California Association for Medical Laboratory Technology

Vitamin D

Course # DL-999

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**COURSE NAME: VITAMIN D**

**COURSE # DL-999**

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VITAMIN D

OBJECTIVES:
Upon completion of this course the participant will be able to:
• Outline the role of vitamin D in calcium homeostasis and its effects in the body.
• Discuss the finding that the role of vitamin D insufficiency is much more widespread than previously thought.
• Discuss the factors that affect activation of vitamin D in the skin by sunlight.
• Outline the diseases vitamin D has been found to ameliorate
• List the current dosing recommendation for vitamin D
• Discuss the status of measuring vitamin D level
• List several dietary sources of vitamin D

ABSTRACT:
Over the last decade, interest in vitamin D has exploded. This vitamin, previously known primarily for its importance in preventing the bone conditions of rickets in children and osteomalacia, the softening of the bones, in adults, has recently been recognized as having a substantially greater role in health. Vitamin D is now known to affect a variety of physiologic systems and increased intake has been linked to a number of favorable health outcomes and even to a reduction in overall mortality.

25-hydroxyvitamin D (25-OHD), vitamin D, determination is of diagnostic importance for the investigation of vitamin D deficiency and much more rarely, intoxication. Despite the name, vitamin D is a pre-hormone, being endogenously synthesized provided there is adequate sunlight. Its biological function, exerted through the active form 1,25 dihydroxyvitamin D₃ (1,25-(OH)₂D) is to maintain calcium and phosphate levels in the blood. In addition, vitamin D has important roles in immune regulation. This presentation will examine the role of vitamin D in calcium homeostasis, discuss recently proposed effects on other diseases and conditions, provide insight into current dosing, discuss vitamin D insufficiency, and describe the measuring of vitamin D.

VITAMIN D
Vitamin D is a fat-soluble compound that is required for normal calcium metabolism. While vitamin D is essential for human health, it is not obtained primarily from the diet and therefore, is not technically a vitamin. In actuality vitamin D is a hormone. Further, its biologic actions occur after it is metabolized to 1,25-dihydroxyvitamin D. This active form of vitamin D is a steroid hormone structurally related to estradiol, cortisol, and aldosterone. (1)

The major form of vitamin D circulating in the body is 25-hydroxyvitamin D [25-(OH)D]. It has a half-life of about 2 weeks, which makes its serum concentration a good biomarker of vitamin D status. The recommended minimum level of 25-(OH)D is under debate, but experts have proposed that levels of 30 ng/mL (75 nmol/L) or higher may be optimal. (Table I) This level is linked with a decreased risk for bone fractures.

Vitamin D deficiency appears to affect all age and demographic groups in the U.S. In a recent national survey, the average 25-(OH)D level for both men and women of various ages was similar at 24 ng/mL. In the U.S., about 36% of healthy young adults have levels that are less than sufficient. However, vitamin D deficiency does vary with age, ethnicity (skin color), and geographic location. (2) Among almost 700 men residing in Boston, most (83%) had insufficient vitamin D levels and one third were deficient; virtually all Indian born and Chinese-born men
had low vitamin D levels. (3) Among young women in Texas, white women had levels near sufficient, while Hispanic and black women had deficient levels. The lowest levels were in black women. In a group of older adults (> 55 years) living in Missouri, African Americans had levels lower than whites but the levels for both races were insufficient. (4) Arab-American women in Michigan had deficient levels, with the lowest in those who wore veils and did not take vitamin D supplements. (5)

**HISTORY OF RICKETS AND VITAMIN D**

Rickets has been known since ancient times. Writings from the first and second centuries by Soranus, a Roman physician, and Galen, a Greek physician, described conditions with bony deformities in infants. Although these descriptions can be interpreted as evidence for rickets, it was not until the 17th century that the first clear descriptions were made by Dr. Daniel Whisler in 1645 and then by Professor Francis Glisson in 1650. In the late 1700s rickets became widespread in Europe as people stayed indoors and lived in polluted cities with reduced sunlight. In 1865 Trousseau recommended cod liver oil for treating rickets. He also noted the importance of sunlight.

Vitamin D became classified as a vitamin through a historical accident: In 1921 Sir Edward Mellanby, after experiments with dogs raised indoors and given a restricted diet, wrote, “The action of fats in rickets is due to a vitamin or accessory food factor which they contain, probably identical with the fat-soluble vitamin.” He established that cod liver oil was an antirachitic agent.

In 1923 Goldblatt and Soames established that when a precursor to vitamin D in the skin was exposed to sunlight, a substance equivalent to the fat-soluble vitamin was produced. Laboratories in Germany and England elucidated the chemical structure of the D vitamins in the early 1930s. Vitamin D$_3$ was not characterized until 1936 when it was shown to result from the ultraviolet irradiation of 7-dehydrocholesterol in the skin. About the same time the antirachitic substance in cod liver oil was shown to be identical to vitamin D$_3$.

