

## **How Vitamin D Metabolism Affects Autoimmune and Inflammatory Diseases**

### **Abstract**

Vitamin D has received much attention in recent years because studies found an association between low serum 25-hydroxyvitamin D (hereafter referred to as 25-D) levels and some disease states. This has caused a heated debate among experts regarding the definition of vitamin D deficiency and the amount of vitamin D necessary for health. Concerns about vitamin D deficiency merit a closer look at the current method of determining vitamin D status. Because the serum level of 25-D doesn't always reflect the level of the hormone 1,25-dihydroxyvitamin-D (hereafter referred to as 1,25-D), measuring both of these vitamin D metabolites may provide a better assessment. An analysis of the active metabolite, as well as its inactive precursor, may reveal low 25-D in the presence of elevated 1,25-D and lead to a diagnosis of abnormal vitamin D endocrine function. The definition of Vitamin D deficiency needs re-evaluation in view of the fact that low serum 25-D is often found in healthy persons. New research contradicts current explanations of vitamin D deficiency and studies used as evidence of the need for vitamin D supplementation are inconclusive. It's theorized that low vitamin D is a consequence of an inflammatory process and increasing 25-D via supplementation could have negative effects. A bacterial etiology pathogenesis of chronic inflammation appears to provide a rationale for an inflammatory disease process that causes low serum 25-D. There are indications treatment directed at eradicating persistent intracellular infection can correct dysregulated vitamin D metabolism and restore vitamin D metabolites to normal levels.

## **Vitamin D**

The five forms of vitamin D are collectively known as calciferol; the primary forms are vitamin D<sub>2</sub> and vitamin D<sub>3</sub>. "Vitamin D" is a generic term that can mean either D<sub>2</sub> or D<sub>3</sub> and may also be used to refer to the metabolites formed from calciferol. Chemically, vitamin D is a steroid that functions as a hormone. 1 It was identified as a vitamin when it was discovered early in the 20<sup>th</sup> century because it cured rickets. 2 The vitamin moniker is not a complete misnomer because vitamin D is an essential nutrient for those not exposed to adequate sunlight. But errors may occur in research studies when authors use vitamin D as a synonym for 1,25-D because there are very significant structural and biological differences between 1,25-D and vitamin D<sub>3</sub>. 3

Both vitamin D<sub>2</sub> and D<sub>3</sub> require ultraviolet (UV) radiation. Emissions from the sun include light, heat and UV radiation; 60% of solar UV radiation is received between 10am and 2pm daily. Sunlight is the main source of vitamin D worldwide. Artificial sources also emit UV radiation (e.g., tanning beds, halogen lights, lasers, fluorescent lights). 4 Vitamin D<sub>2</sub> is photosynthesized in some invertebrates (e.g., yeasts, higher fungi, and phytoplankton, etc.) from ergosterol (an organic molecule in the steroid class). Vitamin D<sub>2</sub> isn't naturally present in humans because we lack ergosterol. Because it doesn't come from an animal product, vitamin D<sub>2</sub> is the form of vitamin D supplementation preferred by vegans who aren't exposed to enough sunlight. 5 Vitamin D<sub>3</sub> is called the "sunshine vitamin" because it's photosynthesized, non-enzymatically, in the skin of vertebrates (i.e., animals with backbones) from 7-dehydrocholesterol (a steroid

molecule). 6 Photochemical regulation mechanisms in the skin prevent the formation of toxic levels of vitamin D<sub>3</sub>. During prolonged exposure to the sun, the accumulation of previtamin D<sub>3</sub> is limited to about 10 to 15% of the original 7-dehydrocholesterol content because the previtamin D<sub>3</sub> photoisomerizes to two biologically inert photoproducts, lumisterol and tachysterol. 7 Vitamin D<sub>3</sub> is photosynthesized when the UV index is greater than 3; this occurs daily in the tropics and daily in the spring/summer of temperate regions. Brief casual exposure of the arms and face is equivalent to ingestion of 200 IU/day. 8

Animal products are the primary dietary source of vitamin D<sub>3</sub>, although there is no dietary requirement for those exposed to adequate sunlight. Fatty fish (e.g., salmon, mackerel, tuna, sardines, herring, etc.) have the highest amounts of D<sub>3</sub>; other animal products (e.g., eggs, meat, dairy foods, etc.) contain lesser amounts. Vitamin D<sub>3</sub> can also be found in processed food that has been synthetically fortified (e.g., milk, cereals, breads, margarine, juices, etc.). 9 Vitamin D<sub>3</sub> is available as a dietary supplement. Because the chemical processes that lead to the formation of vitamin D<sub>3</sub> are non-enzymatic, they can take place in organic solvents (ex vivo), as well as in vivo. Therefore, vitamin D<sub>3</sub> can be synthesized commercially by extracting cholesterol from sheep wool; chemically synthesizing it to 7-dehydrocholesterol and then irradiating it. 10 Supplemental vitamin D<sub>3</sub> is also made from fish oil extract. 11

### **Vitamin D Metabolism**

The metabolic processes that convert biologically inactive, parental vitamin D into active metabolites are called the vitamin D endocrine system. The key elements of this system are photo-conversion, the liver, the kidney as an endocrine gland, the vitamin D receptor (VDR) and the vitamin D binding protein (VDBP). This process begins when vitamin D<sub>3</sub> is photosynthesized in the skin or ingested (or when D<sub>2</sub> is ingested). Vitamin D is then transported to the liver where it's hydroxylated by the enzyme CYP2R1 to produce 25-D. 12 25-D is then transported to the kidneys where it's hydroxylated by the enzyme CYP27B1 to produce the active 1,25-D metabolite. 13 25-D (also known as calcidiol) is the primary circulating metabolite. 14 It has a half-life of two to three weeks but it's stored in the liver and fatty tissues for later use. 15 25-D is the pro-hormonal precursor to 1,25-D. Although 25-D is not an active signaling molecule, it does have some biological activity. 16

1,25-D (also known as calcitriol) is the most potent steroid hormone in the human body. 17 Feedback mechanisms regulate production of 1,25-D in the kidneys via serum levels of parathyroid hormone (PTH), fibroblast-like growth factor-23 (FGF23) calcium and phosphate. 15 1,25-D has a half-life of four to six hours and is also produced in many other tissues (e.g., skin, macrophages, colon, pancreas, blood vessels, etc.) by enzymatic actions. 18 The VDBP is the transport vehicle. VDBP, which is synthesized in the liver, delivers lipophilic (fat soluble) vitamin D to the liver, 25-D to the kidneys and 1,25-D to the vitamin D receptor. 19 VDBP also acts as a storehouse for D metabolites and prevent vitamin D deficiency. 20 VDBP maintains stable serum stores of vitamin D metabolites and modulates the rates of its bioavailability, activation, and end-organ responsiveness. These properties may have evolved to stabilize and maintain serum levels of vitamin D in environments with variable D availability. 21

The most important function of 1,25-D is to bind to the VDR nuclear receptor and mediate the transcription of DNA, triggered by signaling proteins, like nuclear factor kappa-B (NF- $\kappa$ B). 22 Located in the nucleus of cells, the VDR has been identified in 37 different tissues throughout the body (including the nucleus of phagocytic cells of the immune system). 23 The effects of 1,25-D are pleiotropic (producing more than one effect); it controls expression of over 1000 genes, 24 and transcribes numerous proteins. 25 When 1,25-D binds to the VDR, it heterodimerizes with the retinoid X receptor (RXR). This duo (VDR/RXR) then binds to cellular DNA (deoxyribonucleic acid) via the vitamin D response element (VDRE) to initiate a cascade of molecular actions. Transcription factors separate the DNA double helix at the correct gene location and make complementary mRNA (messenger RNA). The mRNA then returns to the cytoplasm to be translated into a specific protein by ribosomes. Consequently, genomic expression is usually activated, but it may also be repressed. 26 VDR-activated genomic expression mediates many tissue-specific biological effects. Classical effects (e.g., calcium transport and bone health, etc.) are well known. The non-classical, extra-skeletal effects (e.g., cell differentiation, central nervous system, skin/hair, immune regulation, hormone secretion, etc.) have only been recognized for about 25 years. 27 In addition to the classical VDR-mediated genomic pathway, 1,25-D also has been shown to elicit rapid responses. The term rapid response is used to describe the biological effects of 1,25-D that occur within a few minutes after hormone treatment and are considered too rapid to be explained by a VDR-mediated genomic pathway. Rather, the rapid responses are thought to be mediated by a direct action of 1,25-D on the plasma membrane of target cells stimulating a signal transduction pathway involving the rapid opening of voltage-sensitive Ca<sup>2+</sup> channels and activation of protein kinases. 28

***1,25-D regulates the immune system.*** VDR are present in most cell types of the immune system, particularly in antigen-presenting cells (APCs) such as monocyte, macrophages and dendritic cells. The influence of 1,25-D on the immune system is one of its most important roles. In general, the innate system is enhanced and the adaptive system is inhibited by 1,25-D. 29 Many cells outside the kidneys contain VDR and express CYP27B1, the enzyme that catalyzes 25-D to 1,25-D. In monocytes and macrophages (innate immune system), synthesis of 1,25-D from 25-D promotes an antibacterial response to infection. 18 Monocytes sense pathogen-associated molecular patterns (PAMPs) by utilizing pattern-recognition receptors (PRR), such as toll-like receptors (TLRs). 30 Induction of CYP27B1 occurs following PAMP-sensing by TLR2/1. 30 The inflammatory cytokine interferon  $\gamma$  (IFN $\gamma$ ) also stimulates expression of CYP27B1 by macrophages. 31 As a result, 1,25-D production is increased in response to a pathogen immune challenge. 1,25-D activates the VDR to express antimicrobial peptides (AMPs) such as cathelicidin 32 and beta defensins which attack pathogens. 33 VDR immune system regulation also involves cell proliferation, differentiation and apoptosis. 34 The VDR is also expressed in both B and T white blood cells (lymphocytes). 35 1,25-D modulates the adaptive immune system by inhibiting dendritic cell maturation, 35 reducing T helper (Th) cells, 36 and shifting Th<sub>1</sub>/Th<sub>17</sub> cells to the Th<sub>2</sub> and T regulatory pathways. 37 In addition, 1,25-D inhibits Th<sub>1</sub> cytokines that support cell-mediated immunity 38 and promotes Th<sub>2</sub> cytokines that support humoral immunity (antibodies circulating in bodily fluids). 39

## Vitamin D Deficiency

The realization that vitamin D is vital for so many essential biological functions has prompted a number of current trends. There's been an explosion in vitamin D research; more scientific articles have been published about vitamin D in the 21<sup>st</sup> century than about any other vitamin (there were 28,047 listed in MedLine between January 1, 2000 and December, 2012). An Internet search reveals over 600 clinical trials currently underway concerning vitamin D. Research has led to concerns about vitamin D deficiency 40 and increased use of serum 25-D testing. Karen Lusky reported, in the June 2009 Cleveland Clinic newsletter, an increase from 1,500 tests/month in 2006 to 12,000/month in 2009. Another trend is the increased use of vitamin D supplements, as reported by Alex Williams April 4, 2009 in the New York Times.

