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## Editorial

**Rickets** is a condition that results in weak or soft bones in children. Symptoms include bowed legs, stunted growth, bone pain, large forehead, and trouble sleeping. Complications may include bone fractures, muscle spasms, an abnormally curved spine, or intellectual disability. The most common cause of rickets is a vitamin D deficiency. This can result from eating a diet without enough vitamin D, dark skin, too little sun exposure, exclusive breastfeeding without vitamin D supplementation, celiac disease, and certain genetic conditions. Other factors may include not enough calcium or phosphorus. The underlying mechanism involves insufficient calcification of the growth plate. Diagnosis is generally based on blood tests finding a low calcium, low phosphorus, and a high alkaline phosphatase together with X-rays.

Prevention for exclusively breastfed babies is vitamin D supplements. Otherwise, treatment depends on the underlying cause. If due to a lack of vitamin D, treatment is usually with vitamin D and calcium. This generally results in improvements within a few weeks. Bone deformities may also improve over time. Occasionally surgery may be done to correct bone deformities. Genetic forms of the disease typically require specialized treatment.

Rickets occurs relatively commonly in the Middle East, Africa, and Asia. It is generally uncommon in the United States and Europe, except among certain minority groups. It begins in childhood, typically between the ages of 3 and 18 months old. Rates of disease are equal in males and females. Cases of what is believed to have been rickets have been described since the 1st century, and the condition was widespread in the Roman Empire. The disease was common into the 20th century. Early treatments included the use of cod liver oil.

The **"DISEASE DIAGNOSIS"** segment outlines all details regarding Rickets.

Naturally, **"INTERPRETATION"** section highlights the importance of Vitamin D.

Extrapolating the trend, **"TROUBLESHOOTING"** discusses various modalities employed for Vitamin D assays. Balance left being **"BOUQUET"** has not been ignored.

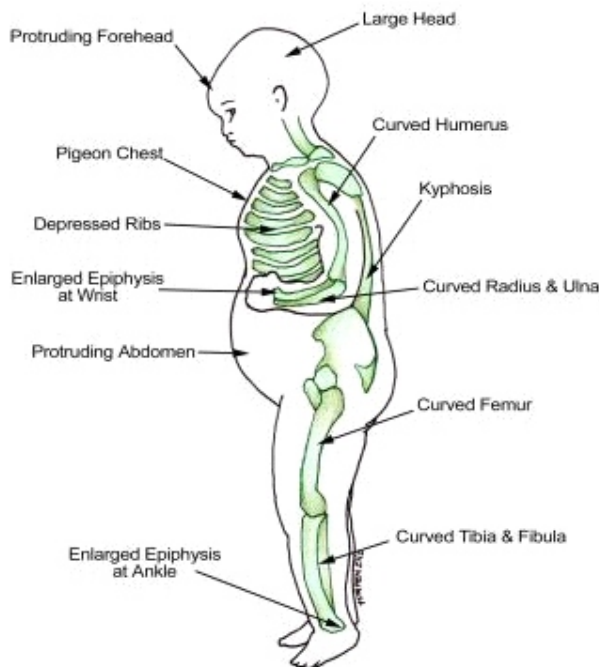


## DISEASE DIAGNOSIS

### RICKETS

#### Background

Rickets is a disease of growing bone that is unique to children and adolescents. It is caused by a failure of osteoid to calcify in a growing person. Failure of osteoid to calcify in adults is called osteomalacia. The image below illustrates findings in a patient with rickets.



Findings in patients with rickets.

Vitamin D deficiency rickets occurs when the metabolites of vitamin D are deficient. Less commonly, a dietary deficiency of calcium or phosphorus may also produce rickets. Vitamin D-3 (cholecalciferol) is formed in the skin from a derivative of cholesterol under the stimulus of ultraviolet-B light. Ultraviolet light or cod liver oil was the only significant source of vitamin D until early in the 20th century when ergosterol (vitamin D-2) was synthesized from irradiated plant steroids. During the Industrial Revolution, rickets appeared in epidemic form in temperate zones where the pollution from factories blocked the sun's ultraviolet rays. Thus, rickets was probably the first childhood disease caused by environmental pollution. Natural nutritional sources of vitamin D are limited primarily to fatty, ocean-going fish. In the United States, dairy milk is fortified with vitamin D (400 IU/L). Human milk contains little vitamin D, generally less than 20-40 IU/L. Therefore, infants who are breastfed are at risk for rickets, especially those who receive no oral supplementation and those who have darkly pigmented skin, which blocks penetration of ultraviolet light. Rickets may lead to skeletal deformity and short stature. In females, pelvic distortion from rickets may cause problems with childbirth later in life. Severe rickets has been associated with respiratory failure in children.

Findings in rickets are illustrated in the image below.



Radiograph in a 4-year-old girl with rickets depicts bowing of the legs caused by loading.