In the 1930s the Public Health’s recommendation of fortifying milk with vitamin D and giving cod liver oil as a nutritional supplement led to near eradication of rickets in the United Stated and other industrialized nations. Now rickets has made a comeback and also remains common in less well developed countries. (6)

**ACTIVATION OF VITAMIN D**

The formation of vitamin D in the body starts with the conversion, by ultraviolet B (UVB) light, of 7-dehydrocholesterol, which is present in the skin, to vitamin D$_3$ (cholecalciferol). This is followed by hydroxylation in the liver to create 25-hydroxyvitamin D$_3$ (25-(OH)D$_3$) and then a second hydroxylation, primarily by the kidneys, to form 1,25 dihydroxyvitamin D$_3$ (1,25-(OH)$_2$D$_3$), also known as calcitriol, which is the physiologically active form of vitamin D$_3$.

There are two main forms of vitamin D: vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol). Vitamin D$_2$ is obtained by consumption of plant foods and yeasts, while D$_3$ is produced in the skin by UVB radiation or obtained from animal foods such as fatty fish. (Table 2) The 25-(OH)D serum concentration reflects vitamin D produced in the skin as well as that obtained from food and supplements; both 25-(OH)D$_2$ and 25-(OH)D$_3$ are measured as part of the 25-(OH)D serum concentration. Hydroxylated vitamin D (25-(OH)D) is stored in body fat until needed. Similar to D$_3$, D$_2$ is hydroxylated in the liver to form 25-(OH)D$_2$ and then in the kidneys to form active 1,25-(OH)$_2$D$_2$.
Since adequate amounts of vitamin D cannot be obtained from the diet, throughout the history of the species humans have had to get vitamin D from UVB’s activating 7-deoxycholesterol in the skin. The earliest members of *Homo sapiens* evolved in Africa and had darkly pigmented skin adapted to the conditions of UV radiation that existed near the equator. As humans migrated north out of the tropics they encountered environments in which they got less UV radiation during the year, especially during winter-time. The dark pigmentation was detrimental to the production of adequate amounts of vitamin D. Dark skin contains so much melanin, which acts like a natural sun-screen, that very little UV radiation, and specifically very little of the shorter-wavelength UVB radiation, can penetrate the skin. The solution, over evolutionary time, has been for migrants to northern latitudes to lose skin pigmentation.

Another effect of UVB is to destroy the nutrient folate. Anthropologist Dr. Nina Jablonski (7) states, “Throughout the world, human skin color has evolved to be dark enough to prevent sunlight from destroying folate but light enough to foster the production of vitamin D. Recent epidemiological and physiological evidence suggests that the worldwide pattern of human skin color is the product of natural selection acting to regulate the effects of the sun’s ultraviolet radiation on key nutrients crucial to reproduction.” In farther northern latitudes most UVB is absorbed by the atmosphere for most of the year, only reaching the earth during summer. Thus, rickets was prevalent in the northern populations until the problem was recognized and dietary supplementation, such as codfish oil became available. Now pills can supply adequate amounts of vitamin D.

**MECHANISM OF ACTION OF VITAMIN D**

Most of the actions of vitamin D are mediated through a nuclear transcription factor, vitamin D receptor (VDR). When 1,25-dihydroxyvitamin D enters the nucleus of a cell, it associates with the VDR and then complexes with retinoic acid X receptor (RXR). The entire complex starts molecular interactions that modulate the transcription of specific genes. More than fifty genes in tissues in the body are known to be regulated by 1,25-dihydroxyvitamin D.

In small intestinal epithelial cells 1,25-(OH)\(_2\)D upregulates expression of a number of genes that stimulate transepithelial calcium transport from the intestinal lumen into the blood. In the bone 1,25-(OH)\(_2\)D stimulates terminal differentiation of osteoclast precursors to osteoblasts. 1,25-(OH)\(_2\)D also stimulates osteoblasts to influence osteoclasts to mobilize bone calcium. 1,25-(OH)\(_2\)D plays an important role in mineralization of bone, since abnormal bone results when vitamin D is deficient or its metabolism is defective.

The conversion of 25-(OH)D to active 1,25-(OH)\(_2\)D is tightly regulated by the body, primarily by the conversion step in the kidneys. The parathyroid hormone (PTH) and serum calcium and phosphorus levels are the major regulators of 1,25-(OH)\(_2\)D production in the kidneys. The parathyroid glands sense serum calcium levels and secrete PTH if calcium levels drop too low. This increases production of 1,25-(OH)\(_2\)D, which results in

- Increasing intestinal absorption of dietary calcium
- Increasing the reabsorption of calcium filtered by the kidneys
- Mobilizing calcium from bone when serum calcium level is below normal

Elevated PTH is a marker for vitamin D deficiency. Overproduction of 1,25-(OH)\(_2\)D is inhibited by a negative feedback loop: 1,25-(OH)\(_2\)D inhibits PTH release and the CYP (cytochrome) 27B1 enzyme that forms 1,25(OH)\(_2\)D, in addition to activating the CYP enzyme that metabolizes it.