Concerns about vitamin D deficiency arose when studies showed patients with autoimmune diseases have lower levels of serum 25-D 41, 42 and study subjects given vitamin D had lower rates of autoimmune diseases and fewer markers of inflammation. 43 Although more people are being tested, there is no consensus on the definition of vitamin D deficiency or insufficiency and experts haven't agreed on the significance of low 25-D. In the U.S., the leading authority regarding medical research is the prestigious Institute of Medicine (IOM). Established in 1970 by the National Academy of Sciences, the IOM is an independent, non-government, non-profit organization with over 1700 volunteer members who provide evidence-based information on science, medicine, and health. IOM committees are carefully composed to assure expertise, avoid bias or conflict of interest; and their reports are reviewed by external experts. 44 In a New York Times article on August 25, 2011, Gardiner Harris wrote, "The IOM is the most esteemed and authoritative adviser on issues of health and medicine, and its reports can transform medical thinking around the world."

Vitamin D<sub>3</sub> levels in human serum are rarely used to estimate cutaneous synthesis of vitamin D, partly because of the transient nature of this blood parameter and the difficulty of measuring the low levels in serum. 45 In 1997, the IOM defined serum 25-D as the functional indicator of vitamin D status. 46 It is a biomarker of exposure and, thus, a reflection of the supply of vitamin D to the body (the net incoming contributions from cutaneous synthesis and total intake). Serum 25-D can be a useful adjunct to examining the intake level of vitamin D if the measure's variability is kept in mind. However, what is not clearly established is the extent to which 25-D levels serve as a biomarker of effect. Does 25-D relate to health outcomes via a causal pathway and can it serve as a predictor of such health outcomes? 47

In 2006, the Merck Manual listed 25-40 ng/ml as the normal 25-D range. 48 Recently, this range has skyrocketed to 30-74 ng/ml 49, leading some to declare that half the U.S. non-institutionalized adult population is deficient, with 25-D levels between 12-30 ng/ml. 41 Quest Diagnostics now lists the upper limit of normal 25-D as 100 ng/ml. 50 Laboratory reference ranges for serum 25-D levels have long been based upon average values from populations of healthy individuals but many people are now supplementing with vitamin D. The IOM report emphasized that the current measurements, or cut-off points, of sufficiency and deficiency of 25(OH) D in use by laboratories have not been set using rigorous scientific studies. They suggest

that since no central authority has determined which cut-off points to use, reports of deficiency and lab ranges may be skewed and numbers overestimated. 47

Without a consistent normal range for serum 25-D, the definitions of vitamin D insufficiency and deficiency vary greatly; indeed, some definitions of deficiency for adults are based on 25-D levels associated with rickets in children (<10 ng/ml). 51

The Institute of Medicine definition of vitamin D insufficiency and deficiency. 47

- Risk/deficiency = <12 ng/ml
- Risk/insufficiency = 12-20 ng/ml
- Sufficient = 20 ng/ml
- No benefit >30 ng/ml
- Cause for concern >50 ng/ml

The Endocrine Society definition of vitamin D deficiency and insufficiency (slightly higher). 51

- Deficiency = <20 ng/ml
- Insufficiency = 20 to 29 ng/ml

The Vitamin D Council definition of deficiency and insufficiency (much higher). 52

- Deficient: 0-40 ng/ml
- Sufficient: 40-80 ng/ml
- High Normal: 80-100 ng/ml
- Undesirable: > 100 ng/ml
- Toxic: > 150 ng/ml

The IOM responded to the Endocrine Society guideline, stating in part that, "aspects are not well supported and [are] in need of reconsideration." 53 The Vitamin D Council guideline is worrisome because it is so high and refers to both adults and children. Some laboratories quote the IOM definition of deficiency in their reports but list a higher range; according to the IOM, the extent of vitamin D deficiency among the North American population has been overestimated because of inflated cut-points for serum 25-D. 47 Measurements of serum 25-D also have limitations. Samples sent to different labs may yield a wide variation in results, 54 depending on the assay used; ELISA or RIA (both are cheaper) or chromatography (reduces assay drift but isn't standardized). 55 Interpretation of serum 25-D levels must consider factors such as diet, light exposure, illness and the potential for genetic variations (DBP 56, VDR 57, and CYP27B1 58) to influence the physiological and clinical impact. Most importantly, 25-D may not always reflect the level of 1,25-D (the active metabolite). 59 Measuring only 25-D, and accepting it at face value, may result in a false diagnosis of vitamin D deficiency which could lead to inappropriate, and possibly toxic, supplementation.

***Vitamin D recommendations.*** There is no recommended daily allowance (RDA) of vitamin D because it is not an essential nutrient (i.e., a substance the body can't make). Instead, the recommendation for vitamin D is stated as adequate intake (AI) and the need for an AI is based on the absence of adequate sunlight and the presence of adequate calcium. An IOM committee met in 1997 and set the AI standard of 400 IU of vitamin D per day for adults. In 2010, after an

extensive, evidence-based review of the data from all pertinent vitamin D studies, the IOM raised the recommendation: 47

- 600 IU daily for ages 1-70
- 800 IU daily for 70 and over
- 4,000 IU/day (highest safe level)

The 2010 IOM consensus report on vitamin D was endorsed by many organizations such as the American Society for Bone and Mineral Research. Hector DeLuca, one of the most respected vitamin D researchers in the world and a member the National Academy of Scientists, agrees with the IOM guidelines but support is not universal. Proponents of vitamin D supplementation lobbied the IOM, unsuccessfully, to raise the AI much higher (2,000-10,000 IU/day) but their request was denied because the IOM saw many problems with the vitamin D research they reviewed.

## **Vitamin D Studies**

High quality human studies of vitamin D are difficult to conduct because of the difficulty in eliminating the con-founding factor of photosynthesis. Most in vivo studies (taking place in a living organism) are murine (mouse); however, there are significant differences between murine and human biological responses. 60 A recent analysis revealed that genomic responses in mouse models poorly mimic human immune system responses, making it difficult to study basic pathophysiological mechanisms. 61 Using knockout mice (genetically altered to lack a functional VDR or CYP27B1) improves the study quality. 62 The IOM noted that most vitamin D studies are observational; few are randomized or well-controlled. They're short-term and serum 25-D is used as a surrogate marker (i.e., not a true health outcome). No biological plausibility is given to explain study conclusions and often the evidence is ambiguous. Con-founding variables (e.g., health consciousness, sick people remain indoors, access to medical care, etc.) are often not taken into account. The studies only show a link between low 25-D and illness; researchers and clinicians understand that correlation does not equal causation. 63 In a widely studied example, numerous epidemiological studies showed that women who were taking combined hormone replacement therapy (HRT) also had a lower-than-average incidence of coronary heart disease (CHD), leading doctors to propose that HRT was protective against CHD. But randomized controlled trials showed that HRT caused a small but statistically significant increase in risk of CHD. Re-analysis of the data from the epidemiological studies showed that women undertaking HRT were more likely to be from higher socio-economic groups, with better-than-average diet and exercise regimens. The use of HRT and decreased incidence of coronary heart disease were coincident effects of a common cause (i.e. the benefits associated with a higher socioeconomic status), rather than cause and effect, as had been supposed. 64

At the 15th European Congress of Endocrinology April 27 - May 1, 2013 in Copenhagen, Denmark, Mark Cooper, M.D., 65 from the University Hospital, Birmingham, UK, a leading expert in the field of vitamin D, noted that the supposed benefits of vitamin D are exclusively reported in observational studies and there is a huge sector of the scientific community which is "evangelical" in its pro-vitamin-D stance. He commented,

We really need some robust evidence. Physicians have been here before, with many other nutrients that subsequently, in large intervention trials, turned out to have a null effect or even be harmful. In fact, there is already evidence of risks with supplements of vitamin D from randomized clinical trials, with no evidence of benefit. There have been lots of randomized controlled trials, different doses of vitamin D, different groups, all well-designed, all in major journals, and all negative. At least 1 in 20 of these studies should be positive just by chance, but we haven't seen that yet.

Meta-analysis statistics studies have attempted to make sense of multiple vitamin D studies. An analysis was done in 2012 by Loyola University Chicago Stritch School of Medicine, in which researchers explored all-cause mortality rates, across the spectrum of 25-D levels, over an eighteen-year follow-up, among adults with and without kidney disease. They concluded “while significantly higher mortality rates are noted with 25-D levels less than 12 ng/ml, mortality rates are fairly similar across the range of 25-D levels 20–40 ng/ml. 66 In other words, they found no benefit to a higher level of 25-D. A retrospective analysis of over 247,000 subjects in Denmark came to a similar conclusion. At the University of Copenhagen, scientists found the lowest mortality rate when 25-D was 20 ng/ml. 67 A 2013 study found parallel results; 25-D in the 20–36 ng/ml range was associated with the lowest risk for mortality and morbidity. 68 Another study found little association with vitamin D deficiency and autoimmune conditions, challenging the assumption that serum levels of 25-D are a sensitive measure of the autoimmune disease state. 59

### **Vitamin D proponents**

Despite conflicting study results and the IOM recommendation, vitamin D proponents continue to exhort people to take vitamin D supplements. Dr. Joseph Mercola, 69 the founder and editor of an alternative medicine website visited by thousands of people daily, stated, “A lack of this wonder nutrient can set the stage for no fewer than 33 disorders, including autism, cancer, diabetes and infertility.” Referring to vitamin D as a nutrient is a serious error and Dr. Mercola failed to point out that evidence for his claims is inconclusive. Dr. Michael Holick, 70 a leading vitamin D researcher and director of the Vitamin D, Skin and Bone Research Laboratory at Boston University School of Medicine stated, “There's overwhelming evidence ...that increasing your vitamin D intake can make substantial improvement in your overall health and welfare. There is no downside to increasing your vitamin D intake.” However, it's premature to claim there is no downside to taking a steroid that has not been studied long-term because the evidence does not yet support vitamin D supplementation. Proponents may also oversell the study findings. For example, Mercola's website headlined an item on a new study this way: “Vitamin D fights Crohn's disease”. But the lead researcher, Dr. John White 69 of the Research Institute of McGill University Health Center in Montreal, said the data came from a lab study that “will have to be borne out in the clinic, which may be tricky. Data is coming, but there's a good reason to be skeptical — people have been on this bandwagon before. When it gets into the clinic, it often doesn't work out quite as well.”

