

Cell Defenses and the Sunshine Vitamin

Scientists now recognize that vitamin D does much more than build strong bones and that many people are not getting enough of it. Is widespread D deficiency contributing to major illnesses?

By Luz E. Tavera-Mendoza and John H. White

KEY CONCEPTS

- Vitamin D, long associated only with its role in bone formation, is actually active throughout the human body, powerfully influencing immune system responses and cell defenses.
- It can be obtained from food or manufactured by human skin exposed to sunlight. Measures of vitamin D levels show, however, that many people have too little of it circulating in their blood to protect health.
- Clear associations between low vitamin D levels and cancers, autoimmunity, infectious diseases and other conditions suggest that current daily intake recommendations for this critical nutrient need revision.

—The Editors

It was called the sunshine cure, and in the early 20th century, before the era of antibiotics, it was the only effective therapy for tuberculosis known. No one knew *why* it worked, just that TB patients sent to rest in sunny locales were often restored to health. The same “treatment” had been discovered in 1822 for another historic scourge, rickets—a deforming childhood condition caused by an inability to make hardened bone. Rickets had been on the rise in 18th- and 19th-century Europe, coinciding with industrialization and the movement of people from the countryside to the polluted cities, when a Warsaw doctor observed that the problem was relatively rare in rural Polish children. He began experimenting with city children and found that he could cure their rickets with exposure to sunshine alone.

By 1824 German scientists found that cod-liver oil also had excellent antirickets properties, although that treatment did not catch on widely, in part because the possibility that a food might contain un-

seen micronutrients important to health was not yet understood by doctors. And nearly a century would pass before scientists made the connection between such dietary cures for rickets and the beneficial effects of sunshine. Early 20th-century researchers showed that irradiated skin, when fed to rats with artificially induced rickets, had the same curative properties as cod-liver oil. The critical common element in the skin and the oil was finally identified in 1922 and dubbed vitamin D. By then the idea of “vital amines,” or vitamins, was a popular new scientific topic, and subsequent research into the functions of vitamin D in the body was very much shaped by D’s image as one of those essential micronutrients that humans can obtain only from food.

The association with rickets also steered most vitamin D research for the next 50 years toward understanding the molecule’s role in bone building and how it acts in the kidneys, intestines and the skeleton itself to help control the flow of calcium into and