Natural vitamin D levels in humans exposed daily to hours of intense sunlight (e.g., lifeguards) are 50-125 ng/mL. Continued UVB exposure does not result in excessive vitamin D formation. Vitamin D is sensitive to UV radiation and heat; sustained UVB exposure causes its
photodegradation in the skin to an inactive product. No cases have ever been reported of vitamin D intoxication from sun exposure.

**VITAMIN D REQUIREMENTS (see Table III)**

The recommended adequate intake (AI) of vitamin D as established by the Food and Nutrition Board (FNB) of the Institute of Medicine in 1997 was 200 IU for newborn to age 50. This dose is a daily intake, obtained from food and/or supplements, that is minimally sufficient to maintain bone health and normal calcium metabolism in healthy people. Since that time, a considerable amount of new information on vitamin D has become available. In 2008, the FNB established an expert panel to review current information and to revise the AI for vitamin D. The Institute of Medicine issued new recommendations in September 2010. (See Table III)

Many clinicians and researchers consider the current vitamin D normal range is too low for optimal health due to the focus only on vitamin D’s action on calcium and bone. In 2008 both the National Osteoporosis Foundation (NOF) and the American Academy of Pediatrics recommended intakes for vitamin D that are greater than the current AIs. (8,9)

The NOF recommends that adults under 50 years of age consume 400-800 IU/day. (Table III). Other clinicians believe that daily doses will need to be even higher (in the absence of sun exposure) to achieve the desired vitamin D levels linked with health benefits beyond bone health. It has been suggested that to achieve sufficiency levels for 97% of U.S. residents who are at risk for bone loss, the required minimum daily intake of vitamin D₃ would be 2600 IU/day (in the absence of significant sun exposure) or intake from diet and supplements of about 1000 IU/day for every 33 lb (15 kg) of body weight. The presence of obesity, increased vitamin D destruction, or serious illness such as cancer, heart disease, or diabetes may increase the required intake. Further, these are the doses needed for maintenance of adequate serum concentrations. For individuals with vitamin D deficiency or insufficiency (Table 1), a “loading dose” to correct the serum concentration is the first step. The typical loading dose is 50,000 IU of oral vitamin D given weekly for 8 weeks or twice weekly for 5 weeks. It has been suggested that all individuals at risk of vitamin D deficiency have a serum 25-(OH)D level measured twice yearly to guide vitamin D dosing. Prescription vitamin D analogues and active vitamin D (calcitriol) are available for people with fat malabsorption or an inability to produce active vitamin D in the kidney (chronic renal failure).

Vitamin D₂ and D₃ have been regarded as equally effective based on their ability to prevent rickets. However, new evidence shows they are metabolized differently and that D₃ may be more effective at increasing and maintaining vitamin D serum levels, as well as binding to the vitamin D receptor (VDR). Most vitamin manufacturers have replaced ergocalciferol (D₂) with cholecalciferol (D₃) in their products.

**VITAMIN D DEFICIENCY**

**Risk Factors for Deficiency** (1)

- “Exclusively breast-fed infants: Infants who are exclusively breast-fed and do not receive vitamin D supplementation are at high risk of deficiency, particularly if they have dark skin and/or receive little sun exposure. Human milk generally provides 25 IU of vitamin D per liter, which is not enough for an infant if it is the sole source.
- Dark skin: People with dark-colored skin synthesize less vitamin D on exposure to sunlight than those with light colored skin, particularly if they live far from the equator.
• Aging: The elderly have reduced capacity to synthesize vitamin D in skin when exposed to UVB, and the elderly are more likely to stay indoors or use sunscreen. Institutionalized adults who are not supplemented are at extremely high risk of deficiency.
• Covering all exposed skin or using sunscreen whenever outside: Osteomalacia has been documented in women who cover all of their skin whenever they are outside for religious or cultural reasons. The application of sunscreen with an SPF greater than 8 reduces production of vitamin D by 95%.
• Fat malabsorption syndromes: Cystic fibrosis and cholestatic liver disease impair the absorption of dietary vitamin D.
• Inflammatory bowel disease: People with inflammatory bowel disease like Crohn’s disease appear to be at increased risk of deficiency, especially those who have had small bowel resections.
• Obesity: Obesity increases the risk of deficiency—once vitamin D is synthesized in the skin or ingested, it is deposited in body fat stores, making it less bioavailable to people with large stores of body fat.”

Severe deficiency
Rickets: In infants and children severe deficiency results in the failure of bone to mineralize. In the absence of adequate mineralization, weight-bearing arms and legs become bowed. In infants there may be delayed closure of the fontanels in the skull. The rib cage may be deformed due to the pulling action of the diaphragm. Severe hypocalcemia may cause seizures. Since UVB activates 7 dehydrocholesterol in the skin to vitamin D$_3$, when UVB is decreased in the higher latitudes, inadequate amounts of vitamin D$_3$ are produced, resulting in inadequate mineralization of bones. Now fortification of foods and taking of vitamin D pills has led to significant reduction in rickets. However, complacency about vitamin D deficiency has led to continued nutritional rickets in cities throughout the world.