#### Pathophysiology

Cholecalciferol (i.e., vitamin D-3) is formed in the skin from 5-dihydroxycholesterol. This steroid undergoes hydroxylation in 2 steps. The first hydroxylation occurs at position 25 in the liver, producing calcidiol (25-hydroxycholecalciferol), which circulates in the plasma as the most abundant of the vitamin D metabolites and is thought to be a good indicator of overall vitamin D status. The second hydroxylation step occurs in the kidney at the 1 position, where it undergoes hydroxylation to the active metabolite calcitriol (1,25-dihydroxycholecalciferol). This cholecalciferol, which circulates in the bloodstream in minute amounts, is not technically a vitamin but a hormone. Calcitriol acts at 3 known sites to tightly regulate calcium metabolism: (1) it promotes absorption of calcium and phosphorus from the intestine; (2) it increases reabsorption of phosphate in the kidney; and, (3) it acts on bone to release calcium and phosphate. Calcitriol may also directly facilitate calcification. These actions result in an increase in the concentrations of calcium and phosphorus in extracellular fluid. This increase of calcium and phosphorus in extracellular fluid, in turn, leads to the calcification of osteoid, primarily at the metaphyseal growing ends of bones but also throughout all osteoid in the skeleton. Parathyroid hormone facilitates the 1-hydroxylation step in vitamin D metabolism. In the vitamin D deficiency state, hypocalcemia develops, which stimulates excess secretion of parathyroid hormone. In turn, renal phosphorus loss is enhanced, further reducing deposition of calcium in the bone. Excess parathyroid hormone also produces changes in the bone similar to those occurring in hyperparathyroidism. Early in the course of rickets, the calcium concentration in the serum decreases. After the parathyroid response, the calcium concentration usually returns to the reference range, though phosphorus levels remain low. Alkaline phosphatase, which is produced by overactive osteoblast cells, leaks into the extracellular fluids, so that its concentration rises to anywhere from moderate elevation to very high levels. Intestinal malabsorption of fat

and diseases of the liver or kidney may produce the clinical and secondary biochemical picture of nutritional rickets. In such cases, disturbance in calcium homeostasis may be the consequence of renal excretion or may result from intestinal losses, as dietary calcium forms insoluble soaps with malabsorbed fats. Anticonvulsant drugs (e.g. phenobarbital, phenytoin) accelerate metabolism of calcidiol, which may lead to insufficiency and rickets, particularly in children who have darkly pigmented skin and those who are kept primarily indoors (e.g., children who are institutionalized). Calcium and vitamin D intakes are low in infants who are fed vegan diets, particularly in those who are lactovegans, and monitoring of their vitamin D status is essential. Studies have noted that disorders of increased fibroblast growth factor 23 (FGF-23) function are associated with rickets.

## Epidemiology

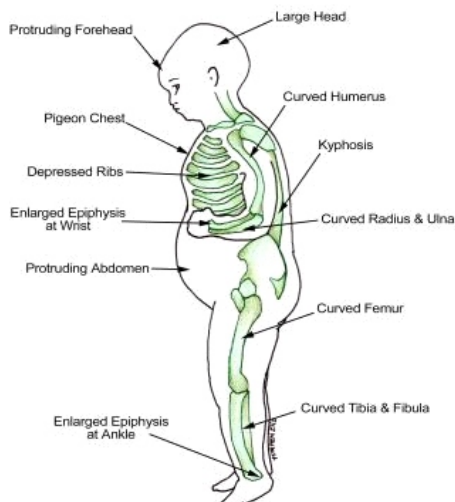
### International statistics

The incidence of rickets in Europe is similar to that in the United States. In sunny areas, such as in the Middle East, rickets may occur when infants are bundled in clothing and are not exposed to sunlight. In some parts of Africa, deficiency of calcium, phosphorus, or both in the diet may also lead to rickets, especially in societies where corn is predominant in the diet. The frequency of rickets has been increasing internationally. Possible reasons include recommendations for children to wear sunscreen while outdoors and a tendency for children to spend more time indoors, watching television or playing electronic games, instead of playing outdoors.

## Clinical Presentation

### Physical Examination

Generalized muscular hypotonia of an unknown mechanism is observed in most patients with clinical (as opposed to biochemical and radiographic) signs of rickets. Craniotabes (areas of thinning and softening of bones of the skull) manifests early in infants with vitamin D deficiency, although this feature may not be present in infants, especially those born prematurely. If rickets occurs at a later age, thickening of the skull develops. This produces frontal bossing and delays the closure of the anterior fontanelle. In the long bones, laying down of uncalcified osteoid at the metaphases leads to spreading of those areas, producing knobby deformity, which is visualized on radiography as cupping and flaring of the metaphyses. Weight bearing produces deformities such as bowlegs and knock-knees. In the chest, knobby deformities result in the so-called rachitic rosary along the costochondral junctions. The weakened ribs pulled by muscles also produce flaring over the diaphragm, which is known as Harrison groove. The sternum may be pulled into a pigeon-breast deformity. In more severe instances in children older than 2 years, vertebral softening leads to kyphoscoliosis. The ends of the long bones demonstrate that same knobby



thickening. At the ankle, palpation of the tibial malleolus gives the impression of a double epiphysis (Marfan sign). Because the softened long bones may bend, they may fracture on one side of the cortex (ie, greenstick fracture). Manifestations of rickets are illustrated in the image.