Thankfully, some experts are advising caution regarding recommendations made based on observational studies. In his August 4, 2009 presentation “Weighing Scientific Evidence” at the IOM Information Gathering Workshop for Dietary Reference Intakes for Vitamin D and Calcium, Dr. Barry Kramer (Office of Disease Prevention, NIH) urged caution by quoting the

Scottish philosopher David Hume (1711-1778) "A wise man proportions his belief to the evidence". In a May 10, 2010 Chicago Tribune article Julie Deardorff 69 quoted two other vitamin D experts. Anastassios Pittas (endocrinologist at Tufts University Medical Center, Boston) stated, "It's premature to go out and make a big deal out of vitamin D supplementation when we don't have the evidence. We've been burned before on nutrition-based interventions." Dr. Ethan Balk (Center for Clinical Evidence Synthesis) cautioned, "There's a potentially large problem with leaping from observational studies to making decisions about interventions." In a Journal of the National Cancer Institute editorial Davis and Dwyer 71 concluded, "While vitamin D may well have multiple benefits beyond bone, health professionals and the public should not, in a rush to judgment, assume that vitamin D is a magic bullet and consume high amounts of vitamin D. More definitive data on both benefits and potential adverse effects of high doses are urgently needed."

Unfortunately, the media have confused the public by only publishing headline-pleasing conclusions instead of reporting the conflicting scientific data surrounding a complicated issue. 72 The Vitamin D Council is the most vocal source of information urging the public to take more vitamin D. This non-profit organization was founded in 2003 by John J. Cannell, M.D., a practicing psychiatrist with a history of activism in various causes. His website ([www.vitaminCouncil.org](http://www.vitaminCouncil.org)) markets vitamin D<sub>3</sub> as a nutrient and enables him to sell his book on vitamin D, as well as vitamin D supplements and vitamin D test kits. Dr. Len Lichtenfeld 73 (Deputy Chief Medical Officer for the national office of the American Cancer Society) countered Vitamin D Council claims in his online blog with this statement,

When we succumb to making every medical decision solely on the basis of the strongest advocate's voice, we run the risk of moving medical practice back into an era similar to that from which we are trying to emerge. If the review and research studies confirm Dr. Cannell's position, that will be welcome. But we need to once and for all establish the science-based evidence that will conclusively answer the question one way or the other, rather than relying on advocacy to establish dietary and medical practice recommendations for the world.

Apparent conflicts of interest are not unusual among the most vocal vitamin D proponents. Some of Holick's research was supported by the UV foundation which is funded by the tanning bed industry. Dr. Robert Heaney (an endocrinologist at Creighton University in Omaha) has authored vitamin D studies funded by Smith/Kline/Glaxo, spoken on behalf of Proctor & Gamble, and sat on the board of a dairy association. Dr. Reinhold Vieth (a biochemist from Toronto) has co-authored vitamin D studies with vitamin D lobbyists and his wife has a business selling vitamin D supplements. Americans spend \$20 billion annually for dietary supplements; it's estimated that if just 1/3 of the Canadian population followed Vieth's recommendation, sales of vitamin D supplements would increase by \$2 billion annually. 74 Other vitamin D proponents have more obvious commercial interests. Dairy farmers' associations fund vitamin D studies. Food manufacturers are eager to add vitamin D to their products and promote them as healthier in order to increase sales. Vitamin D is big business; in 1925, the first vitamin D patent made the University of Wisconsin the richest chemistry department in the world. The Wisconsin Alumni Research Foundation now holds 162 vitamin D patents, which contribute about \$45 million per

year to the University. 75 Many others are eager to share in the profits; the U.S. patent office has over 1,000 patent entries for vitamin D analogs or vitamin D testing. 76

### **Purported Causes of Vitamin D Deficiency**

Vitamin D proponents reiterate many reasons why they believe the general population is unable to obtain enough vitamin D without the aid of supplementation. These beliefs are often based on outdated or limited studies and can be challenged with more recent research.

***Skin pigmentation.*** The ‘early man’ theory of low vitamin D posits that melanin pigmentation evolved in people living near the equator to prevent the excessive production of vitamin D due to constant exposure to sunlight. As people migrated away from the equatorial regions, their sunlight exposure was shortened and, in order to allow adequate production of vitamin D and prevent rickets, the melanin levels in their skin diminished. 77 This evolutionary rationale for low serum 25-D levels is highly speculative. Rick Potts, 78 paleoanthropologist and curator of anthropology at the Smithsonian's National Museum of Natural History, points out, “The study of human evolution has long sought to explain major adaptations and trends that led to the origin of Homo sapiens. Environmental scenarios have played a pivotal role in this endeavor. They represent statements or, more commonly, assumptions concerning the adaptive context in which key hominin traits emerged. In many cases, however, these scenarios are based on very little if any data about the past settings in which early hominins lived.” Robins 79 disputes the idea that light skin evolved to allow more vitamin D production. Other critics of this hypothesis point out there are no reported cases of hypercalcemia secondary to vitamin D toxicity as a sole consequence of prolonged sun exposure (i.e., photosynthesis of D<sub>3</sub> is stopped at normal levels by a protective regulation mechanism in the skin). Many factors (e.g., protection of sweat glands, sunburn, frostbite, skin cancer, defense against microorganisms, etc.) may have played a role in the evolution of skin color. 80 One study found evidence that low vitamin D levels among Africans may be due to genetic variation tied to African ancestry 81 but genetic studies of Caucasians have found no link to skin pigmentation. 82,83 Even prominent experts are in seeming disagreement about the role skin pigmentation plays in vitamin D production. Believing our dark-skinned ancestors produced higher 25-D levels than modern man, Heaney 84 opined, “It is highly likely that values of 32-60 ng/ml reflect the status in which human physiology developed.” While Holick reports 85, “Increases in skin pigmentation markedly diminish the production of vitamin D.”

A number of studies have established the relationship of skin pigmentation to the capacity to produce vitamin D<sup>3</sup> following UVB exposure 86,87 but the results are not consistent. This study concluded, “...in blacks and whites, UVB exposure produces similar elevations of serum 25-D concentrations and unchanged calciotropic hormones concentrations.” 88 Low 25-D levels in Indians living in India 89 and Chinese in China 90 does not support the hypothesis that low levels seen in people with more pigmentation are due to lack of photosynthesis from the sun at higher latitudes. Other studies have shown that the response to UV dose is non-linear and also dependent on genetic factors, 91 dose of UV exposure, and baseline serum 25-D levels. 92 Melanin pigmentation is only one factor that determines the amount of vitamin D<sub>3</sub> which is photosynthesized. As early as 1994, it was known “The skin of some vertebrates contains lower quantities of 7-dehydrocholesterol and its photochemical conversion to cholecalciferol is quite

inefficient." 93 In 2010, researchers at the Department of Dermatology in Copenhagen, Denmark measured the baseline serum 25-D and total cholesterol levels of 182 fair-skinned and dark-skinned subjects; and studied the effect of UV radiation on their serum 25-D levels. They found the amount of serum 25-D produced was determined by the amount of cholesterol in the skin, not on skin pigmentation. 94 Most importantly, skin pigmentation doesn't negatively affect vitamin D status. 95 Persons with dark skin compensate for low 25-D by rapidly converting it to the active 1,25-D metabolite, thus allowing them to maintain adequate vitamin D status. 96 Matsuoka et al 97 investigated the effect of racial pigmentation on vitamin D<sub>3</sub> formation, simulating the process with a fixed dose of UVB radiation and concluded that while racial pigmentation has a photo-protective effect, it does not prevent the generation of normal levels of active vitamin D metabolites. *The concern about dark skin and vitamin D deficiency appears to be misplaced.*

**Latitude.** An early study done in Boston and Canada is often cited to support the conviction that latitude dramatically influences the amount of solar radiation available to synthesize vitamin D<sub>3</sub>. 98 However, authorities who conducted recent studies refute this hypothesis. Kimlin 99 concluded, "It may no longer be correct to assume that vitamin D levels in populations follow latitude gradients." And Lubin 47 stated, "Geophysical surveys have shown that UV-B penetration over 24 hours, during the summer months at Canadian north latitudes when there are many hours of sunlight, equals or exceeds UV-B penetration at the equator." In addition, our bodies have mechanisms for preserving the vitamin D we acquire during the summer; which have evolved to stabilize and maintain serum levels of vitamin D in environments with variable vitamin D availability. The DBP optimizes and stores 25-D for later use; it also binds 1,25-D, as well as the parental vitamin D itself. DBP sequesters vitamin D sterols in the serum, prolongs their serum half-lives, and provides a circulating store of vitamin D to meet transient periods of deficiency. 21 The IOM states, "*Ample opportunities exist to form vitamin D (and store it in the liver and fat) from exposure to sunlight during the spring, summer, and fall months even in the far north latitudes.*" 47

**Clothing and sunscreen lotion.** Clothing is a barrier to ultraviolet radiation 100 but this is an issue only for people who cover themselves from head to toe (e.g., woman who wear a burka may not be exposed to sufficient sunlight). It takes relatively little sunlight exposure to acquire adequate stores of vitamin D and few people wear enough clothes to prevent that from happening. The National Institutes of Health (NIH) advises, "Ten to 15 minutes of sunlight or daylight exposure to a small area of skin (e.g., the forearm or face, etc.) twice a week (without sunscreen) supplies all the vitamin D necessary for health." 9

The belief that sunscreen lotion blocks vitamin D production is based on a 1987 study funded by the ultraviolet foundation, which is supported by the tanning bed industry. 101 More accurate information was provided by a 2010 study which concluded: "Emerging evidence suggests that although sunscreens are effective, many may not actually be blocking UV-B because they are improperly or inadequately applied. *Thus, sunscreen use may not actually diminish vitamin D synthesis in real world use.*" 102 As reported by ABC News on May 21, 2009, (according to a survey of 1,000 adults by the Consumer Reports National Research Center) 31 percent of Americans reported not using sunscreen, while 69 percent were occasional users. In the ABC News report, Dr. Michele McDonald, a dermatologist and assistant professor of medicine at