Osteomalacia (soft bones) in adults: Although bones are no longer growing in adults, there is a constant state of turnover. In adults with severe vitamin D deficiency bone mineral is progressively lost, resulting in bone pain and osteomalacia.

Symptoms of Deficiency
The symptoms of vitamin D deficiency in adults are bone discomfort (in the low back and lower extremities), myalgia (muscle aches), and weakness. These symptoms may be misdiagnosed as arthritis, fibromyalgia, or chronic fatigue syndrome. Due to muscle weakness, there is an increased risk of falls. Vitamin D deficiency can also exacerbate or cause osteoporosis. Myalgia is also a common adverse effect experienced by individuals taking statin medications to lower cholesterol. Some evidence suggests that individuals with low vitamin D levels may be more likely to experience myalgia, and that increasing vitamin D levels may resolve statin induced myalgia in a large percentage of patients.

Toxicity
Vitamin D toxicity is rare. Too much sun exposure does not cause toxicity because the body regulates the amount of vitamin D produced; even fortified foods do not contain large amounts. Short-term use of even very large doses of vitamin D (e.g., 50,000 IU/week for 8 weeks) does not cause toxicity. Toxicity is unlikely in adults unless more than 10,000 IU/day is taken for many months or years. Vitamin D toxicity results in hypercalcemia. Increased serum calcium levels can cause nausea and vomiting, constipation, mental status changes, and heart
arrhythmias; deposition of calcium in the kidneys and soft tissues may also occur. A 25-(OH)D level consistently above 200 ng/mL (> 500 nmol/L) is considered potentially toxic.

**Effects of Vitamin D in the Body**

A growing volume of research shows that vitamin D has diverse effects throughout the body. As mentioned previously, researchers have discovered that the vitamin D receptor (VDR) is found in numerous tissues. Once activated, this receptor can work on DNA to produce a wide variety of effects. A summary of some of the most intriguing findings about the emerging roles for vitamin D follows.

**Bone Fracture**

Osteoporosis-related fractures are a major health risk for the elderly, particularly older women, and vitamin D may reduce this risk. A recent analysis of 9 clinical trials found that vitamin D, in doses greater than 400 IU daily, reduced the risk of nonvertebral and hip fractures by 20% and 18%, respectively, in adults aged 65 or older. These results were independent of calcium supplementation. Lower doses did not appear to provide the same benefit. An earlier analysis of 13 trials found that overall vitamin D in doses of 300-800 IU/day did not significantly reduce fractures. However, high-dose vitamin D (700-800 IU/day) reduced the risk of both nonvertebral and hip fractures, particularly in institutionalized elders, who may have greater compliance with daily vitamin D intake. Not surprisingly, a substantial body of evidence indicates that the combination of vitamin D and calcium may provide a greater benefit in reducing bone fractures than vitamin D (400-800 IU daily) alone. Overall, the current body of evidence suggests the combination of vitamin D, in doses greater than 400 IU daily, with calcium (at least 1000 mg daily) is a reasonable regimen for elders interested in reducing the risk of fractures.

**Muscle Strength and Falls**

With the aging population of the U.S., the impact of falls and injuries including bone fracture on health care utilization is becoming increasingly important. Each year, about one-third of people who are 65 or older experience one or more falls. About 10% of these lead to emergency room visits and more than 5% are linked with at least one fracture. Vitamin D is an emerging therapy for fall prevention. The positive effect of vitamin D on muscle strength appears to be mediated by the vitamin D receptor (VDR) present in skeletal muscle tissue. Vitamin D supplementation has been noted to improve muscle strength, function, and balance.

A number of clinical trials have shown that vitamin D supplementation reduces the risk of falls in older adults, while others have found no benefit. Differences in study design may have contributed to the inconsistent results. For example, the definition of a fall was not consistent across studies, and some studies may not have recorded all falls. In addition, the dose of vitamin D appears to be an important variable. The strongest evidence supporting vitamin D supplementation for fall prevention comes from studies using at least 700 IU per day. A recent meta-analysis of 8 randomized, controlled trials showed about a 20% reduction in the risk of fall with doses of 700 to 1000 IU daily; little or no effect was seen with daily doses of 400 IU or less. The analysis suggested that supplementation must result in 25-(OH)D levels of at least 24 ng/ ml to prevent falls. In addition, vitamin D₃ appeared to provide a greater benefit than vitamin D₂. The dosing regimen also appears to have an important effect on falls. In a recent study using once yearly administration of a very high vitamin D dose (500,000 IU), an increase in risk of falls was observed in the first 3 months after administration. Daily, weekly or monthly dosing regimens may be more likely to be beneficial. Preliminary evidence suggests the benefit of
vitamin D supplementation for fall prevention may be more pronounced in older, than in healthier individuals. For example, one study in ambulatory elders who were younger and healthier, on average, than the populations in previous studies did not find a significant reduction in fall risk with a dose of 800 IU of vitamin D. (10)

Although further study is needed, the evidence to date suggests that higher vitamin D doses reduce the risk of falls in elders. Cholecalciferol (vitamin D₃), in doses of at least 700 IU daily, is a reasonable recommendation for older individuals interested in taking a vitamin D supplement to help prevent falls.