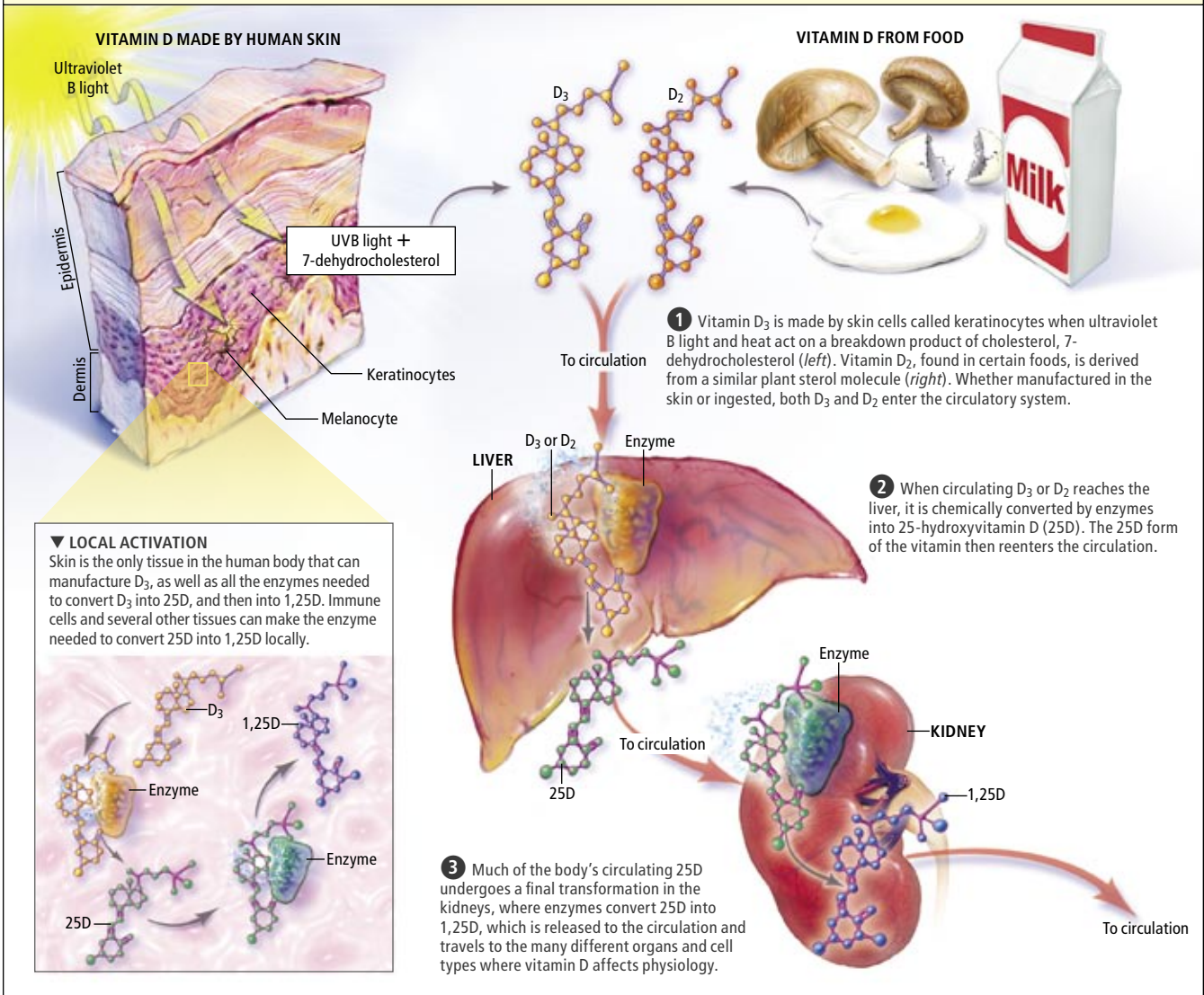


JAMES PORTO



THE MAKING OF AN ACTIVE VITAMIN

The term "vitamin D" refers to two slightly different molecules— D_3 , manufactured by human skin, and D_2 , which originates in plants and which people can obtain from food. Both versions of D must undergo stages of conversion by enzymes to reach a biologically active form, known as 1,25D.



out of bones from the bloodstream. In the past quarter century, however, studies of vitamin D's function have broadened, revealing that the so-called sunshine vitamin does far more than build bones. Extensive evidence now shows that D has potent anticancer actions and also serves as an important regulator of immune system responses. Moreover, many of D's newly recognized benefits are maximized when it is present in the bloodstream at levels considerably higher than those found in many populations. These findings, together with epidemiological data linking low vitamin D levels to disease, support the possibility that widespread vitamin D deficiency is contributing to a number of serious illnesses.

A Versatile Switch

To make sense of new findings about vitamin D, it pays to first review what D actually is and how it is used in the human body. People can obtain the molecule known as vitamin D from limited food sources, such as fatty fish and fish oil, and, today, from dietary supplements. But we can also make it ourselves, through a chemical reaction that happens in the skin when it is exposed to ultraviolet B (UVB) light. Strictly speaking, then, vitamin D is not a vitamin at all, because with moderate UVB exposure, we do not need to get it from food. In temperate regions of the world, however, UVB light is insufficient to induce adequate vitamin D synthesis in the skin

for up to six months of the year, and then dietary sources of vitamin D become essential [see box on opposite page].

The term “vitamin D” generally refers collectively to the two very similar molecules that come from each of those sources. Vitamin D₃, which is also known as cholecalciferol, is created by skin cells called keratinocytes from a breakdown product of cholesterol, 7-dehydrocholesterol, in response to UVB light. Vitamin D₂, or ergocalciferol, is derived from a similar plant sterol, and the resulting molecule has slight structural differences that distinguish it from D₃. Neither version has any biological activity in the body, however. First, either molecule must be modified by a series of related enzymes in a process called hydroxylation, which adds two thirds of a water molecule to generate 25-hydroxyvitamin D (25D).

That conversion takes place primarily in the liver, but various cell types within the skin are also capable of performing the transformation locally. The 25D made by the liver is nonetheless the major form of vitamin D circulating in the bloodstream. When it is needed in the body, a final conversion to the biologically active form is required—25D is further hydroxylated and becomes 1,25-dihydroxyvitamin D (1,25D). The enzyme that performs this task, 1 α -hydroxylase, was first discovered in the kidney, and renal processing is responsible for generating much of the body’s circulating 1,25D supply.