## Differential Diagnoses

### Diagnostic Considerations

Rare metabolic bone diseases, including hypophosphatasia, have been confused with rickets in infancy. Jansen syndrome is a rare autosomal dominant form of short-limbed dwarfism in which infants present with metaphyseal chondroplasia. Hereditary disorders of vitamin D metabolism have also been described, such as hypophosphatemic vitamin D-resistant rickets. Severe calcium deficiency can also cause a syndrome that is confused with vitamin D deficiency rickets. Premature infants who are breast fed and do not receive mineral supplements may develop severe phosphorus deficiency that presents as rickets.

## Workup

### Approach Considerations

Serum measurements in the workup for rickets may include the following:

- Calcium
- Phosphorus
- Alkaline phosphatase
- Parathyroid hormone
- 25-hydroxy vitamin D
- 1,25-dihydroxyvitamin D

Radiography is indicated in patients with rickets

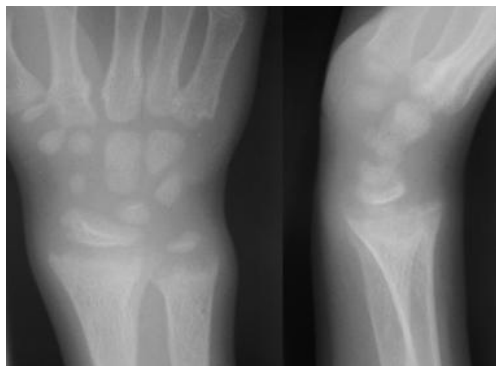
## Serum Chemistry

Early in the course of rickets, the calcium (ionized fraction) is low. However, this level is often within the reference range at the time of diagnosis, as a consequence of increased parathyroid hormone secretion. Although calcidiol (25-hydroxy vitamin D) is low and parathyroid hormone is elevated, determining calcidiol and parathyroid hormone levels is typically not necessary in order to establish a diagnosis. Calcitriol levels maybe normal or elevated because of increased parathyroid activity. The phosphorus level is invariably low for age, unless recent partial treatment or recent exposure to sunlight has occurred. Alkaline phosphatase levels are uniformly elevated. A generalized aminoaciduria occurs from the parathyroid activity. However, aminoaciduria does not occur in familial hypophosphatemia rickets (FHR).

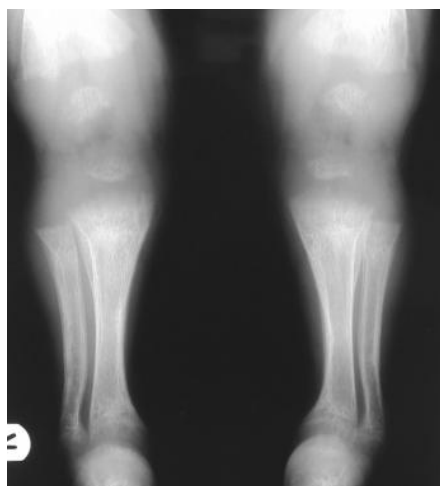
## Radiography

The best single radiographic view for infants and children younger than 3 years is an anterior view of the knee that reveals the metaphyseal end and epiphysis of the femur and tibia. This site is best because growth is most rapid in this location, thus the changes are accentuated. The metaphyses exhibit widening and cupping because of their exaggerated normal concavity and irregular calcification. Because calcified osteoid is abundant, the provisional calcification zone of the metaphysis is much more distant from the calcification center of the epiphysis than is normal for age. Along the shaft, the uncalcified osteoid causes the periosteum to appear separated from the diaphysis. Generalized osteomalacia occurs (observed as osteopenia), with visible coarsening of trabeculae in contrast to the ground-glass osteopenia of scurvy.

Examples of radiographic findings are shown in the images below.



Anteroposterior and lateral radiographs of the wrist of an 8-year-old boy with rickets demonstrates cupping and fraying of the metaphyseal region.



Radiographs of the knee of a 3.6-year-old girl with hypophosphatemia depict severe fraying of the metaphysis.

### Treatment & Management

#### Approach Considerations

Treatment for rickets may be administered gradually over several months or in a single-day dose of 15,000 mcg (600,000 U) of vitamin D. If the gradual method is chosen, 125-250 mcg (5000-10,000 U) is given daily for 2-3 months until healing is well established and the alkaline phosphatase concentration is approaching the reference range. Because this method requires daily treatment, success depends on compliance. [If the vitamin D dose is administered in a single day](#), it is usually divided into 4 or 6 oral doses. An intramuscular injection is also available. Vitamin D (cholecalciferol) is well stored in the body and is gradually released over many weeks. Because both calcitriol and calcidiol have short half-lives, these agents are unsuitable for treatment, and they bypass the natural physiologic controls of vitamin D synthesis. [The single-day therapy avoids problems](#) with compliance and may be helpful in differentiating nutritional rickets from familial hypophosphatemia rickets (FHR). In nutritional rickets, the phosphorus level rises in 96 hours and radiographic healing is visible in 6-7 days. Neither happens with FHR. [A study by Thacher et al sought to determine the optimal dose of calcium](#) for treatment of children with rickets. The authors reported that a daily calcium intake of 1000 mg or 2000 mg resulted in more rapid radiographic healing than 500 mg per day dosing.