**Cancer**

The association between cancer and sunlight exposure has been investigated by medical researchers for almost 75 years. Early investigators noted that patients with skin cancers attributed to sun exposure had a reduced incidence of non-skin cancers. Studies have looked at the relationship between latitude, a surrogate measure for sun exposure and vitamin D levels, and the risk of cancers. The strongest data to date come from epidemiological and observational studies. While these studies cannot establish a causal relationship between low vitamin D levels and cancers, they have identified an association. Several approaches have been taken to look at vitamin D status, including the evaluation of dietary intake, sun exposure, and serum levels of both 25-(OH)D and 1,25-(OH)₂D. Colorectal cancer and breast cancer are the most well-studied. Although the evidence to date is mixed, there is sufficient positive evidence linking higher 25-(OH)D levels to lower cancer risk to warrant further investigation. (11)

**Colorectal Cancer**

Over 30 observational studies of colon cancer or pre-cancerous polyps and vitamin D have been published. The majority found a statistically significant link between increasing vitamin D status and reduction in cancer risk. A recent meta-analysis of studies looking at colorectal cancer risk and 25-(OH)D levels over time (rather than single measurements) also supports an inverse relationship. One large clinical trial, the Women’s Health Initiative, did not show any correlation between taking a vitamin D supplement and the incidence of colon cancer. However, the dose of vitamin D used in this study was only 400 IU, which is now generally believed to be inadequate to produce a protective effect.

**Breast Cancer**

Numerous studies have shown a link between higher levels of sun exposure and a lower incidence of breast cancer. In contrast, 6 observational studies did not find a link between breast cancer risk and vitamin D intake from food and supplements. Some evidence suggests the preventive effects of vitamin D may be limited to specific groups (e.g., premenopausal women) or tumor types (e.g., estrogen receptor positive), although further study is needed. The results of studies evaluating vitamin D blood levels have been inconsistent. Thus, while some evidence suggests higher vitamin D status is linked with lower breast cancer risk, the relationship remains unclear.

**Prostate Cancer**

Observational studies identified a correlation between higher sunlight levels and lower prostate cancer (PC) mortality, leading to the hypothesis that vitamin D affects the risk of developing this cancer. However, analysis of 4 studies evaluating sunlight exposure and PC risk found no relationship. Similarly, the analysis of 10 studies of serum vitamin D levels and PC
incidence found no decrease in incidence with increased 25-(OH)D levels. The strongest evidence to date does not support a link between vitamin D and reduced PC risk.

Other Cancers

Associations between increased vitamin D and decreased risk for some other cancers including pancreatic, other GI tract cancers, and some hematologic cancers have also been reported.

Potential Mechanisms

How might vitamin D affect cancer growth? The bottom line is that there is no clear consensus, and the effects may vary by tumor type. Several potential mechanisms have been identified. Most of the tissues of the body contain the vitamin D receptor (VDR), and vitamin D affects cell growth and proliferation by activation of this receptor.

Vitamin D promotes cell differentiation, modulates cell growth, and induces apoptosis (programmed death) in cancer cells. Vitamin D also has an increasingly recognized role in immune modulation, which can impact the body’s ability to respond to cancer. Its role in hormonal and cellular signaling is also beginning to be recognized. Vitamin D has an anti-angiogenic effect on tumors, blocking the growth of new blood vessels needed to supply growing tumors. Many types of tumor cells express the VDR, including melanoma and breast cancers. Many healthy tissues, including colon, breast and prostate, tissue, contain an enzyme that activates circulating pre-vitamin D, which allows local tissue levels of active vitamin D to be higher than circulating levels.

Cardiovascular Disease

The body of evidence supporting the role of vitamin D in cardiovascular risk reduction is also increasing. Most studies to date have been epidemiological and observational, but unlike the evidence for cancer, a number of clinical trials have also been published. While the observational evidence has not been uniformly positive, overall it suggests an inverse relationship between vitamin D status and the incidence of cardiovascular disease (CVD). For example, an analysis of 3 studies evaluating 25-(OH)D levels and hypertension incidence found a statistically significant association between low levels and new onset hypertension after 7 to 8 years. Nine observational studies have investigated the relationship between 25-(OH)D levels and new onset CVD (e.g., heart attack, cardiovascular-related death, stroke). Five of these studies, or slightly more than half, found an inverse relationship between the two.

Type 2 Diabetes

Pancreatic β cells have vitamin D receptors, and pancreatic tissue contains the enzyme needed to convert 25-(OH)D to 1, 25-(OH)₂D. Vitamin D has been theorized to play a role in insulin secretion, insulin sensitivity, and inflammation. All these effects potentially influence the development and control of type 2 diabetes mellitus (DM).