Once again, however, scientists now realize that many other tissues, including cells of the immune system and the skin, can also make that enzyme and perform the 25D conversion themselves. Skin is therefore unique among organs in that it is capable of manufacturing biologically active 1,25D in the presence of UVB light from start to finish, although local production of 1,25D from circulating 25D in other tissues is a substantial source of vitamin D’s biological activity in the body that was not appreciated until very recently. Once the breadth of D’s activity is considered, though, it becomes easy to see why the ability to make its active form for local use might be important to certain types of cells.

The 1,25D molecule functions as a switch that can turn genes “on” or “off” in virtually every tissue in the human body. This form of D acts by attaching to a protein known as the vitamin D receptor (VDR), which serves as a so-called transcription factor inside a cell’s nucleus. Once bound by 1,25D, the VDR protein seeks out a companion protein, the retinoid-x recep-

tor (RXR), and the complex they form binds to a specific region of the cell’s DNA adjacent to a target gene. Their attachment to the DNA induces cellular machinery to begin transcribing the nearby gene into a form that the cell will translate into a protein [see box on page 68].

By causing a cell to make a particular protein, 1,25D alters cellular function, and this ability to trigger gene activity in different cells is the basis of vitamin D’s widespread physiological effects. Because D is a substance manufactured in one tissue and circulates through the body influencing many other tissues, it is also technically a hormone. In fact, the VDR belongs to a family of proteins known as nuclear receptors that respond to powerful steroidal hormones such as estrogen and testosterone.

At least 1,000 different genes are believed to be regulated by 1,25D, including several involved in the body’s calcium processing that account for D’s well-known role in bone formation. Over the past two decades, however, scientists have identified many other genes influenced by vitamin D activity in the body, including genes with critical roles in a variety of cellular defenses.

Fortification by D

Since the 1980s various lines of evidence have pointed to vitamin D’s protective effect against cancer. Many epidemiological studies have shown a strong inverse relation between exposure to sunlight and the incidence of certain types of cancer. Studies in animals and cell cultures have supported that association and helped to pinpoint the mechanisms that may be involved.

In a mouse model of head and neck cancer, for example, a compound called EB1089, which is a synthetic analogue of 1,25D, reduced tumor growth by 80 percent. Similar results have been attained in animal models of breast and prostate cancer. Identifying the genes activated by this synthetic version of D has helped explain these responses. Uncontrolled proliferation, or growth, is a hallmark of tumor cells, and EB1089 was shown to suppress the cells’ ability to multiply by altering the activity of a number of different genes. One gene ramped up by the compound—*GADD45 α* —is well known for triggering growth arrest in normal cells whose DNA is damaged, thereby reducing their risk for becoming cancerous. In addition, EB1089 activates



SOURCES OF VITAMIN D

Vitamins D₃ and D₂ occur naturally in some foods, and both versions of the vitamin are added to certain “fortified” products. Foods provide relatively small doses of D compared with amounts made by the skin in response to UVB light. (IU = international units.)