However no clinical or radiographic differences were found between daily calcium supplements of 2000 mg and 1000 mg. The study also found that complete healing of nutritional rickets may take some children longer than 24 weeks. [If severe deformities have occurred](#), orthopedic correction may be required after healing. Most of the deformities correct with growth. A consultation with a pediatric endocrinologist is recommended.

### Deterrence/Prevention

Human milk contains little vitamin D and contains too little phosphorus for babies who weigh less than 1500 g. Infants weighing less than 1500 g need special supplementation (ie, vitamin D, calcium, phosphorus) if breast milk is their primary dietary source. Recommending a vitamin D supplement from the first week of life for susceptible infants who are breastfed is safe and effective and, therefore, should be considered. [The United States Institute of Medicine recommends](#) an upper level of intake of 1000 IU/d and 1500 IU/d in infants aged 0-6 months and 6-12 months, respectively. An adequate intake of 400 IU/d has been suggested for infants aged 0-12 months. The recommended daily allowance is 600 IU/d thereafter. The US Endocrine Society's Clinical Practice Guideline suggests 400-1000 IU/d may be needed for children younger than 1 year; they also recommend 600-1000 IU/d for children aged 1 year or older. Internationally, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition also suggests an oral supplement of 400 IU/d until age 1 year. [Adequate ultraviolet light or 10 mcg \(400 IU\) orally \(PO\) daily of a vitamin D preparation](#) and an adequate dietary supply of calcium and phosphorus prevent rickets. As little as 20 min/d of ultraviolet light to the face of a light-skinned baby is sufficient; however, significantly longer periods of exposure are necessary for children with increased skin pigmentation.

### Medication

#### Medication Summary

Treatment for rickets is with cholecalciferol, which may be gradually administered over several months or in a single-day dose. The single-day therapy avoids problems with compliance and may be helpful in differentiating nutritional rickets from familial hypophosphatemia rickets (FHR). In nutritional rickets, the phosphorus level rises in 96 hours and radiographic healing is visible in 6-7 days. Neither happens with FHR. [Vitamin D is well stored in the body](#) and is gradually released over many weeks. Because both calcitriol and calcidiol have short half-lives, they are unsuitable; they would bypass the natural physiologic controls of vitamin D synthesis.

### Vitamin D

#### Class Summary

Vitamin D is a fat-soluble vitamin used to prevent or treat vitamin D deficiency.

### Cholecalciferol (Vitamin D3, D drops Kids, Delta-D3)

For treatment of rickets, cholecalciferol can be given in a single-day dose of 15,000 mcg (600,000 U), which is usually divided into 4 or 6 oral doses. An intramuscular injection is also available. [An alternative regimen is to give 125-250 mcg \(5000-10,000 U\) daily](#) for 2-3 months until healing is well established and the alkaline phosphatase concentration is approaching the reference range. Because this gradual method requires daily treatment, success depends on compliance.



## INTERPRETATION

### Vitamin D Deficiency

**Also called:** Hypovitaminosis D, Low Vitamin D

#### What is vitamin D deficiency?

Vitamin D deficiency means that a person is not getting enough vitamin D to stay healthy.

#### Why does one need vitamin D and how does one get it?

Vitamin D helps your body absorb calcium. Calcium is one of the main building blocks of bone. Vitamin D also has a role in your nervous, muscle, and immune systems.

You can get vitamin D in three ways: through your skin, from your diet, and from supplements. Your body forms vitamin D naturally after exposure to sunlight. But too much sun exposure can lead to skin aging and skin cancer, so many people try to get their vitamin D from other sources.

#### How much vitamin D does one need?

The amount of vitamin D you need each day depends on your age. The recommended amounts, in international units (IU), are

- Birth to 12 months: 400 IU
- Children 1-13 years: 600 IU
- Teens 14-18 years: 600 IU
- Adults 19-70 years: 600 IU
- Adults 71 years and older: 800 IU
- Pregnant and breastfeeding women: 600 IU

People at high risk of vitamin D deficiency may need more. Check with your health care provider about how much you need.

#### What causes vitamin D deficiency?

You can become deficient in vitamin D for different reasons:

- You don't get enough vitamin D in your diet
- You don't absorb enough vitamin D from food (a malabsorption problem)
- You don't get enough exposure to sunlight.
- Your liver or kidneys cannot convert vitamin D to its active form in the body.
- You take medicines that interfere with your body's ability to convert or absorb vitamin D

#### Who is at risk of vitamin D deficiency?

Some people are at higher risk of vitamin D deficiency:

- Breastfed infants, because human milk is a poor source of vitamin D. If you are breastfeeding, give your infant a supplement of 400 IU of vitamin D every day.
- Older adults, because your skin doesn't make vitamin D when exposed to sunlight as efficiently as when you were young, and your kidneys are less able to convert vitamin D to its active form.