Vanderbilt University Medical Center in Nashville, Tenn., stated that recent concerns about regular sunscreen use limiting vitamin D intake are unfounded. "If you're out and you're getting sun, you're getting vitamin D; after 30 to 40 minutes, it's getting through [sunscreen]." Although dermatologists express concern about UV radiation leading to skin cancer, the amount of sun exposure required to produce adequate vitamin D is unlikely to damage skin cells. 103 Vitamin D compounds have a protective effect against DNA photo damage. 104 Dr. Robyn Lucas, 105 an Australian epidemiologist stated, "*Far more lives are lost to diseases caused by lack of sunlight than by those caused by too much.*" 106,107

***Pollution, tall buildings, shade and clouds.*** Although pollution can block some ultraviolet radiation, even in urban areas of high pollution 50% of UV rays reach the ground. 4 This study concluded, "Reductions in atmospheric ozone, due to pollution, are expected to result in higher amounts of UV-B radiation reaching the earth's surface." 108 A significant amount of UV radiation exposure can be obtained in dense metropolitan areas. Tall buildings provide shade but shade gives up to 50% of UV rays. Indoor workers receive 10% to 20% of outdoor workers' yearly UV exposure; 4 and for many, this may be adequate, especially if sunlight exposure is higher when they are not working. UV radiation is reflected or scattered to varying extents by different surfaces. For example, fresh snow can reflect as much as 80% of UV radiation, sand about 25% and UV radiation is still 40% as intense at half a meter under water. 4 UV radiation levels are highest under cloudless skies but even with cloud cover, UV levels can be high. The scattering and absorption of light by clouds may not significantly reduce natural light exposure because over 90% of UV rays may penetrate clouds. Scattering can have the same effect as the reflectance by different surfaces and thus increase total UV radiation levels. 4 *These environmental factors are rarely an impediment to photosynthesis of adequate vitamin D.*

***Elderly skin and diet.*** As the skin ages, there is a decline in the cutaneous levels of 7-dehydrocholesterol and alteration of skin morphology, resulting in a marked reduction of the skin's capacity to produce vitamin D<sub>3</sub>. 109 However, despite the up to fourfold reduction in vitamin D<sub>3</sub> production in a 70-year-old compared to a 20-year-old, the skin has such a high capacity to make vitamin D<sub>3</sub>, *elders exposed to sunlight will produce an adequate amount of vitamin D<sub>3</sub> to satisfy their vitamin D requirement.* 110, 111

Natural processes favor photosynthesis but, in many developed countries, it's possible to obtain the recommended vitamin D AI, or a substantial portion of it, from food. A typical Western diet contains many foods that are fortified with vitamin D and some that naturally contain vitamin D (e.g., eggs, fatty fish, cheese, etc.), making it relatively easy to ingest a significant amount. For example, food commonly eaten in one day (e.g., 3 cups of milk, 1 serving of fortified cereal, 1 cup of fortified juice and 3 ounces of cheese) supplies 600 IU of vitamin D – the AI for those who are not exposed to sunlight in the Spring or Summer.

## **Hypovitaminosis-D**

Studies of healthy subjects have found levels of 25-D that, by some vitamin D expert's standards, are declared deficient (hypovitaminosis-D). 112

- In a 2009 meta-analysis of studies on healthy subjects worldwide, the mean 25-D was 21.62 ng/ml. 113
- In a 2006 study, 80% of healthy Bangladeshi women had a 25-D level under 16 ng/ml. 114
- A 2011 study found healthy young female hospital employees in Oman had 25-D levels below 20 ng/ml. 115
- Among healthy young adults in India, a 2011 study found the average level of 25-D was 17.4 ng/ml. 116
- A 1992 study found healthy full-term infants in China had 25-D levels of 5-14 ng/ml and none developed rickets. 117
- A 2012 study of Saudi medical students found the mean 25-D level was 10.74 ng/ml in males and 6.42 ng/ml in females. 118
- The average 25-D level of a cross-section of healthy Calgary citizens, in 2002, was 23 ng/ml. 119

Vitamin D levels that are considered deficient have even been found in persons who are exposed to abundant sunlight. In 2007, a study showed a mean 25-D level of 31.6 ng/ml among healthy young adult Hawaiian surfers. 120 This finding is a good illustration of the action of regulatory mechanisms in the skin which prevent overproduction of vitamin D<sub>3</sub>.

### **Low Vitamin D May be Normal**

It's clear that low levels of 25-D are found in both healthy persons and those with autoimmune or chronic inflammatory diseases. Opposing reasoning can be used to explain this contradiction. One explanation reasons that healthy persons with low 25-D will become sick; however, studies don't support this hypothesis. The more logical explanation may be that, in the absence of disease, low 25-D is normal.

A couple conundrums also support the thesis that low 25-D is normal in healthy persons. Breast milk, unarguably the perfect food, is low in vitamin D and remains relatively low even when lactating mothers take very high doses of vitamin D<sub>3</sub>. 121 However, the vitamin D biological activity in breast milk may be higher than the analyzed values, because human milk contains small amounts of 25-D in addition to vitamin D<sub>3</sub>; 122 further, the biological activity of 25-D is approximately 50 percent higher than that of vitamin D. 123 Secondly, studies show blacks have higher adjusted bone mineral density than whites and a slower age-adjusted annual rate of decline in bone mineral density. 124 In data from the National Health and Nutrition Examination Survey (NHANES), very few white children aged 1-12 years are vitamin D deficient, using the classic threshold of 15 ng/ml. In contrast, about 10% of black 1-6 year olds are vitamin D deficient, as are close to 30% of those in the 7-12 years old age bracket. 125 If vitamin D deficiency is rampant in blacks, why do they have greater bone strength and muscle mass, on average, than whites? Perhaps the answer to both these puzzles is that *low vitamin D is not a sign of deficiency in healthy individuals.*



**Fig. 1** Since low 25-D is found in both healthy persons and those with autoimmune or chronic inflammatory diseases, assessing vitamin D status with measurement of 1,25-D may be helpful

***Low vitamin D in the presence of inflammatory disease.*** Since low 25-D is found in both healthy persons and those with autoimmune or chronic inflammatory diseases, assessing vitamin D status with the measurement of an additional clinical marker may be helpful. (Fig. 1) It is assumed that low levels of 25-D accurately indicate vitamin D status; (i.e., vitamin D storage and VDR-mediated control of calcium metabolism and innate immunity). However, measurement of 1,25-D often demonstrates a positive correlation of elevated 1,25-D to inflammatory diseases. This is illustrated by a study of 100 patients with autoimmune and chronic disease which found 85% of subjects had levels of 1,25-D higher than 46.2 pg/ml without hypercalcemia. 59 Although this serum level may be considered normal by some, lab ranges for 1,25(OH)<sub>2</sub> (e.g., 18-72 pg/ml) may have been skewed high by the presence of patients with unrecognized dysregulated vitamin D metabolism. The Danish 1,25-D population data (from a large and reliable study) found the mean value for 1,25-D in a normal population was 29 pg/ml with a standard deviation of 9.5. 126 *More frequent measurement of both D-metabolites in the clinical and research settings, may shed light on the real meaning of low 25-D.*

## Vitamin D Supplementation

In recent years, dietary supplements containing vitamin D have been more frequently consumed. In the United States, vitamin D can now be found in multi-vitamin/multi-mineral formulations as well as a single supplement in a range of dosage levels; including 1,000 to 5,000 IU of vitamin D<sub>3</sub> per dose and even up to 50,000 IU of vitamin D<sub>2</sub> per dose. 47 The impetus for the upsurge in supplementation is the hope that it might protect against a broad range of chronic diseases; including cancer, cardiovascular disease, osteoporosis and autoimmune disease. However, that hope is driven mostly by epidemiologic data, which should be viewed as hypothesis-generating rather than definitive. 127

### Vitamin D and Cancer

Evidence for a beneficial effect of vitamin D supplementation in cancer is lacking. 128,129 In 2011, the U.S. Preventive Services Task Force analyzed 28 observational studies for cancer outcomes and stated, “Evidence is not sufficiently robust to draw conclusions regarding the benefits or harms of vitamin D supplementation for the prevention of cancer.” 130 This statement was based on the results of a variety of cancer and vitamin D studies. For example, the Iowa Womens' Health study showed protection against breast cancer in the first five years but noted the effect was lost after ten years; illustrating the importance of long-term studies before conclusions are made. 131 The findings of a large prospective study in 2008 do not support the claim that vitamin D is associated with decreased risk of prostate cancer; in fact, higher circulating 25-D concentrations may be associated with increased risk of aggressive disease. 132 On March 5, 2013, the U.S. Food and Drug Administration (FDA) advised that marketing of calcitonin nasal spray for the treatment of osteoporosis be stopped, citing lack of benefit and concerns about a possible cancer risk. 133

### Vitamin D and Cardiovascular Disease

There's no conclusive evidence vitamin D supplementation affords protection against heart disease. 134, 135 A randomized, placebo-controlled study, done at Rockefeller University in New York, found vitamin D supplementation failed to improve lipid profiles 136 and elevated 25-D was associated with carotid and aorta artery plaque in a 2012 study. 137 At a symposium sponsored by the American College of Rheumatology Dr. Lenore Buckley 138 (professor of internal medicine at Virginia Commonwealth University) commented on the study:

One of these concerns is that not all of the extra calcium absorption promoted by boosting vitamin D is going into bone to prevent fractures. Some of it may actually be taken up by atherosclerotic plaque, increasing the risk of cardiovascular events. The question we have to ask is: What does that low serum vitamin D level mean? Is it the thing that predisposes, or is it somehow a byproduct of illness?

Regarding supplementation to prevent cardiovascular disease, Dr. Wayne 139 (Professor of Medicine-Cardiology, Gill Heart Institute, University of Kentucky, Lexington, KY) concluded, "...potential benefit of supplementation must be weighed against the current absence of definitive outcomes studies."