Cod-liver oil (1 tbsp):
1,360 IU D₃

Cooked tuna, sardines, mackerel or salmon (3–3.5 oz):
200–360 IU D₃

Shiitake mushrooms (fresh, 3.5 oz):
100 IU D₂
(dried, 3.5 oz):
1,600 IU D₂

Egg yolk:
20 IU D₃ or D₂

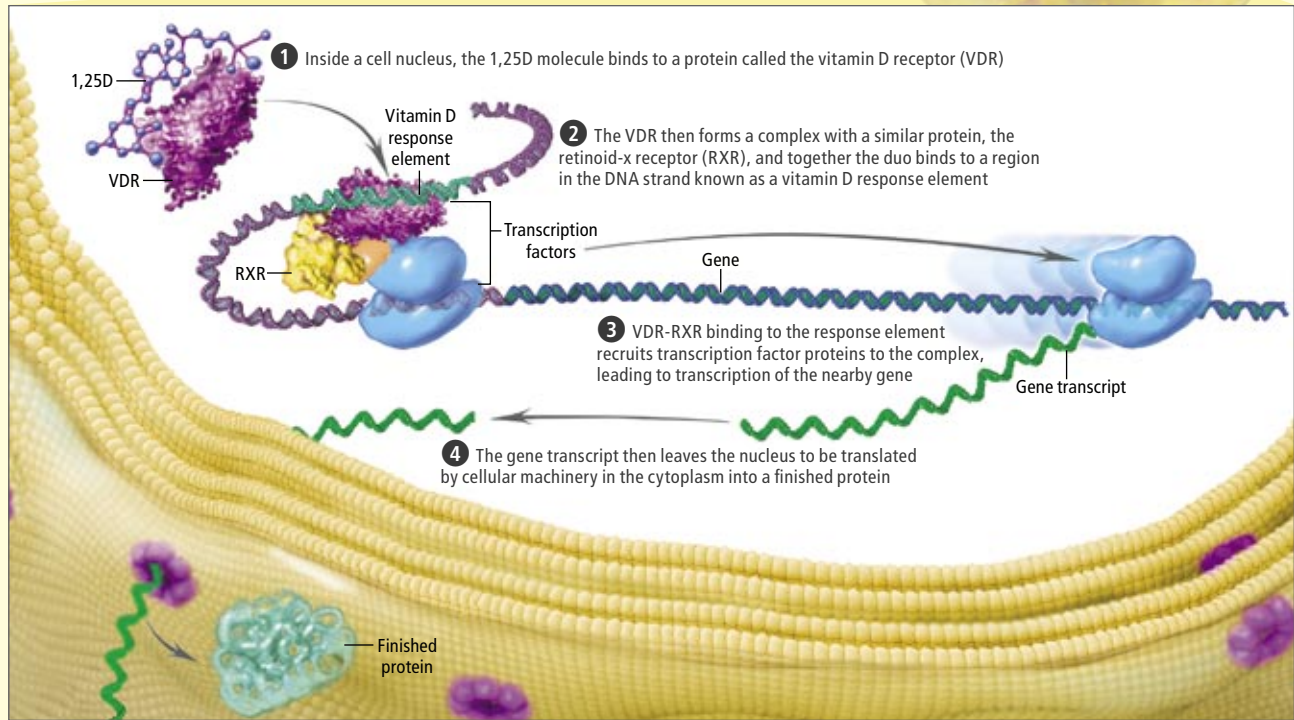
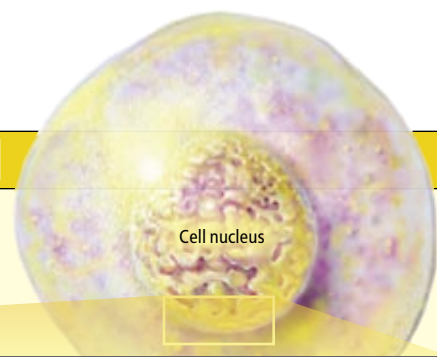
Fortified dairy products, orange juice or cereals (one serving):
60–100 IU D₃ or D₂

Full-body exposure to UVB (15 to 20 minutes at midday in summer, fair skin):
10,000 IU D₃



A BROAD-ACTING GENE SWITCH

The biologically active form of vitamin D—1,25D—turns “on” certain genes, leading to the manufacture of the proteins they encode. Those proteins may have local or widespread physiological effects. More than 1,000 different genes, in at least a dozen tissues and cell types throughout the body, are thought to be regulated by 1,25D.



genes that direct tumor cells to become more differentiated, a mature state that limits a cell's ability to proliferate.

Another dozen genes involved in a cell's energy management and self-detoxification have also been linked to EB1089's antitumor actions. That experimental compound, which was chemically designed to have 1,25D-like activity without causing toxic levels of calcium to build up in the bloodstream and body tissues, is one of several potential cancer therapies developed by pharmaceutical companies to harness vitamin D's powerful antitumor properties.

Indeed, our laboratory group at McGill University was also investigating D's cancer-related actions in 2004 when we inadvertently hit on a completely different form of physiological defense governed by 1,25D. Many of the genes regulated by vitamin D have been discovered in recent years by scientists scanning parts of the human genome, looking for vitamin D response elements (VDREs)—the distinctive sequences of DNA code that lie adjacent to genes, to which the VDR-RXR protein complex binds. In col-

laboration with Sylvie Mader of the University of Montreal, we used a computer algorithm designed to scan the entire genome, seeking out VDREs and mapping their positions relative to nearby genes.