- People with dark skin, which has less ability to produce vitamin D from the sun.
  - People with disorders such as Crohn's disease or celiac disease who don't handle fat properly, because vitamin D needs fat to be absorbed.
  - People who have obesity, because their body fat binds to some vitamin D and prevents it from getting into the blood.
  - People who have had gastric bypass surgery
  - People with osteoporosis.
  - People with chronic kidney or liver disease.
  - People with hyperparathyroidism (too much of a hormone that controls the body's calcium level).
  - People with sarcoidosis, tuberculosis, histoplasmosis, or other granulomatous disease (disease with granulomas, collections of cells caused by chronic inflammation)
  - People with some lymphomas, a type of cancer.
  - People who take medicines that affect vitamin D metabolism, such as cholestyramine (a cholesterol drug), anti-seizure drugs, glucocorticoids, antifungal drugs, and HIV/AIDS medicines.
- Talk with your health care provider if you are at risk for vitamin D deficiency. There is a blood test which can measure how much vitamin D is in your body.

#### What problems does vitamin D deficiency cause?

Vitamin D deficiency can lead to a loss of bone density, which can contribute to osteoporosis and fractures (broken bones).

Severe vitamin D deficiency can also lead to other diseases. In children, it can cause rickets. Rickets is a rare disease that causes the bones to become soft and bend. African American infants and children are at higher risk of getting rickets. In adults, severe vitamin D deficiency leads to osteomalacia. Osteomalacia causes weak bones, bone pain, and muscle weakness.

Researchers are studying vitamin D for its possible connections to several medical conditions, including diabetes, high blood pressure, cancer, and autoimmune conditions such as multiple sclerosis. They need to do more research before they can understand the effects of vitamin D on these conditions.

#### How can one get more vitamin D?

There are a few foods that naturally have some vitamin D:

- Fatty fish such as salmon, tuna, and mackerel
- Beef liver
- Cheese
- Mushrooms
- Egg yolks

You can also get vitamin D from fortified foods. You can check the food labels to find out whether a food has vitamin D. Foods that often have added vitamin D include

- Milk
- Breakfast cereals
- Orange juice
- Other dairy products, such as yogurt
- Soy drinks

FOOD	SERVING SIZE	VITAMIN D, IU
Cod liver oil	1 Tablespoon	1360
Salmon, cooked	3 1/2 ounces	360
Mackerel, cooked	3 1/2 ounces	345
Sardines, canned in oil and drained	1 3/4 ounces	250
Tuna fish, canned in oil	3 ounces	200
Milk (skim, low fat, whole), vitamin D fortified	1 cup	98
Margarine, fortified	1 Tablespoon	60
Pudding (from mix, with vitamin D-fortified milk)	1/2 cup	50
Cereal, vitamin D fortified	3/4 to 1 cup	40
Egg	1 whole	20
Liver or beef, cooked	3 1/2 ounces	15
Cheese, Swiss	1 ounce	12

Vitamin D is in many multivitamins. There are also vitamin D supplements, both in pills and a liquid for babies.

If you have vitamin D deficiency, the treatment is with supplements. Check with your health care provider about how much you need to take, how often you need to take it, and how long you need to take it.

### Can too much vitamin D be harmful?

Getting too much vitamin D (known as vitamin D toxicity) can be harmful. Signs of toxicity include nausea, vomiting, poor appetite, constipation, weakness, and weight loss. Excess vitamin D can also damage the kidneys. Too much vitamin D also raises the level of calcium in your blood. High levels of blood calcium (hypercalcemia) can cause confusion, disorientation, and problems with heart rhythm.

Most cases of vitamin D toxicity happen when someone overuses vitamin D supplements. Excessive sun exposure doesn't cause vitamin D poisoning because the body limits the amount of this vitamin it produces.

### Why Is Vitamin D So Important for Your Health?

Vitamin D is one of the most important vitamins for our overall health, but many people in the United States, as well as worldwide, are not getting enough of this vitamin. And the only way to know if you're getting enough of this vitamin is by doing an expensive test. And every day it seems; new studies show new reasons why this vitamin is so important to our health.

Although vitamin D can be stored in your body fat until it is needed, the problem is that it's not so easy to get enough vitamin D into your body.

The main job of vitamin D is to keep the right amount of calcium and phosphorus in your blood? these are the 2 nutrients that work together to make your bones strong. If you don't have vitamin D in your body, only a small amount of the calcium from your diet can be absorbed by your body, and only a little more than half of phosphorus is absorbed. Without enough calcium and phosphorus being absorbed in your body, your bones would become brittle and break easily.

Until recently, experts believed that the main role of vitamin D was to keep our bones healthy and prevent them from breaking up. But new research has shown many other reasons why this vitamin is so important for our health.