### **Vitamin D and Rickets**

Rickets is a softening of bones in children due to deficiency or impaired metabolism of vitamin D, phosphorus, or calcium. 140, 141, 142 Hypophosphatemia is the common denominator of all rickets; low calcium intake leads to hyperparathyroidism, which leads to high phosphorus excretion and, thus, phosphorus deficiency. 143 *Adequate vitamin D is essential to prevent rickets, but adequate calcium is equally important*; if either calcium or vitamin D is deficient, bone health suffers. Rickets is rare in the developed world; 144 however, children in developing countries, who usually photosynthesize enough vitamin D from sunlight, develop rickets if poverty prevents them from eating enough calcium rich food. 145 Studies found rickets occurs in sunny countries due to poor calcium intake 146, 147 and is cured with increased calcium ingestion. 148 149

### **Vitamin D and Osteoporosis**

Osteoporosis is a bone disease characterized by a decrease in bone mineral density and the appearance of small holes in bones due to loss of minerals. 150 Vitamin D is an important factor in maintaining bone health to avoid osteoporosis. 47 Precise maintenance of the physiologic levels of both extracellular and intracellular ionized calcium is essential to life 151; 1,25-D maintains calcium homeostasis between blood, cells and bones by stimulating calcium absorption from the intestines, reabsorption in the kidneys, and resorption in bones. 1,25-D up-regulates VDR in the small intestine, which then transcribes genes that shuttle calcium and phosphorus through the intestinal epithelium. *However, mucosal response and calcium/phosphorus absorption is dependent on a competent VDR and elevated 1,25-D reduces VDR competence.* 152 Thus, calcium and phosphorus absorption may be inhibited if VDR function is impaired by elevated 1,25-D.

Although some studies show vitamin D and calcium supplements increase bone density slightly and decrease the risk of falls and fractures in certain populations, the quality of evidence is poor. 153 A 2013 report by the U.S. Preventive Services Task Force recommends against vitamin D supplementation for the primary prevention of fractures in non-institutionalized, pre or post-menopausal women or older men. 154 The 2005 RECORD study concluded, "...routine supplementation with calcium and vitamin D<sub>3</sub>, either alone or in combination, is not effective in the prevention of further fractures in people who had a recent low-trauma fracture." 155 A similar study stated, "We found no evidence that calcium and vitamin D supplementation reduces the risk of clinical fractures in women with one or more risk factors for hip fracture." 156 A 2008 study found, "Vitamin D supplementation adds no extra short-term skeletal benefit to calcium citrate supplementation even in women with vitamin D insufficiency." 157 And a study at the Bone Mineral Research Center, Winthrop University Hospital, Mineola, NY showed that "Additional intake of 100 mcg vitamin D<sub>3</sub> did not lower PTH or markers of bone turnover." 158

***Elevated 1,25-D may cause osteoporosis.*** In fact, there is ample evidence that elevated 1,25-D leads to bone loss. In 1999, Brot et al 159 found "...elevated levels of 1,25-D were strongly associated with decreased bone mineral density and content, and increased bone turnover." When levels are above 42 pg/ml 1,25-D stimulates bone osteoclasts. This leads to osteoporosis, dental fractures and calcium deposition into the soft tissues: lungs, breasts, muscle bundles, kidneys." 160 An earlier study warned, "Vitamin D is a toxic compound, and excessive amounts can cause soft-tissue calcification. There is a narrow leeway between the amount required and that initiating tissue damage." 161 Kawamori et al 162 found that, "Elevated 1,25-D induces increased production of osteoclasts from stem cells." The EMAS study found that "A combination of high 1,25-D and low 25-D is associated with the poorest bone health." 163 *This significant evidence regarding bone loss should motivate medical practitioners and researchers to measure both 25-D and 1,25-D to determine vitamin D status.*

### **Vitamin D and Autoimmune Disease**

The cause of autoimmune disease is unknown; the prevailing theory states that an overactive immune system produces auto-antibodies against self. A logical alternate hypothesis posits a bacterial etiology in which a persistent intracellular infection causes a cytokine release that induces signals to T cells and B cells, 164 and the antibodies they produce (to the intracellular invader) include some that attack human proteins, as well as target the pathogens. 165 In other words, when an innate immune system is forced to respond to a persistent infection, the resulting cascade of chemokines and cytokines will also stimulate an adaptive response. Hintermann explored this hypothesis: "In theory, a structural similarity or identity between the host and an invading pathogen might cause the immune system of the host to react not only to the pathogen but also to self-components." 166 Infections can act as environmental triggers inducing or promoting autoimmune disease in genetically predisposed individuals; 167 researchers have shown how antinuclear antibodies (ANA) are created in response to infectious agents. 168, 169 170, 171 Microbial and human metabolites often have very similar structures; and interactions between human and bacteria genomes may make it difficult for the body to distinguish "foreign" from "self". Infections (bacterial, viral and parasitic) are known to induce and exacerbate autoimmune diseases, mainly by the mechanism of molecular mimicry." 172

Vitamin D reduces autoimmune disease symptoms via immune system suppression. 173 Vitamin D inhibits pro-inflammatory processes by suppressing the enhanced activity of immune cells that take part in the autoimmune reaction. 174 Exposure to ultraviolet light, especially UV-B wavelengths, can impair immune responses in animals and humans. 175 Seasonal variation can have an impact on the immune response; in the summer, when vitamin D<sub>3</sub> is highest, 1,25-D down-regulates the immune system. 176 Reduced immunity following exposure of skin to UV radiation may explain the positive latitude gradient measured for a number of autoimmune diseases (decreased incidence of disease with residence at lower latitudes). 177 As a result of vitamin D immunosuppression inflammation, clinical disease markers and symptoms of autoimmune disease are reduced but this doesn't treat the underlying cause and relapse is common. 178 Unfortunately, some researchers believe immunosuppression is a good thing. 179 Much of current research focuses on finding drugs to suppress inflammation but, according to Francis S. Collins 180 (NIH director), 95% of these studies have failed. It seems clear a better

direction is needed.

### **Effect of Vitamin D Supplementation**

Despite vitamin D supplementation chronic diseases have increased. More foods than ever before are fortified with vitamin D; the Nutrition Business Journal reported sales of vitamin D supplements have skyrocketed to \$425 million in 2009 from just \$40 million in 2001. 181 Vitamin D supplement proponents promised double digit declines in chronic disease yet, between 2000 and 2010, the percentage of adults aged 45-64 (and 65+) with two or more (of nine selected) chronic conditions, increased for both men and women, all racial and ethnic groups examined, and most income groups. 182 As reported by the Partnership to Fight Chronic Disease, more than one in four Americans lives with multiple chronic conditions, including one in 15 children. 183 Almost \$2 out of \$3 spent on health care in the U.S. is directed toward care for the 27% of Americans with multiple chronic conditions 184 and chronic illness is expected to continue increasing. 185

The research evidence supports a positive role for vitamin D supplementation in bone health but not in other health outcomes. According to our most respected medical experts, “Outcomes related to autoimmune disorders, cancer, cardiovascular disease and hypertension, diabetes and metabolic syndrome, falls and physical performance, immune functioning, infections, neuropsychological functioning, and preeclampsia could not be linked reliably with calcium or vitamin D intake and were often conflicting.” 47 The majority of the findings concerning vitamin D, calcium, or a combination of both nutrients on the different health outcomes were inconsistent. 186 Genetic findings in those predisposed to longevity cast doubt on whether low levels of vitamin D cause age-related diseases and mortality. 187 A study done at Tufts Medical Center’s Division of Rheumatology concluded, “Vitamin D supplementation for two years at a dose sufficient to elevate 25-D plasma levels to higher than 36 ng/ml, when compared with placebo, did not reduce knee pain or cartilage volume loss in patients with symptomatic knee osteoarthritis.” 188 Subjects supplemented with high doses of vitamin D to increase 25-D levels from 20.9 ng/ml to 40.1 ng/mg, saw “...no improvement in serum lipids, HbA1c, or HS-CRP with high dose vitamin D supplementation. If anything, the effect was negative.” 189

Vitamin D deficiency or insufficiency can occur in certain situations. Genetic defects in the VDR may result in vitamin D deficiency; a number of mutations have been identified that lead to hereditary vitamin D resistance. 190 Disorders that limit vitamin D absorption and conditions that impair conversion of vitamin D into active metabolites (e.g., certain liver, kidney & hereditary disorders) may cause deficiency. 191 Sick or elderly people who rarely go outdoors and have poor diets are also at risk. 109 Vitamin D supplementation may be appropriate in these special conditions but the evidence indicates it’s not appropriate to supplement the general population.

**Experts urge caution.** More experts are beginning to reconsider vitamin D supplementation among the general population. 192 In the Leiden Longevity study, low levels of 25-D "cast doubt on the causal nature of previously reported associations between low levels of vitamin D and age-related diseases and mortality." 187 This 2009 study 193 found that depressive symptoms were not associated with low vitamin D and researchers have expressed concern that high

vitamin D levels in pregnancy and at birth may contribute to a higher risk for food allergy in children. 194 Recommending higher vitamin D intake to large populations carries the potential risk of overdosing certain individuals; for example, vitamin D supplementation increased mortality among institutionalized elderly women. 195

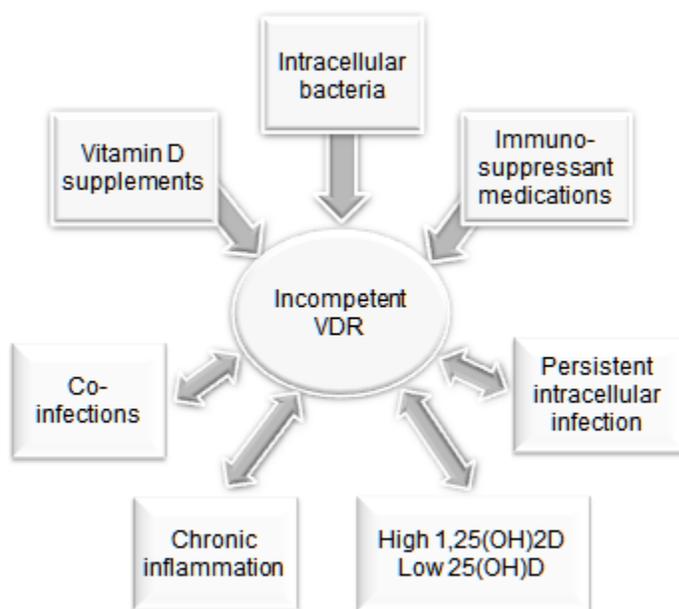
Excessive vitamin D can be toxic to humans and to animals. This is dramatically illustrated in the use of rodenticides with a high vitamin D content (e.g., True Grit Rampage & Ortho Rat-B-Gone) and when livestock die following ingestion of plants that contain 1,25-D (e.g., *cestrum diurnum*). 196 DeLuca et al 197 concluded that 25-D is the metabolite responsible for vitamin D toxicity because high concentrations can bind the VDR and induce transcription of CYP27B1. The immediate effects of vitamin D toxicity (hypervitaminosis-D) are hypercalcemia, hypercalciuria, bone resorption, and calcification of soft tissues. 197 People with high blood calcium or phosphorus levels, heart problems, kidney disease, sarcoidosis, and tuberculosis are at highest risk. 198 It would be difficult to ingest too much vitamin D from food, and natural mechanisms regulate the amount of vitamin D<sub>3</sub> photosynthesized from sunlight; within about 20 minutes of ultraviolet exposure in light-skinned individuals (3–6 times longer for pigmented skin), the concentrations of vitamin D precursors produced in the skin reach an equilibrium, and any further vitamin D that is produced is degraded. 199 However, elevated 25-D and vitamin D toxicity may occur due to vitamin supplementation. 191