Although these mapping studies did help us to better understand some of the anticancer actions of vitamin D, they also revealed VDREs lying close to two genes that encode antimicrobial peptides called cathelicidin and defensin beta 2. These small proteins act as natural antibiotics against a wide spectrum of bacteria, viruses and fungi. We pursued this lead with studies in cultured human cells and found that exposure to 1,25D caused a relatively modest increase in the cells' manufacture of the defensin beta 2 peptide. But in a number of different cell types—including immune system cells and keratinocytes—the rise in cathelicidin production was dramatic. We next demonstrated that immune cells treated with 1,25D, when exposed to pathogenic bacteria, released factors—presumably cathelicidin—that killed off the bacteria.

TISSUES AFFECTED BY VITAMIN D

The VDR receptor protein (above) is found in many body tissues as well as circulating immune cells, indicating a role for active vitamin D in regulating gene activity in those locations. The list below includes some of the tissues and cells where 1,25D action has been established.

Bone	Liver
Brain	Nerves
Breast	Pancreas
Fat	Parathyroid gland
Intestine	Prostate
Immune cells	Skin
Kidneys	keratinocytes

Philip Liu and Robert Modlin of the University of California, Los Angeles, and their collaborators substantially advanced this line of investigation last year by showing that human immune cells respond to bacterial cell walls by manufacturing both VDR proteins and the enzyme that converts circulating 25D into the biologically active 1,25D. In the group's experiments, these events induced the immune cells to start producing cathelicidin and to demonstrate antimicrobial activity against a variety of bacteria, including one that is perhaps the most intriguing: *Mycobacterium tuberculosis*. Thus, for the first time, the group revealed a plausible basis for the mysterious efficacy of the tuberculosis sunshine cure: the sun-soaked convalescents' vitamin D boost could have provided their immune cells with the raw material needed to generate a natural antibiotic that fought off the TB bacteria.

As gaps in our understanding of vitamin D physiology are filled in, researchers have come to appreciate that a number of D's protective actions in the body might have evolved from functions originating at the molecule's source, in the skin. The growth-arresting influence of 1,25D on cancer cells makes sense in this light because excess UVB exposure is known to damage the DNA of skin cells, which can lead them to become cancerous. Some have also speculated that the antimicrobial response regulated by vitamin D is an adaptation that might have evolved to compensate for D's role in suppressing certain other immune system reactions—specifically, those that lead to excessive inflammation. As many of us know too well from experience, excessive UVB exposure causes sunburned skin, which at the tissue level results in fluid buildup and inflammation. Although a limited amount of inflammation is a beneficial mechanism for wound healing and helps the immune system fight off infection, too much inflammation causes its own havoc.

Perhaps not surprisingly, then, an impressive body of work now shows that 1,25D also acts as an anti-inflammatory agent that functions by influencing immune cell interactions. For example, different subtypes of immune cells communicate by secreting factors called cytokines to initiate a particular type of immune response. Vitamin D has been shown to repress exaggerated inflammatory responses by inhibiting that cytokine cross talk.

Direct evidence of vitamin D's natural role in preventing inflammation came first from animal

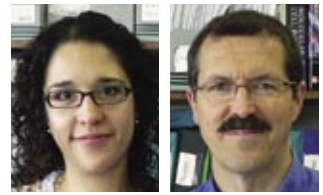
experiments in the early 1990s, which showed that mice treated with 1,25D were protected from the inflammation normally associated with wounds and the chemical irritant dinitrobenzene, whereas vitamin D-deficient mice were hypersensitive to those same insults. This immune-suppressing function of vitamin D immediately suggested a range of new therapeutic possibilities for using vitamin D or its analogues in the control of autoimmune diseases thought to be caused by overactive cytokine responses, such as autoimmune diabetes, multiple sclerosis (MS) and inflammatory bowel disease.

Since that time, scientists have realized that many cell types, including immune cells, are capable of using circulating 1,25D and of converting circulating 25D to the active form of the vitamin, confirming that the anti-inflammatory actions of 1,25D are not restricted to skin cells nor simply to the context of sunburn.

Epidemic Deficiency?