### The Reasons Why Vitamin D Is So Important

It helps to:

- Prevent bone fractures
- Prevent falls in older people and osteoporosis
- Reduce the risk of cancer, especially colon cancer, prostate cancer, and breast cancer
- Reduce the risk of diabetes, especially in young people and in those living in high altitude
- Protect against heart disease, including high blood pressure and heart failure
- Reduce your risk for multiple sclerosis
- Improve your mood
- Improve your lung function.

### If You Don't Get Enough Vitamin D

- Your bones can become weak and can break
- Children can get "rickets," a disease that prevents their bones from growing properly, delays their growth, and causes problems with their immune system
- Adults can develop "osteomalacia," a disease that weakens the bones and makes them hurt, and causes fractures
- Older adults can get osteoporosis, which doesn't cause pain, but makes the bones thin and easy to fracture

The best way to know if you are getting enough vitamin D is to have a specific blood test. Otherwise, you may not know that you're not getting enough vitamin D until you start having symptoms associated with vitamin D deficiency. The vitamin D test is very expensive, so it's not routinely done.

### What Are the Best Sources?

The best source for vitamin D is sunlight. Only few foods supply vitamin D in significant amounts. The best source for this vitamin for humans is the sun, not supplements. The problem is that too much exposure to the sun can cause skin cancer, but too little sun exposure is the reason that many people today don't get enough vitamin D.

Because it's difficult to get all the vitamin D you need from food, and since you don't want to be exposed to too much sunlight, most people need short-time sun exposures and daily vitamin D supplements. Spending a short time in the sun each day, without sun block, may not be a bad idea, unless you have a special sensitivity to sun exposure. Check with your doctor if you're not sure.

You can get vitamin D supplements over the counter at the supermarket, a drugstore, or any health food store. Some vitamin D supplements are available only by prescription and are given to people who are vitamin D deficient.

If your skin is exposed to summer sunlight for about 10 to 15 minutes at least twice a day, you're probably getting enough vitamin D. But when you use sunscreen, it prevents the skin from soaking up enough vitamin D.

And in the winter, the sun isn't strong enough to give us enough amounts of vitamin D. People who live in northern regions might not get enough vitamin D from sunshine, even in the summer. People who live in big cities not get enough vitamin D, because pollution can block the sun's rays.

## TROUBLESHOOTING

### VITAMIN D ASSAY

#### Insight into Vitamin D Assays In Clinical Laboratory

The status of vitamin D is usually assessed by measuring the serum concentration of 25-hydroxyvitamin D (25-OH-D).

Over the recent years there has been a dramatic increase in 25-OH-D requests, prompting many laboratories to consider the use of automated immunoassays. In this article, the two major techniques that are used for measuring vitamin D will be discussed and compared (binding assay and chemical assay techniques).

#### Dramatic Increase in Vitamin D Testing

Vitamin D was first recognized as a very important component of the diet back in the late 1800s when rickets was initially described. Presently, rickets has been eradicated from most developed countries. However, it is still a very common problem in areas of the world where food is scarce.

The recent dramatic increase in vitamin D testing is primarily due to two causes. First, there has been a marked rise in vitamin D deficiency throughout the world. The second reason is that vitamin D has increasingly being used as general health marker and several diseases were linked to vitamin D deficiency.

#### Metabolism



What we commonly refer to as vitamin D actually comes in two different forms: vitamin D2 and vitamin D3. Vitamin D2 is also known as ergocalciferol, calciferol, or just vitamin D. Vitamin D3 is also known as cholecalciferol (it derives from cholesterol). There are two main ways via which vitamin D gets into the body: through the skin and through diet. In the intestine, either previtamin D or vitamin D is absorbed and trapped in the chylomicron molecules. In the skin, under the effect of UV rays of sunlight, 7-dehydrocholesterol is converted to cholecalciferol (Vitamin D3). Vitamin D from the two sources is subjected to hydroxylation in the liver to form 25-OH-D. The hydroxylated vitamin is then alpha hydroxylated in the kidney to form 1,25(OH)<sub>2</sub> vitamin D, the active form of vitamin D. 1,25 (OH)<sub>2</sub> vitamin D increases the absorption of calcium and phosphorous in the intestine. It also interacts with the parathyroid gland as feedback in the production of parathyroid hormone, therefore acting

as a regulator of new bone formation. Vitamin D is also being recognized as a very important player in the signal transduction mechanisms in several organs like the brain, prostate, breast and colon tissue, as well as the immune cells. The cells in these organs have vitamin D receptors and respond to 1, 25 (OH)<sub>2</sub> vitamin D.

In the circulation, vitamin D is transported by the vitamin D - binding protein, which belongs to the albumin and alpha-fetoprotein gene family. The concentration of vitamin D - binding protein in the plasma greatly exceeds that of 25-OH-D (9 nM versus 50 nM), with less than 5% of available binding sites being occupied.

#### Measurement of 25-OH-D

The analytical measurement of vitamin D is performed for two major reasons: to determine the nutritional status of vitamin D, and to monitor its therapeutic level. As mentioned before, there are two different types of vitamin D. To adequately monitor therapy, we need to be aware of which vitamin D entity is the one measured in the different assays. Specifically, if an immunoassay or protein-binding assay is to be used, is the antibody reacting equally with both types of vitamin D? The answer to this question is that if the intention of measuring vitamin D is to monitor vitamin D2 therapy, then the assay must measure vitamin D2.