The observational evidence for a link between low vitamin D status and the risk of type 2 DM is mixed. Among men, 2 of 4 longitudinal observational studies identified a link between higher vitamin D levels and lower risk of new-onset type 2 DM. Among women, only 1 of 4 studies found a similar link. The randomized clinical trials (RCTs) to date have shown some interesting results. Three trials showed no effect of vitamin D on fasting glucose levels among participants who had normal fasting glucose levels when the trials began. In 2 short-term trials of participants with type 2 DM, vitamin D supplementation had no effect on measures of glucose control. A more recent trial in women with vitamin D deficiency and insulin resistance showed
improvement in insulin resistance with supplementation. Although the evidence to date is intriguing, further study is needed to establish a role for vitamin D in type 2 DM.

Autoimmune Diseases

Vitamin D is known to play a role in both the innate and the acquired immune systems. Low vitamin D levels have been linked with an increased risk of autoimmune diseases such as type 1 diabetes, multiple sclerosis, and rheumatoid arthritis. Vitamin D receptors are present in cells involved in these diseases, including macrophages, chondrocytes, pancreatic β cells, and synoviocytes. In addition, the enzyme for converting 25-(OH)D to 1,25-(OH)₂D is present in macrophages and dendritic cells. The mechanisms underlying the link between vitamin D and reduced autoimmune disease risk are largely unknown, but may be due to vitamin D’s immunomodulatory effects.

Type 1 Diabetes Mellitus

Animal studies suggest that the immunomodulatory effects of Vitamin D decrease autoimmune destruction of pancreatic β-cells and increase insulin secretion and glucose tolerance. Studies investigating vitamin D supplements for the prevention of type 1 DM have been largely observational. In a recent meta-analysis of observational studies, vitamin D supplementation in children and infants was associated with a 29% reduction in the risk of type 1 DM. It appeared to be a dose dependent effect, with higher doses resulting in greater risk reduction. This preliminary evidence suggests an important role for vitamin D supplementation in type 1 DM; however, clinical trials are needed to establish a benefit.

Multiple Sclerosis

Some of the strongest evidence for a link between multiple sclerosis (MS) risk and serum 25-(OH)D levels comes from a large observational study of over 7 million U.S. military personnel. Cases of MS were identified along with 25-(OH)D levels. Compared with matched controls, white participants with higher vitamin D levels had a significantly lower risk of developing MS. This relationship was not significant in blacks and Hispanics. Based on the results, Ascherio, Munger, and Simon, authors of a recent review, estimated that 70% of MS cases in the U.S. and Europe could be prevented by increasing vitamin D serum levels in adolescents and young adults to a level greater than 40 ng/mL. Studies of the effect of vitamin D on preventing progression or prolonging remission in MS patients have been small and vary in quality. One study designed to examine the tolerability of oral calcitriol (target dose: 2.5 mcg/d) noted a reduction in the exacerbation rate during the 48 week supplementation period compared with the pre-treatment rate. Further study of vitamin D supplementation for both preventing and slowing the progression of MS are needed to clarify its role. (12)

Rheumatoid Arthritis

The evidence supporting a role for vitamin D in rheumatoid arthritis (RA) is developing, but remains relatively scarce. One observational study of older women suggested a link between higher vitamin D intake and a lower risk of developing RA. However, another observational study found no link between vitamin D levels and the onset of RA. In patients with established RA, the relationship between higher vitamin D serum levels and disease activity is also unclear. The impact of vitamin D in studies of RA is often confounded by other RA treatments that could affect disease activity, and by the overlap between RA and other conditions where vitamin D has known or likely effects (e.g., bone fractures and falls). Until a clear benefit for individuals with
RA is established, the most reasonable recommendation is to correct vitamin D deficiency in individuals at high risk for RA, fractures or falls, such as postmenopausal women. (13)

**Infectious Diseases**

Early research showing an antimicrobial effect of vitamin D in cells infected with *M. tuberculosis* led to studies investigating the role of vitamin D in various infectious diseases. Studies have largely been observational, but 2 recent RCTs are of interest. The first examined the effects of vitamin D supplementation in an African population with tuberculosis, and failed to show any benefit. However, the dosing regimen may not have been optimal. The other trial compared the incidence of influenza A among school-aged Japanese children taking vitamin D 1200 IU daily with those who were taking placebo. The results suggested that vitamin D₃ supplementation in winter may reduce the incidence of influenza A. (14) Additional clinical trials are needed to confirm a role for vitamin D supplementation in these or any other infections.

**Measuring Vitamin D status**

The serum concentration of 25-hydroxy-vitamin D is typically used to determine vitamin D status. It reflects vitamin D produced in the skin as well as that acquired from the diet, and has a fairly long circulating half-life of 15-21 days. It does not, however, reveal the amount of vitamin D stored in other body tissues. The level of serum 1,25-dihydroxy-vitamin D is not usually used to determine vitamin D status because it has a short half-life of 15 hours and is tightly regulated by parathyroid hormone, calcium, and phosphate, such that it does not decrease significantly until vitamin D deficiency is already well advanced. Although cholecalciferol and 1,25-(OH)₂D can be measured in the circulation, the best estimates of vitamin D status are provided by measurement of 25-(OH)D₃.