Recognition that 1,25D has a broad range of biological activities far beyond its role in calcium homeostasis has thrown into sharp relief a large body of epidemiological evidence that low vitamin D levels correlate strongly with certain types of disease, among them cancers, autoimmune conditions and even infectious diseases, such as influenza, as well as with seasonal variations in illness rates. In addition, many of the noted physiological responses to vitamin D seen both in the laboratory and in clinical studies are optimized only when circulating concentrations of 25D are higher than is typical in many populations. Members of the vitamin D research community are therefore coming to a widespread consensus that substantial numbers of people in temperate regions of the world have

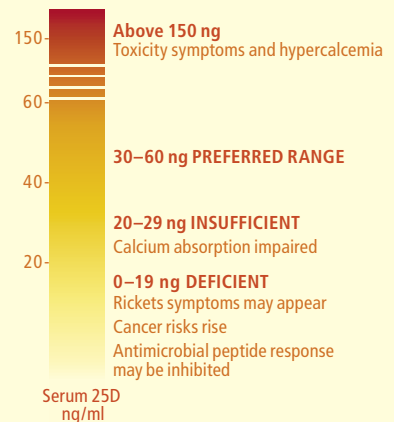
[THE AUTHORS]



Luz E. Tavera-Mendoza and John H. White worked together in White's laboratory at McGill University investigating the molecular activities of vitamin D in human cells. They have uncovered aspects of the vitamin's role in cancer prevention and discovered, with their collaborators, that D regulates certain genes involved in cell responses to microbial invaders. Tavera-Mendoza is now a postdoctoral fellow at Harvard Medical School studying vitamin D and breast cancer. Having witnessed vitamin D's health-protecting actions firsthand in the lab, both authors take supplements during the parts of the year when sunlight is too weak in the northern cities where they reside to produce adequate amounts in skin. White takes 4,000 international units of D₃ daily during those "vitamin D winter" months, and Tavera-Mendoza takes 1,000.

HOW MUCH 25D IS DESIRABLE?

Estimates of vitamin D available to the body are based on measurements of 25D concentrations in blood serum. Levels between 30 and 45 nanograms per milliliter of serum are considered minimally sufficient for bone health, although some beneficial cellular responses to D are optimized at higher concentrations. Below 30 ng/ml, health risks increase; above 150 ng/ml, excess calcium buildup in blood and tissues and symptoms of toxicity are possible.



levels of vitamin D that are well below optimal concentrations for health, particularly during winter months.

UVB light penetrates the atmosphere more directly in the tropics than in more temperate regions of the planet, which receive substantial amounts of it only during the summer. Because most people obtain vitamin D mainly through UVB exposure, circulating 25D levels in populations generally diminish with increasing latitude, although variations at a given latitude do arise because of differing ethnicities and diets, as well as variations in local climate and elevation. Consistent with vitamin D's observed gene-regulatory activities, a clear association is seen between increasing latitude and increased risk of several illnesses, most conspicuously autoimmune diseases such as MS.

A chronic progressive illness, MS is caused by immune cell assaults on the protective myelin sheath that surrounds nerve fibers of the central nervous system. Its incidence is significantly higher in areas farthest from the equator in North America, Europe and Australia, and convincing evidence suggests that this pattern results from decreased UVB exposure. Disease progression and symptom flare-ups in MS sufferers also display well-established seasonal variations, with the highest disease activity in the spring (when circulating 25D levels would be lowest following the winter) and the least disease activity in the fall, after the summertime boost of D₃. For example, scientists at the University of Southern California found an inverse relation among 79 pairs of identical twins between increased sun exposure during childhood and a lifetime risk of developing MS. The twins who spent more time outdoors as children had as much as 57 percent lower risk of developing the condition.

Similar patterns of risk for illness have been documented for autoimmune diabetes and for Crohn's disease, an inflammatory autoimmune intestinal condition, as well as for certain types of malignancy. Population rates of cancers of the bladder, breast, colon, ovary and rectum increase twofold from south to north in the U.S., for instance.

In addition to the many studies correlating sun exposure with disease incidence, recent investigations have made similar connections between disease risk and direct measurements of circulating 25D concentrations in blood serum. An enormous survey by researchers at the Harvard School of Public Health looked at the

stored serum samples of some seven million U.S. Army and Navy personnel as well as their health records to see which individuals had developed MS between 1992 and 2004. The researchers found a significantly lower risk of later developing the disease in the group with high serum 25D levels at the time the sample was taken. Soldiers with serum 25D concentrations above 40 nanograms per milliliter had a 62 percent lower risk than the soldiers whose concentrations were 25 ng/ml or below.