The assays currently available in the market (US and EU) can be classified as binding assays and chemical assays. Chemiluminescence immunoassays (CLIA), ELISA, radioimmunoassay (RIA), and binding protein assay belong to the binding assays group, while chemical assays include high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The specificity and accuracy of these methods are very variable. Both RIA and CLIA are immunoassays in which the accuracy of the method will depend on the specificity of the antibody used (how well the antibody recognizes D2 and D3). The binding assays are affected by the matrix effects due to the tight binding of the vitamin D - binding protein to vitamin D.

The first automated vitamin D assay was based on Competitive-Protein Binding Assays (CPBA) for the Nicholis Advantage analyzer. It has the advantages of being inexpensive, can be performed on small sample size, and is co-specific for 25-OH-D2 and 25-OH-D3. This assay underestimated 25-OH-D at low levels and overestimated it at high levels. Immunoassay methods were first reported in the 1980s with a radioimmunoassay (RIA). This assay formed the basis for a subsequent chemiluminescent detection – based system. The Radioimmunoassay (RIA) requires a small sample size and the incorporation of Iodine – 125 as a tracer. It is not subjected to nonspecific interference, and in addition to being rapid it is inexpensive and accurate. Nevertheless, it still requires the use of radionuclides, and some RIA assays discriminate between 25-OH-D2 and 25-OH-D3.

Chemical assays have been originally more technically involved but are also now able to accommodate a large number of tests per day. Chemical methods (HPLC and LC-MS/MS) can report vitamin D2 and D3 independently. Ultraviolet quantitation following HPLC is a very stable, repeatable assay, and provides separate quantitation of 25-OH-D2 and 25-OH-D3. Nevertheless, it requires a larger sample size, needs a preparation step before chromatography and sometimes is subject to interferences with other compounds measured in the ultraviolet spectrum. Also, this assay requires a high level of technical expertise.

LC-MS/MS has been referred to as a "Gold Standard" technique for 25-OH-D3, although results can also be erroneous. This technique requires the skills of an experienced analyst. Another caveat with LC-MS/MS is the presence of the 25-OH-D2 and C3 epimers of vitamin D3 in pediatric specimens. If the assay is not optimised, vitamin D2/D3 result may be higher than expected in the pediatric population due to this epimer. Publications have shown that the C3 epimer may be present in adults as well.

#### Standardization and External Quality Control Assessment:

With the availability of many vitamin D assays, differences in the reported 25-OH-D values for the same samples were observed among different assays.

These differences could impact the classification of patients' vitamin D status and therefore affect the clinical management of some patients. The question is if it is appropriate to have a clinical decision limit without assay standardization. To address this issue, the Vitamin D Standardization Program (VDSP), an initiative of the National Institutes of Health Office of Dietary Supplements (NIH ODS), was launched in 2010 in collaboration with the National Institutes of Health, the Center for Disease Control and Prevention (CDC), the National Institute for Standards and Technology (NIST), and Ghent University in Belgium.

Following, the CDC has introduced a Vitamin D Standardization-Certification Program to ensure reliable clinical measurement of vitamin D. It was recommended that all assay manufacturers should participate in the CDC's Standardization-Certification Program. This is especially important for the in-house reference method of the manufacturers and for the assay measurement systems as they are being developed. The primary steps to standardization are as follows (fig. 1):

- (1) develop a reference system,
- (2) establish metrological traceability and
- (3) verify "end-user" test performance.

When participants pass four consecutive surveys, they are awarded a certification for one year, which can be renewed annually.

#### Controversies Regarding Vitamin D Testing

**Past:** Over the past decade, a big number of studies linking low vitamin D levels to cancer, heart disease, diabetes, and other diseases have led many doctors to routinely test vitamin D levels for their healthy patients. Consequently, laboratory professionals are confronted with the challenge of helping clinicians navigate the complexities of vitamin D assays.

The current evidence suggest that the main beneficial effects of vitamin D supplementation relate to musculoskeletal, rather than extraskeletal. Moreover, the exponential increase in vitamin D testing and supplements used in the past few years, have raised justifiable concerns if many vitamin D measurements are being undertaken without evidence-supported indications and if many individuals are being supplemented with little evidence of the benefit.

**Present:** In response to these concerns in 2013, the Royal College of Pathologists of Australasia (RCPA) published a position statement to clarify the role of vitamin D testing in the context of vitamin D deficiency, with guidelines about who should be tested and when repeat testing should be performed. Also, the U.S. Preventive Services Task Force (USPSTF) published a new recommendation in November 2014, which stated that there is no practical reason for most people to get a vitamin D test. Testing for vitamin D might be indicated in patients with osteoporosis or other bone-related problems, conditions that affect absorption of fat, including celiac disease or weight-loss surgery or who are taking medications that interfere with vitamin D activity, including anticonvulsants and glucocorticoids.