Definitive methods employ GC coupled with mass spectrometry detection. Recently a candidate reference method using LC-tandem mass spectrometry was published. While these reference methods are suitable for validating the recovery and accuracy of routine methods, their complexity and derivatisation requirements mitigate against regular use. Vitamin D has also been measured by High Pressure Liquid Chromatography (HPLC); Immunoassay, both RIA and Chemiluminescent. These include the Diasorin RIA; the Nichols Advantage; the IDS Gamma B and the Diasorin Liaison.

Serum is the preferred specimen although plasma (EDTA and Li-heparin) samples are satisfactory. Vitamin D analytes have been shown to be stable for up to 2 weeks at 30°C and to be unaffected by up to 4 freeze-thaw cycles. Storage of frozen serum samples at −20°C for up to one year has also been reported to cause no loss in vitamin D metabolites. However, it seems that the measurement of 25-(OH)D by immunoassay will remain the method of choice for reasons of convenience, speed, turnaround and cost. Beginning in July 2009 a standard reference material became available which allowed laboratories to standardize their procedures.
Dietary sources of Vitamin D

Listed below are a few sources of vitamin D and the amounts of vitamin D contained:

- Fatty fish species, such as:
  - Catfish, 85 g (3 oz) provides 425 IU (5 IU/g)
  - Salmon, cooked, 100 g (3.5 oz) provides 360 IU (3.6 IU/g)
  - Mackerel, cooked, 100 g (3.5 oz), 345 IU (3.45 IU/g)
  - Sardines, canned in oil, drained, 50 g (1.75 oz), 250 IU (5 IU/g)
  - Tuna, canned in oil, 100 g (3.5 oz), 235 IU (2.35 IU/g)
  - Eel, cooked, 100 g (3.5 oz), 200 IU (2.00 IU/g)

- A whole egg provides 20 IU if egg weighs 60 g (0.33 IU/g)
- Beef liver, cooked, 100 g (3.5 oz), provides 15 IU (0.15 IU/g)
- Fish liver oils, such as cod liver oil, 1 Tbs. (15 ml) provides 1360 IU (90.6 IU/ml)
- UV-irradiated mushrooms and UV-irradiated yeast are the only vegan sources of vitamin D from food stuffs. A 100g portion provides: (regular) 14 IU (0.14 IU/g), (exposed to UV) 500 IU (5 IU/g). Both yeast and mushroom materials, when irradiated with UV, produce vitamin D₂, but it is not known whether the D₂ is biologically fully equivalent to the D₃ vitamin in humans.

Nutrition Facts labels on food products in the U.S. are not required to list vitamin D content unless a food has been fortified with this nutrient.

Conclusion

Vitamin D is not a true vitamin but has been found to be a steroid hormone. It is obtained primarily by action of UVB on a precursor substance in the skin and lesser amounts from the diet. Vitamin D was formerly thought to be associated only with bone health (prevention of rickets and osteomalacia) but in recent years evidence has accumulated indicating that it is important for the immune system and for help in preventing cancers and other diseases.

Epidemiological studies suggest that achieving and maintaining a serum 25-(OH)D level of 34 ng/mL may be associated with a 50% reduction in the relative risk of colorectal cancer. Similarly, a level of 42ng/mL may be linked with a 40% reduction in breast cancer risk, and a level of 52ng/mL may be associated with a 50% drop in breast cancer risk. Supplementation may be a safe and effective way to achieve these levels, although daily consumption of 2,000-4,000 IU would be necessary to achieve the indicated levels in 90% or more of the population. These doses exceed the upper limit for a daily dose in current guidelines, but revisions to these guidelines are expected soon. Recommended intakes and the upper limit are anticipated to be adjusted upward.
Table I. Vitamin D [25-(OH)D] Serum Levels

<table>
<thead>
<tr>
<th>Level</th>
<th>Serum Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>&lt; 20 ng/ml (&lt;50 nmol/L)</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>21-29 ng/mL (51-74 nmol/L)</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>&gt; 30 ng/mL</td>
</tr>
</tbody>
</table>

Table II. Definition of Term

<table>
<thead>
<tr>
<th>Vitamin D Form</th>
<th>Physiologically Active?</th>
<th>Major Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-(OH)D₂ (ergocalciderol, vitamin D₂)</td>
<td>No</td>
<td>Plant foods</td>
</tr>
<tr>
<td>25-(OH)D₃ (cholecalciferol, D₃)</td>
<td>No</td>
<td>Animal foods, fortified foods produced in the skin with UVB</td>
</tr>
<tr>
<td>1,25-(OH)₂ D</td>
<td>Yes</td>
<td>Produced in the body from D₂ and D₃</td>
</tr>
</tbody>
</table>