Measuring circulating levels of 25D is the usual method of gauging vitamin D availability in the body. Generally agreed on health standards, based largely on bone-forming needs, hold circulating 25D levels of 30 to 45 ng/ml to be minimally sufficient. Serum vitamin D concentrations below between 21 and 29 ng/ml are considered insufficient and often accompanied by decreased bone density. Some symptoms of rickets may appear when concentrations fall below 20 ng/ml, and the risk of colon cancer rises.

Such low concentrations are unfortunately all too common, especially in winter. In February and March of 2005, for example, a survey of 420 healthy females across northern Europe—in Denmark (Copenhagen: 55° latitude), Finland (Helsinki: 60°), Ireland (Cork: 52°) and Poland (Warsaw: 52°)—found that 92 percent of adolescent girls in these countries had 25D levels less than 20 ng/ml and that 37 percent of the girls were severely deficient, with 25D levels of less than 10 ng/ml. Among the older women tested, 37 percent were found to be vitamin D deficient and 17 percent were severely deficient.

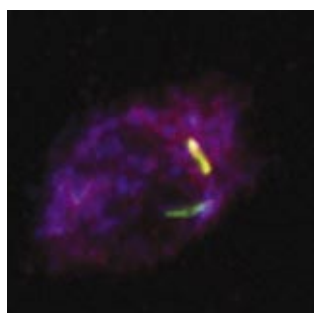
Beyond latitude, several factors can contribute to vitamin D deficiency, and primary among these is race. White skin synthesizes vitamin D six times faster than dark skin because higher levels of melanin in darker skin block UV penetration [see "Skin Deep," by Nina G. Jablonski and George Chaplin; *SCIENTIFIC AMERICAN*, October 2002]. As a result, African-Americans generally have levels of 25D that are approximately half those of whites in the U.S. In fact, data gathered for the U.S. National Health and Nutrition Examination Survey showed that 42 percent of African-American women tested were seriously 25D deficient, with serum concentrations of less than 15 ng/ml.

Increased public awareness that excessive exposure to sunlight causes skin damage is undoubtedly contributing to vitamin D deficiency as well. When properly applied, topical sunscreens reduce vitamin D produced in the skin

D MAKES A DIFFERENCE

Growing evidence suggests that chronically low levels of vitamin D raise a person's risk for certain major illnesses. Examples of findings based on a population's blood serum D levels or UV exposure include:

- 30% to 50% higher risk for breast, prostate and colon cancers at serum 25D levels below 20 ng/ml
- Five times higher risk of ovarian cancer among women living at high latitudes (for example, Norway and Iceland) than women living at equatorial regions
- 77% lower risk for all cancers among Nebraska women age 55 and older taking 1,100 IU of D₃ daily over a three-year period compared with a placebo group
- 62% lower risk for multiple sclerosis at serum 25D levels above 40 ng/ml than at 25 ng/ml or less
- 80% lower lifetime risk for autoimmune (type 1) diabetes in Finnish children given 2,000 IU of D₃ daily during first year of life