## BOUQUET

### Brain Teasers

1. Deficiency of which vitamin causes Rickets in Children.
  - A. Vitamin A
  - B. Vitamin C
  - C. Vitamin E
  - D. Vitamin D
2. What is the daily recommended allowance for a person 50 years of age
  - A. 200 IU
  - B. 400 IU
  - C. 600 IU
  - D. 1200 IU
3. Of the following which is the best source of Vitamin D
  - A. Cod Liver Oil
  - B. Mango
  - C. Egg
  - D. Cheese
4. Vitamin D toxicity causes \_\_\_\_
  - A. Hyperglycemia
  - B. Hypercalcemia
  - C. Hypercholesterolemia
  - D. Hypercupremia
5. Which of the following natural activities is going to help prevent vitamin D deficiency?
  - A. Walking
  - B. Bathing
  - C. Sunbathing
  - D. Running

ANSWER: 1.D, 2.C, 3.A, 4.B, 5.C



**BOUQUET****Wisdom Whispers**

Don't cry over  
the past, it's  
gone. Don't  
stress about the  
future, it  
hasn't arrived.  
Live in the  
present and make  
it beautiful.

DO NOT TRY TO FIX  
WHATEVER COMES  
IN YOUR LIFE.  
FIX YOURSELF IN  
SUCH A WAY THAT  
WHATEVER COMES,  
YOU WILL BE FINE.

A  
meaningful  
SILENCE  
is always  
better than a  
meaningless  
WORDS.

DON'T JUDGE  
EACH DAY BY THE  
HARVEST YOU REAP  
BUT BY THE SEEDS  
THAT YOU PLANT.

**In Lighter Vein**

**Santa**-Oye!what R U doing?

**Banta**-Recording this babys voice.

**Santa**-Why?

**Banta**- When he grows up,

I shall ask him what he meant by this..

Height of addiction:

In a college form,

when asked about the permanent

address a student wrote

[www.facebook.com](http://www.facebook.com).



Two friends visit a Stadium.

**First**: Why are all these  
people running?

**Second**: This is a race,  
the winner will get the cup.

**First**: If only winner will  
get the cup, why are others running?



Husband came home drunk. To avoid  
wife's scolding, he took a laptop

& started working.

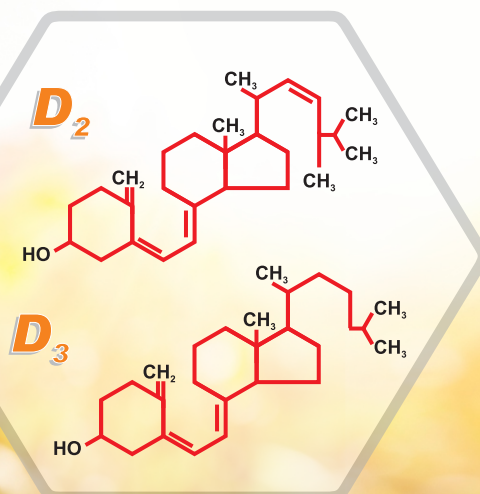
Wife: did u drink

Husband : no

Wife: Idiot then y u r typing  
on suitcase



# Qualisa 25-OH Vitamin-D



**Tulip Introduces** ▶ **India's First indigenised  
25-OH Vitamin - D kit based on  
Sandwich Assay Technology**

## Short Assay Procedure

Total assay procedure of 40 mins.

## User Friendly Protocol

Unique packaging of microwells 3x8=24 microwells for better stability.  
Ready to use Calibrators, Controls, Conjugate and Substrate.

## Detects both Vitamin D<sub>2</sub> and D<sub>3</sub>

Qualisa 25-OH Vitamin-D uses monoclonal antibodies to detect both 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub>.

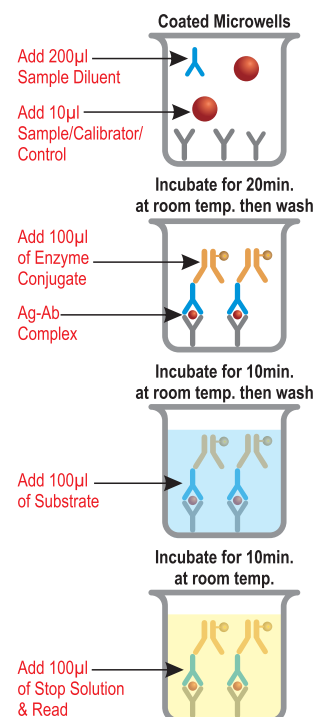
## No interference of Biotin (Vitamin B7)

Sandwich Assay does not use Biotin, hence eliminates possibilities of interference of Biotin (Vitamin B7)

## Correlation with LC-MS/MS

Qualisa 25-OH Vitamin-D demonstrated excellent correlation with Gold Standard LC-MS/MS results.

## Test Procedure



**Innovative Technology backed up with Tulip's Service**

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Fax: (0832) 2458544. E-mail: sales@tulipgroup.com Website: www.tulipgroup.com

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