Researchers are beginning to realize excess vitamin D may cause other problems in addition to toxicity. Muhammad Amer, M.D., 200 an assistant professor in the division of general internal medicine at the Johns Hopkins University School of Medicine, said “People taking vitamin D supplements need to be sure the supplements are necessary. Those pills could have unforeseen consequences to health even if they are not technically toxic.” When cancer studies (breast and colorectal) from the Womens' Health Initiative, showed no protective effects of vitamin D supplementation, the researchers suggested the dose of vitamin D might have been too low. However, the results of the studies actually showed trends toward potential harmful effects of high vitamin D intake. 201 Researchers like Dr. Anja Olsen 202 are concerned that the discussion on potential negative effects of vitamin D supplementation (besides those of toxicity) is very limited. “...the past has shown us (with the history of  $\beta$ -carotene and lung cancer as the scariest lesson) that observational studies on micronutrient blood levels cannot always be extrapolated to positive effects of high-dose supplementation.” Dr. Michael J. Glade 203 believes 25-D may appear to be low for reasons totally independent of sunlight exposure or vitamin D intake. He expresses concern that local tissue vitamin D intoxication may be present in individuals with much lower serum 25-D concentrations than are currently appreciated and that prolonged routine consumption of megadoses of vitamin D may interfere with the regulation of phosphate homeostasis by fibroblast growth factor-23 (FGF23) and the Klotho gene product, with consequences that are detrimental to health. This 1983 study sounded an early alarm,

The requirement for vitamin D is normally met by its synthesis in the skin. In the United States, various foods are fortified with vitamin D to ensure that deficiencies do not occur. As a result, most individuals consume and synthesize more vitamin D than they require. As most individuals appear to be at risk of obtaining too much vitamin D rather than too little, we suggest that fortification of foods with vitamin D should be curtailed, preferably abolished, that excessive fortification of animal foods be reduced to the level required,

and that the use of dietary supplements be restricted. Populations at risk could be monitored closely and counseled to prevent vitamin D deficiency. 204

Because adverse effects of vitamin D supplementation may take decades to be realized, more clinicians (mindful of the precept 'First, do no harm') are erring on the side of caution and waiting for the results of long-term vitamin D studies.



**Fig. 2** Cell-wall-deficient (CWD) bacteria invade nucleated cells and use strategies to avoid destruction. Excess 1,25-D is produced in an effort to up-regulate the VDR to transcribe AMPs; and 25-D is rapidly metabolized in the process, resulting in a low serum level. The resulting elevated 1,25-D causes chronic, systemic inflammation and its accompanying symptoms

## Putative Etiology of Low Vitamin D

Vitamin D proponents use a disease deficiency model to explain low levels of 25-D. Their hypothesis states low 25-D causes chronic diseases; however, a pathogenesis has not been elucidated. Low serum 25-D in the presence of disease can also be explained with a dysregulated vitamin D model. This hypothesis proposes that low vitamin D is the consequence of a chronic inflammatory process caused by persistent infection.

## **Bacterial Pathogenesis**

The bacterial pathogenesis theorizes that intracellular bacteria cause abnormal vitamin D endocrine function, resulting in low vitamin D. Specifically, cell-wall-deficient (CWD) bacteria invade nucleated cells and use strategies to avoid destruction. Excess 1,25-D is produced in an effort to up-regulate the VDR to transcribe AMPs; and 25-D is rapidly metabolized in the process, resulting in a low serum level. The resulting elevated 1,25-D causes chronic, systemic inflammation and its accompanying symptoms. (Fig. 2) Gerald J Domingue, PhD., Professor Emeritus of Tulane University School of Medicine commented, “This might translate into an etiology for chronic inflammatory diseases, when the stressed bacteria increase in numbers and overwhelm the normal biological functions of the host.” 205 Gabriel Nunez, M.D., 206 Professor of Pathology at the University of Michigan Medical School, was quoted in the university newsletter, “In our study, the presence of bacterial microbes inside the cell is what triggers the immune response.” 207 In 2007 French researchers observed, “...the presence of pathogenic invasive bacteria could be the link between innate immune response to invasive bacteria and the development of the inflammation.” 208 Dr. Siobhan O’Connor, 209 assistant to the director of the National Center for Infectious Diseases, stated, “The epidemiologic, clinical, and pathologic features of many chronic inflammatory diseases are consistent with a microbial cause. Infectious agents likely determine more cancers, immune-mediated syndromes, neurodevelopmental disorders, and other chronic conditions than currently appreciated.”

## **Immune System Suppression**

A known effect of vitamin D is suppression of the immune system. In a study of a pro-inflammatory molecule, lipopolysaccharide (LPS), Lemire 210 found elevated 25-D reduced the inflammatory cascade. Low levels (below 30 ng/ml) failed to inhibit the LPS inflammatory cascade but higher levels (30 ng/ml) inhibited inflammatory signaling. The highest levels of inflammatory inhibition occurred at 50 ng/ml. 25-D can also be indirectly immuno-suppressive by being converted to excess 1,25-D. 211 A 2007 study concluded, “On the whole, vitamin D confers an immunosuppressive effect.” 174 Theoretically, immune system suppression allows parasitic microbes to persist and proliferate in host phagocytes, successfully compete for nutritional resources and displace commensal organisms from their niche. 212

## **Intracellular Infection**

The existence of bacteria which are capable of invading human cells has been known about for over a century and a number of studies suggest disease associations. 213, 214, 215, 216 These novel bacteria are identified by several names: nanobacteria, pleomorphic, stealth, coccoid bodies and L-forms. L-forms are bacterial variants with defective cell walls and irregular growth and multiplication. (Fig. 3) They arise after the exoskeleton of the bacterial cell wall has been either degraded by bacteriolytic enzymes, or its biosynthesis has been disturbed by antibiotics and other inhibitors, or by defect mutations in essential genes for cell wall synthesis. L-forms were discovered in 1935 by Emmy Klieneberger and subsequently described by many authors. 217, 218 The cell wall deficient (CWD) bacteria are pleomorphic (i.e., they can change size and

shape) and many are smaller than viruses (0.01 microns in diameter). 219 They're too small to be seen with normal optical microscopes 220 and they can survive under extreme conditions. 221



**Fig. 3** Peptidoglycan sacculi isolated from L-form spheroplasts of *P. mirabilis*. Electron micrograph of Pt-Ir-shadowed sample. 20,000X [ 222]

CWD bacteria should be of interest for their putative pathogenic role but many microbiologists are unfamiliar with them or complain about the unusually labor-intensive and time-consuming process of L-form isolation and cultivation. However this can be overcome by patient determination. 222 Diagnosing the presence of CWD bacteria is problematic because they're very slow-growing 223 and difficult to culture; 221 conditions must be similar to those in the human body (some labs, such as Mattman's at Wayne State University, have been successful using blood agar at very specific pH and temperatures). 224 CWD bacteria aren't detected by antibody tests because they're able to persist for a long time inside cells (in the presence of inflammation, the life-span of a macrophage may be months) 225 so very few antibodies are created in response to their presence. 226 When the compromised phagocytes die (CWD bacteria use biochemical mechanisms to delay apoptosis) 227 it might be possible to see CWD bacteria with PCR (polymerase chain reaction) testing if the probe sequence is general enough, but there are many limitations. For example, a particular sample may not be infected and the lab may look for a limited number of species (there are many strains of CWD bacteria). The application of PCR to clinical specimens has many potential pitfalls due to the susceptibility of PCR to inhibitors, contamination and experimental conditions. For instance, it's known that the sensitivity and specificity of a PCR assay is dependent on target genes, primer sequences, PCR techniques, DNA extraction procedures, and PCR product detection methods. 228 Biopsy testing doesn't usually look for CWD bacteria and they would be difficult to identify because they're destroyed when taken out of the body; their protective homeostasis is lost and lysosomes in the immune system destroy them. 229

The inability of most research labs to culture CWD bacteria 229 has been an obstacle to their acceptance, and reliance on Koch's postulates has made it difficult to correlate CWD bacteria to specific diseases. But some researchers believe Koch's postulates may have to be redefined in terms of molecular data when dormant and non-culturable bacteria are implicated as causative agents of mysterious diseases. 219 In *Emerging Infectious Determinants of Chronic Diseases*, the authors report "microbes can now be irrefutably linked to pathology without meeting Koch's

postulates”.... and “...powerful tools of molecular biology have exposed new causal links by detecting difficult-to-culture and novel agents in chronic illness settings”. 209

CWD bacteria are ubiquitous and easily acquired throughout life. They're found in water, 230 food, 231 air, 232 soil, 233 and blood products. 234 They may pass the placental barrier 235 and some may be small enough to pass through filters of injectable medications. Acquisition of a variety of CWD bacteria, from a variety of sources, may begin in utero. 235 CWD bacteria grow very slowly but the process may be accelerated by exogenous immuno-suppressants. 236 High levels of stress can also lower immunity by inhibiting lymphocyte populations, natural killer cell activity, and antibody production. 237 The authors of *Emerging Infectious Determinants of Chronic Diseases* state,

A spectrum of diverse pathogens and chronic syndromes emerges, with a range of pathways from exposure to chronic illness or disability. Complex systems of changing human behavioral traits superimposed on human, microbial, and environmental factors often determine risk for exposure and chronic outcome. 209

