levels. As kidney disease progresses, the ability of the kidneys to produce 1,25(OH)$_2$D decreases; supplementation with vitamin D is thus no longer effective, and patients require administration of 1,25(OH)$_2$D or one of its analogs. Although 1,25(OH)$_2$D may be increased or decreased in a number of other disorders, levels are typically used for confirmation rather than diagnosis of these conditions **(Table 3)**.

TEST INTERPRETATION

Optimal 25(OH)D levels are at least 30 ng/mL and preferably 40 ng/mL to 60 ng/mL, whereas levels between 21 ng/mL and 29 ng/mL indicate vitamin D insufficiency and levels ≤20 ng/mL indicate deficiency.[6] It is important to note that the effect of 25(OH)D levels between 21 ng/mL and 29 ng/mL on long-term bone health in children is not yet well understood.[39] Vitamin D insufficiency and deficiency may both lead to elevated PTH levels (secondary hyperparathyroidism),[1] and the most severe forms of deficiency may be associated with hypocalcemia, hypophosphatemia, and elevated alkaline phosphatase.

In children and adults, low 25(OH)D levels are most commonly associated with lack of dietary intake and/or lack of sun exposure. They are also associated with disorders that are characterized by decreased absorption or excessive loss in the gastrointestinal tract, increased vitamin D metabolism, or impaired conversion of vitamin D to 25(OH)D.[1,2] **Table 3** lists expected 25(OH)D and 1,25(OH)$_2$D levels associated with various disorders and medications. In infants, low 25(OH)D levels are associated with dark skin pigmentation, maternal vitamin D deficiency, and breastfeeding without vitamin D supplementation.[6,10]

High 25(OH)D levels are suggestive of vitamin D toxicity, but expert opinions vary regarding an appropriate toxicity threshold. The IOM cites reports of adverse events not related to calcium and bone metabolism in adults at 25(OH)D levels ≥50 ng/mL and recommends lower vitamin D intakes.[11] However, many experts such as the authors of the Endocrine Society Clinical Practice Guideline consider levels up to 100 ng/mL safe and assert that vitamin D toxicity only occurs at 25(OH)D levels ≥150 ng/mL.[6,39]

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