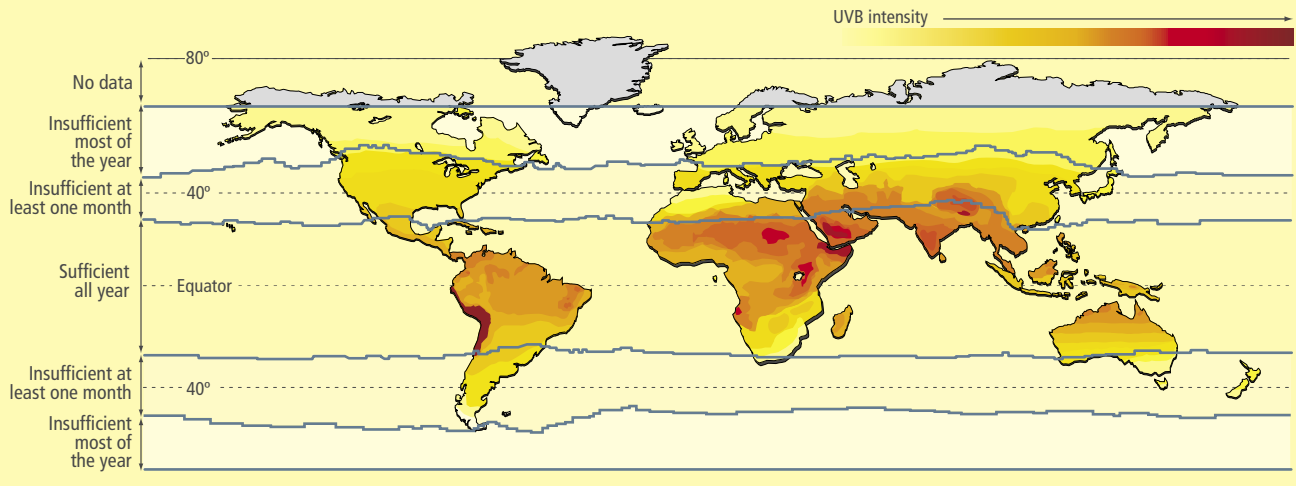


▲ IMMUNE CELL treated with 1,25D responds to infection by tuberculosis bacteria (green and yellow) by making antimicrobial cathelicidin peptides (red).

VITAMIN D WINTER

Exposure to UVB radiation in sunlight is the single greatest source of vitamin D for most individuals, so location and season affect a population's risk of deficiency. For periods of the year known as vitamin D winter, UVB intensity is too weak at some latitudes even to induce vitamin D synthesis in the skin. Because ozone blocks UVB rays, the rays

are most intense nearest the equator, where sunlight travels the least distance through the earth's atmosphere, and vitamin D synthesis is possible year-round. An increasing angle of penetration at higher latitudes weakens UVB intensity until it is insufficient, especially during winter, for making vitamin D.



by more than 98 percent. Enough vitamin D for good health can be synthesized in skin with sun exposure that might produce at most a slight pinkness, however. For most fair- and medium-skinned people in North America, this takes five to 15 minutes of sunlight between 10:00 A.M. and 3:00 P.M. during the summertime.

Vitamin D supplements could address the high prevalence of vitamin D deficiency in temperate zones, but how much people should take is still a subject of debate. The American Academy of Pediatrics recommends a minimum daily intake (RDI) of 200 international units (IU) for children, which many researchers have argued is suboptimal, even for rickets prevention. The RDI for adults in North America and Europe currently ranges between 200 IU and 600 IU, depending on age. After reviewing multiple studies comparing vitamin D intake and the serum concentrations of 25D produced, Harvard School of Public Health researchers and others concluded last year that the current RDIs are inadequate. They suggested that no less than half of U.S. adults needed to consume at least 1,000 IU of vitamin D₃ daily to raise their serum 25D concentrations to the minimum healthy level of 30 ng/ml. No rule of thumb exists for calculating the serum 25D levels generated by supplements, because individual responses can vary and may depend in part on the extent of deficiency. A study of pregnant women showed, for example, that daily doses of 6,400 IU raised se-

rum 25D levels dramatically until they reached about 40 ng/ml and then leveled off. Vitamin D₂ has also been found to be less effective than D₃ at raising and sustaining serum 25D concentrations over time.

Toxic vitamin D overdose through supplementation is certainly possible, although it is generally seen when doses of 40,000 IU or more of D have been taken daily for an extended period. Sunshine-induced vitamin D toxicity has never been observed, however. To put this in perspective, an adult woman with white skin exposed to summer sun while wearing a bikini generates about 10,000 IU of vitamin D in 15 to 20 minutes. Longer exposures do not generate higher amounts of vitamin D, because UVB light also degrades the vitamin, preventing too much of it from building up in the skin.

Accumulating evidence suggests that the subtle and longer-term effects of even slight vitamin D deficiency may be multifold and manifested later in life, in the form of increased frequency of bone fractures and enhanced susceptibility to infection and autoimmune diseases, as well as elevated frequencies of certain cancers. The research strongly implies that at the very least the general public would benefit substantially from greater awareness of the broad physiological benefits of vitamin D, a sound medical consensus on sensible sun exposure and a clear indication of optimal recommended daily intakes of vitamin D from dietary sources. ■

MORE TO EXPLORE

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