Table III. Daily Vitamin D Intake Recommendations

<table>
<thead>
<tr>
<th>Institute of Medicine Food &amp; Nutrition Board – Issued in 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth - 50 years</td>
</tr>
<tr>
<td>51 – 70 years</td>
</tr>
<tr>
<td>71 + years</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Institute of Medicine Food &amp; Nutrition Board – Issued in 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants-1 year</td>
</tr>
<tr>
<td>1-70 years</td>
</tr>
<tr>
<td>71 + years</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>American Academy of Pediatrics – Issued in 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months – adolescence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>National Osteoporosis Foundation – Issued in 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescents – 50 years</td>
</tr>
<tr>
<td>All adults &gt; 51 years</td>
</tr>
</tbody>
</table>
REFERENCES

1. Linus Pauling Institute at Oregon State University
   http://lpi.oregonstate.edu/infocenter/vitamins/vitaminD/
REVIEW QUESTIONS:
Course #DL-999 Vitamin D
Choose the one best answer

1. The primary role of vitamin D is to regulate:
   a. calcium and phosphorous
   b. phosphorous and magnesium
   c. parathyroid hormone
   d. all of above

2. The half life of vitamin D is:
   a. 1 week
   b. 2 weeks
   c. 1 month
   d. 2 months

3. In the U.S. about _______% of healthy young adults have vitamin D levels that are less than sufficient.
   a. 24%
   b. 36%
   c. 50%
   d. 54%

4. Vitamin D is an essential molecule for human health. Vitamin D is technically a:
   a. mineralcorticoid
   b. glucocorticoid
   c. hormone
   d. hydroxyl vitamin

5. The main form(s) of vitamin D is/are:
   a. vitamin D₄
   b. vitamin D₃
   c. vitamin D₂ and D₃
   d. vitamin D₁

6. The National Osteoporosis Foundation recommends that adults who are under 50 years of age should consume:
   a. 100-200 IU/day
   b. 200-400 IU/day
   c. 400-800 IU/day
   d. >800 IU/day

7. Individuals with vitamin D deficiency or vitamin D insufficiency need a “loading dose of ________________ to correct the serum vitamin D concentration.
   a. 25,000 IU per week for 8 weeks
   b. 35,000 IU per week for 8 weeks
   c. 50,000 IU per week for 8 weeks
   d. 60,000 IU per week for 8 weeks
8. Which of the following is not a symptom of vitamin D deficiency in adults:
   a. bone discomfort
   b. myalgia
   c. weakness
   d. headaches

9. Vitamin D toxicity results from:
   a. long-range high dose vitamin D supplementation
   b. too much sun exposure
   c. eating a diet high in vitamin D-containing food
   d. increased activity of the hydroxylation step in the kidney

10. A deficiency of vitamin D is important in which of the following diseases?
    a. rickets
    b. intestinal absorption
    c. osteoporosis
    d. all of above

11. Some recent studies have suggested that there may be a reduction of falls in elderly patients who are receiving vitamin D doses of:
    a. <200 IU/day
    b. 300-400 IU/day
    c. 400 IU/day
    d. 700 IU/day

12. Which of the following is not a risk factor for vitamin D deficiency?
    a. exclusively breast fed infants without supplements
    b. dark skin at higher latitudes
    c. light skin near the equator
    d. elderly age

13. Low vitamin D levels have been linked with increased risks of all but which of the following autoimmune diseases:
    a. type 1 diabetes
    b. multiple sclerosis
    c. rheumatoid arthritis
    d. myasthenia gravis

14. The authors of a recent review estimated that 70% of multiple sclerosis cases in the U.S. and Europe could be prevented by increasing vitamin D serum levels in adolescent and young adults to levels greater than:
    a. 10 ng/ml
    b. 20 ng/ml
    c. 30 ng/ml
    d. 40 ng/ml
15. The preferred specimen for vitamin D analysis is?
   a. EDTA plasma
   b. serum
   c. sodium citrate plasma
   d. li-heparin plasma

16. The current method of choice for vitamin D analysis for reasons of convenience, turn
   around time, and cost is?
   a. immunoassay
   b. High Pressure Liquid Chromatography (HPLC)
   c. atomic absorption
   d. mass spectrometry-gas chromatography (MS-GC)

17. Epidemiological studies suggest that a serum level of approximately 42ng/ml may be
   associated with a decrease in colorectal and breast cancers. Dietary supplementation to
   achieve this level should be approximately:
   a. 200-400 IU/day
   b. 800-1200 IU/day
   c. 2000-4000 IU/day
   d. 4000-6000 IU/day

18. Patients are considered to have a vitamin D deficiency when their serum levels are less
   than:
   a. 45ng/ml
   b. 40 ng/ml
   c. 30 ng/ml
   d. 20 ng/ml

19. Of the following, which is the best source of dietary vitamin D?
   a. fish liver oils
   b. beef liver
   c. spinach
   d. whole eggs

20. Research studies have suggested that there is a relationship between vitamin D and
    cardiovascular disease. They suggest that the relationship is a/an
    a. direct relationship
    b. inverse relationship
    c. 2:1 relationship
    d. equal relationship