CWD bacteria are considered communicable but not contagious; protective immunity depends on an effective cell-mediated immune response. 238 It's now well appreciated that pathologic processes caused by infectious agents may emerge clinically after an incubation of decades. 239 Among the speculated causes of the increase in chronic infection are overuse of Beta-lactam antibiotics (which force bacteria into CWD form) 240, 241 and immunosuppression via medications or excess vitamin D. Almost 60 L-forms have been identified since they were first discovered in the late nineteenth century and research continues. 242, 243 Many microbiologists now believe at least some, if not all, of the inflammation which drives the chronic disease process is caused by the presence of pathogens. 244 In a book review John McDougal 245 writes, “The editors of *Infection and Autoimmunity* boldly state that reading the chapters in this book brings one to the conclusion that all autoimmune diseases are infectious, until proven otherwise.” A considerable body of experimental and clinical evidence supports the concept that difficult-to-culture and dormant bacteria are involved in latency of infection and that these persistent bacteria may be pathogenic. 219, 246, 247, 248 The Center for Disease Control and Prevention states, "Evidence now confirms that non-communicable chronic diseases can stem from infectious agents." 245

### **Effects of Intracellular Pathogens**

The human body is colonized by a vast number of microbes, and microbial colonies gain many advantages by parasitizing immune cells and altering nuclear receptor activity. Tissue invasion provides a privileged niche with access to host protein and iron; sequestration from immune response and a means for persistence. 242 Microbicidal mechanisms of macrophages are thought to be responsible for the control and elimination of intracellular pathogens; 249 1,25-D production and action in human macrophages activates toll-like receptors to increase expression of the AMP cathelicidin and kill the infectious invaders. 250 As a result, immune defenses are stimulated; Th<sub>17</sub> cells mobilize host immunity and contribute to the development of harmful inflammatory conditions. 251

Microbial cells outnumber human cells by a factor of ten 252 and increasing pathogens in the microbiome may ultimately cause pathogenic interferences between intracellular bacteria and host cells; resulting in various clusters of disease symptoms. Phagocyte-inflicted tissue damage plays an important role in many chronic diseases. 253 Intracellular pathogens have developed innovative strategies to evade immune responses and persist in cellular cytoplasm. 254 They induce stress responses that protect the cell from lethal factors, 255 express proteases that degrade AMPs, 256 use biofilms as a shield, 257 and secrete substances that block the VDR. 258 Genetic foreign and host protein interactions alter gene transcription and translation mechanisms 259 and many species survive by horizontal gene transfer. 252 Autoimmune patients acquire a distinct pathogenic microbiota 260 and multi-morbidity often results. 261 Intracellular bacteria modulate cytokine production; 262 and in human monocytes and macrophages, cytokine activation markedly inhibits 1,25-D/VDR gene transcription. 263 When the immune system is fighting a persistent microbe, it continually releases inflammatory molecules in an effort to kill the pathogen. 264 This dysfunctional immunological response produces low-grade inflammation which is a component of many chronic and autoimmune diseases.

## **Dysregulated Vitamin D Metabolism**

Theoretically, the immune system detects and responds to the presence of CWD bacteria by producing more 1,25-D to activate the VDR and express the crucial endogenous AMPs which allow the innate immune system to target intracellular pathogens. Renal production of 1,25-D is tightly self-regulated with the end product down regulating its own further production; by contrast some forms of extra-renal production from diseased tissues show no such tight self-regulation. 265 Extra-renal tissues known to synthesize 1,25-D include uterine decidua and placenta, bone cells, keratinocytes, colon, breast, prostate, spleen, melanoma cells, synovial, and pulmonary monocytes and macrophages. Extra-renal production of 1,25-D is regulated by cytokines (e.g., interferon-gamma), lipopolysaccharide, nitric oxide and intracellular VDBP, which activate the enzyme CYP27B1 to stimulate conversion of 25-D to 1,25-D. 266 In this way, excess production of 1,25-D may contribute to low levels of 25-D.

Because extra-renal production of 1,25-D is primarily dependent on the availability of 25-D, 267 supplementation with vitamin D to increase 25-D may promote the production of 1,25-D in non-renal tissues that are sites of intracellular infection and result in hypervitaminosis-D. Sunlight appears to play a part in this process. The skin (dermal fibroblasts and keratinocytes possess VDR) has the capacity to synthesize 1,25-D, and represents an important target tissue for 1,25-D. 268 If keratinocytes in the skin are infected, natural regulation of photosynthesis may be thwarted and solar energy may overstimulate cellular activity, resulting in an increase in cutaneous production of vitamin D<sub>3</sub>, 25-D and 1,25-D following sun exposure.

When nuclear cells are parasitized by CWD bacteria, known mechanisms 269 are believed to cause extra-renal production of 1,25-D to increase, the kidneys to lose control of 1,25-D production, and pro-hormone 25-D to decrease due to rapid conversion. Several mechanisms may play a part in the process:

- Inflammatory cytokines activate CYP27B1 (formerly 1 $\alpha$ -hydroxylase), an enzyme that causes more 25-D to be converted to 1,25-D. 269

- The microbial-repressed VDR can't transcribe CYP24A1 (formerly 24-hydroxylase), an enzyme that breaks down excess 1,25-D.
- Excess 1,25-D binds the PXR receptor, to inhibit conversion of D<sub>3</sub> to 25-D so 25-D is down-regulated. 270
- 1,25-D inhibits the hepatic synthesis of 25-D. 271

*Thus, low 25-D may be a consequence of the inflammatory process. More studies are concluding that suboptimal circulating levels of vitamin D appear to be caused by the disease process. This 2013 study found serum 25-D was decreased following an acute inflammatory insult (i.e., orthopedic surgery) and concluded “Hypovitaminosis D may be the consequence rather than cause of chronic inflammatory diseases.”* 272 Another recent study stated:

...there may be a relationship between inflammatory processes induced by chronic overstimulation of the renin angiotensin system (RAS) and the worldwide vitamin D deficiency... In fact, the pandemic of vitamin D deficiency could be the other face of increased RAS activity, which could potentially cause a lower activity or lower levels of Vitamin D. 273

In an essay on RAS and immune response, Smith postulates that unresolved cellular stress may be caused by infectious agents with the deliberate intent to avoid adaptive immune responses. 274

### **The Compromised Immune System**

The host immune response has developed many mechanisms to neutralize and remove pathogen bacteria. In turn, pathogenic bacteria have developed mechanisms to alter and evade the host immune response. 275, 276 For example, several studies demonstrate that microbes have the ability to repress the VDR:

- Mycobacterium tuberculosis down-regulates VDR activity. 277
- Epstein-Barr Virus lowers VDR activity. 278
- HIV completely shuts down VDR activity. 279
- In VDR knockout mice, a circumstance that closely mimics extreme VDR dysregulation, 1,25-D levels increase by a factor of ten. 280

Studies also point to immune system depression and elevated 1,25-D 281 in chronic diseases:

- Sarcoidosis patients are deficient in cathelicidin despite healthy vitamin D<sub>3</sub> levels. 282
- 1,25-D is high (>60 pg/ml) in 42% of Crohn's patients. “Treatment of the underlying inflammation may improve metabolic bone disease...” 283
- 1,25-D is elevated in the synovial fluid of patients with RA. 284
- Crohn's disease decreases expression of cathelicidin. 285

Elevated 1,25-D further reduces VDR competence, 211 suppresses macrophage function, 279 and blocks the Nuclear Factor kappa-B pathway; 286 thus inhibiting immune system function. Slowing the ability of the VDR to express elements of innate immune function allows intracellular bacteria to persist in the cytoplasm of nucleated cells and increases susceptibility to extracellular co-infections that are commonly found in chronic illnesses (e.g., viruses, fungi,

parasites and cell-walled bacteria). 287 In conclusion, high levels of 1,25-D may result when dysregulation of the VDR by bacterial ligands prevents the receptor from expressing enzymes necessary to keep 1,25-D in a normal range. 59 Elevated 1,25-D appears to be evidence of a disabled immune system's attempt to activate the VDR to combat infection.

### Diagnosis of Dysregulated Vitamin D Metabolism

Vitamin D status is currently determined by measuring the level of 25-D which, presumably, reflects the levels of other D metabolites (e.g., vitamin D<sub>3</sub>, vitamin D<sub>2</sub> and 1,25-D, etc.). This measurement may not, however, provide enough information to assess vitamin D endocrine function. Measurement of both the active metabolite and its precursor is essential to diagnose dysregulated vitamin D metabolism; assays of 25-D and 1,25-D provide valuable tools to assess vitamin D status of patients. 288 Vitamin D status encompasses more than vitamin D intake. 1,25-D formation isn't directly regulated by parental vitamin D and may be affected by the same factors that cause a decrease in serum 25-D. 1,25-D has not been used as a measure associated with vitamin D nutritional status or as an intermediate marker related to health outcomes, or even routinely in vitamin D research. In the context of solving the puzzle of low 25-D, the reasons cited for this lapse fail to consider the possibility of abnormal levels in the presence of chronic inflammation:

- 1,25-D has a short half-life (hours) and fluctuates rapidly.  
*However, a high result may be discovered even at trough level.*
- 1,25-D levels are regulated by PTH, calcium, phosphate.  
*This isn't true in chronic illness when extra-renal production is prevalent.*
- 1,25-D doesn't decrease until 25-D is very low.  
*A low 25-D may be a sign that 1,25-D is abnormally high.*
- 1,25-D is only over-produced in hypercalcemic disease states such as sarcoidosis.  
*Studies show this isn't true. 59*
- 1,25-D may be elevated as a result of up-regulation of the CYP27B1 enzyme. *This begs the question "why is this enzyme elevated?"*

Measuring both 25-D and 1,25-D (and PTH, calcium, phosphate when indicated) as clinical markers in chronic disease is more likely to provide a true picture of vitamin D status, than measuring 25-D alone. 289 Measuring 1,25-D should be considered in patients with low 25-D, abnormal lab results (especially inflammatory markers), a diagnosis of autoimmune disease or other chronic inflammatory illness, or signs of chronic systemic inflammation. (Table 1.) Each test has specific parameters that must be followed to ensure accurate results. In written correspondence (2013), vitamin D researcher Martin Hewison (Professor in Residence at the David Geffen School of Medicine UCLA), stated, "I agree that 1,25-D is a forgotten component of the vitamin D and human health story - I think measurement of serum 1,25-D will be more common as LC:MS techniques improve."

**Table 1. D-metabolites Tests**

## Serum 25-D

- CPT code: 82306
- Performed at most labs
- No special handling needed
- Lowest mortality reported at 20 ng/ml
- Immunosuppression reported when higher than 30 ng/ml

## Serum 1,25-D

- CPT code: 82652
- ICD-9 codes:
  - 733.00 Osteoporosis, unspecified
  - 733.90 Osteopenia
  - 780.9 Fatigue
- A specialized lab is required 290
- Special handling of the sample is necessary- freeze for transport to avoid degradation due to agitation
- A low result may be inaccurate due to sample mishandling
- A high result is always accurate 48
- Maximum normal = 45 pg/ml

**Restoring VDR Competence**

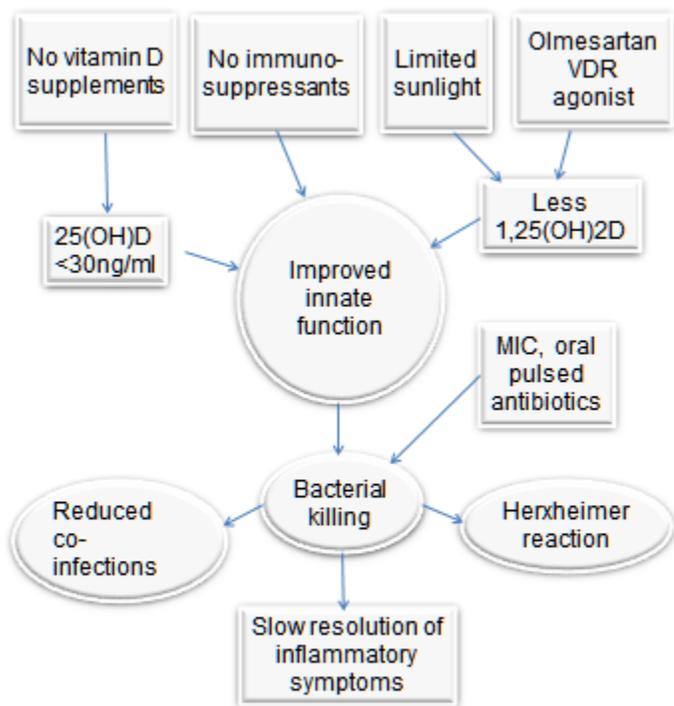
The ability to mount an appropriate response to intracellular infection is highly dependent on a competent VDR. 291 When it appears that 1,25-D is unable to up-regulate the VDR due to microbial activity, another ligand may be able to act as an agonist (an agonist increases the signal transduction activity of a cell when bound to a receptor on that cell) and restore VDR competence. Over 3000 synthetic VDR ligands have been identified but most have no clinical use because of their undue disruption to calcium regulation. 292

A number of non-vitamin D VDR ligands have been identified: curcumin, 293 omega-6 fatty acids such as arachidonic acid and linoleic acid, and lithocolic acid (LCA). 26, 294 In silico molecular modeling found evidence that angiotensin receptor blockers (ARBs) modulate the activation of two key nuclear receptors-VDR and PPARgamma. The ARB olmesartan medoxomil (brand name Benicar®) was estimated to have a Ki value in the low nanmolar rang, not too dissimilar from the Ki values of the natural ligands. 295 In the clinical setting, olmesartan is being used off-label as a novel VDR ligand and it appears to function as a VDR agonist. 296 In patients with autoimmune and inflammatory disorders, olmesartan provokes inflammatory symptoms that suggest the expression of endogenous antimicrobials under VDR control. Olmesartan also lowers angiotensin II (a peptide that's implicated in the inflammatory process). 297 The endogenous VDR ligand 1,25-D has a similar effect in that it represses renin gene

expression to down-regulate the renin angiotensin system (RAS) and reduce inflammation via NF-kb pathway. 298 VDR and RAS receptors are distributed in almost the same tissues. This 2013 study found a link between RAS activity and activation of the VDR:

...the inappropriate stimulation of the RAS has been associated with the pathogenesis of hypertension, heart attack, stroke, and hypertrophy of both the left ventricle and vascular smooth muscle cells. Changes in RAS activity and activation of VDR seem to be inversely related, making it possible to speculate that both systems could have a feedback relationship. [The researchers conclude] the combination of RAS blockade and VDR stimulation appears to be more effective than each one used individually. 273

This is what olmesartan appears to accomplish (blocking angiotensin II and stimulating the VDR) and is consistent with a theory of VDR incompetence. The multiple beneficial effects of olmesartan, including the ability to reduce cardiovascular 299 and kidney disease, 300 prevent migraines, 301 and reduce oxidative stress, 302 suggest it could play a key role in the resolution of chronic systemic inflammation.



**Fig. 4** Restoring VDR competence is the key to improving innate immune system function and eliminating persistent intracellular bacteria to resolve inflammatory symptoms. This may be accomplished with vitamin D avoidance and a non-vitamin D ligand to reduce 25-D and 1,25-D to normal levels; and select antibiotics

## Clinical Response to Treatment

Olmesartan causes a dramatic reduction in elevated 1,25-D within weeks of initiation, which provides further evidence of its ability to up-regulate the VDR. 303 Olmesartan is believed to decrease excess 1,25-D by several VDR-mediated effects:

- The up-regulated VDR transcribes CYP24A1 and CYP3A4 (enzymes which reduce 1,25-D production).
- CYP27B1 (the enzyme that hydroxylates 25-D to 1,25-D) is repressed so less 1,25-D is made.
- Consequently, renal control of 1,25-D production is restored and extra-renal production of 1,25-D is reduced.

A decrease in elevated 1,25-D means less systemic inflammation, as these studies of olmesartan indicate:

- "Improvement of glycemic control and insulin resistance was only observed in olmesartan group. ...these effects of olmesartan might be mediated by an anti-inflammatory action." 304
- "Olmesartan treatment ...significantly reduced serum levels of inflammatory markers; h-CRP, h-TNF $\alpha$ , IL-6, MCP-1 after 6 weeks of therapy..." 305
- Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH<sub>1</sub>- and TH<sub>17</sub>-mediated autoimmunity. 306
- Blocking angiotensin II receptor increases bone mass. 307, 308

Following administration of olmesartan, a Jarisch-Herxheimer reaction (JHR) is usually seen. JHR is a cascade of reactions including inflammation, cytokine release, and endotoxin release as part of the immune response to the disintegration of infected cells) 309. This is evidenced by waxing and waning inflammatory symptoms and/or inflammatory lab markers, suggesting a robust immune response and transcription of AMPs by an activated VDR. 310, 311 This clinical response provides additional evidence that olmesartan is a VDR agonist. 306 The adaptive immune system may also respond to the presence of fragments of DNA generated by pathogenic and cellular debris, generating antibodies in the process (as reflected by laboratory markers). Theoretically, olmesartan restores VDR competence and, thus, phagocytosis leads to bacterial death; consequently, inflammation is increased by cytokine reaction to microbial endotoxins and cellular debris from dead host cells and bacteria. 312 The eminent German researcher Friedrich Flachsbart is often quoted regarding this effect, "Jarisch-Herxheimer is in fact the maximum of evidence possible in search of occult microbes." To eradicate the intracellular pathogens, it's also necessary to use intracellularly active antibiotics which often cause an exacerbation of symptoms (JHR) with each dose. [ 313] Treatment utilizes select sub-inhibitory 314, 315 oral antibiotics, 316 in a pulsed fashion; for their ability to weaken bacterial ribosomes, penetrate cell walls, 317, 318 accumulate in phagocytes, 319 or interfere with folate synthesis. 320 Sunlight exposure, foods high in vitamin D and vitamin D supplements are avoided to maintain serum 25-D at a level (20-30 ng/ml) that isn't likely to suppress the immune system and inhibit bacterial elimination.

In the absence of clinical trials, the determination of when to use olmesartan and low-dose antibiotics as immunotherapy is made on the basis of diagnosis, severity of symptoms, history, potential disease course, previous treatments attempted, efficacy of traditional treatments and risk versus potential benefit. Treatment requires several years (to avoid intolerable JHR) but dramatic improvement has been seen (e.g., reduction in inflammatory symptoms, decrease in viral and antibody titers, normalization of lab work, improvement in bone density and correction of hormonal imbalances) in a wide variety of chronic inflammatory and autoimmune diseases. 296, 321, 322 (Fig. 4)

### **Table 2. Key Points**

A consensus regarding the definition of vitamin D deficiency has been elusive.

Photosynthesis of vitamin D<sub>3</sub> may provide adequate vitamin D stores for most individuals.

Low levels of 25-D are seen in healthy individuals, as well as those with chronic inflammatory conditions.

Studies are inconsistent regarding the health benefits of increasing vitamin D stores; supplementation may have negative effects.

25-D may not always reflect the level of 1,25-D; accurate assessment of vitamin D status may depend on measuring both metabolites.

Intracellular cell wall deficient bacteria may cause impaired immune system function.

A novel non-vitamin D VDR ligand appears to reactivate the immune system, restore VDR competence, resolve dysregulated vitamin D metabolism and reduce inflammatory symptoms.

## **Discussion**

Vitamin D is essential for many important biological processes and most people get an adequate supply from exposure to sunlight. Long-term studies are needed to determine if low vitamin D in healthy individuals leads to disease. Evidence that vitamin D supplementation cures or prevents chronic disease is inconsistent; despite increased supplementation chronic inflammatory diseases are on the rise. Attention to the alternate hypothesis - low 25-D is a consequence of the chronic disease process provoked by persistent intracellular infection – may be crucial to reversing this trend.

The prevailing dogma that the level of serum 25-D provides an accurate assessment of vitamin D status needs closer examination. Circulating levels of 25-D may not be an accurate reflection of vitamin D status in those with an autoimmune disease or chronic inflammatory symptoms; 1,25-D may be elevated, which can lead to osteoporosis and cause inhibition of innate immunity,

which is contraindicated in the presence of infection. Advocating vitamin D supplementation without adequate evidence of efficacy is, in effect, a mass experiment using the general population without their informed consent. The resulting immunosuppression may promote chronic infection which has been implicated in chronic inflammatory diseases. Studies using more advanced cell culture and molecular techniques are confirming the presence of previously undetected bacteria, including cell wall deficient bacteria. A greater understanding of the defense mechanisms that intracellular bacteria use to persist and proliferate is being gained. Pathogen-induced VDR dysfunction which causes the release of pro-inflammatory cytokines may be at the root of chronic disease and low vitamin D. Improving vitamin D receptor activation may be the key to treating autoimmune disease. A treatment which has demonstrated efficacy in reversing vitamin D metabolism dysfunction and reducing inflammatory symptoms is currently being used by clinicians and warrants formal study.

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