Vitamin D Deficiency
And its Role in Viral and Bacterial Infections

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Most people see an overcast sky and do not think twice about not getting their proper amount of vitamin D that day. Vitamin D is an essential, fat-soluble vitamin that has many functions in the human body. Many people are aware that vitamin D helps build strong bones by aiding in calcium absorption; however, vitamin D also plays a role in combating viruses, bacteria, and fungi as well. Without adequate amounts of vitamin D the body becomes deficient and white blood cells are not able to combat viruses and bacterial as well, leaving the body more susceptible to infection.

The United States Department of Health and Human Services recommends a minimum amount of 600 IU of Vitamin D a day for individuals age 1-70, however, for many people this is not possible. Vitamin D is difficult to get from dietary sources alone and most fruits and vegetables are not a source. It can, however, be found in fatty fish, such as salmon or tuna, and in smaller amounts in cheese, eggs and milk. Most of the body's intake of vitamin D is achieved through photochemical conversion of cutaneous 7-dehydrocholesterol into pre-vitamin D3. The amount of UVB rays that can make contact with human skin depend on the absorption of the rays by the ozone, the time of year, and the angle of the sun. In northern latitudes, especially during winter season, it is difficult for individuals to achieve sufficient absorption of Vitamin D from the sun. One study, that took place in Finland, stated that the most important source of Vitamin D for the people living there was through the diet, because sunlight exposure is inadequate. Since vitamin D is difficult to achieve through diet and is varied due to climate, location, and personal limitations, vitamin D deficiency is becoming a problem in both developed and underdeveloped countries. It has been estimated that fifty percent of the population worldwide is insufficient in Vitamin D and about 1 billion people worldwide are Vitamin D deficient. This growing problem is making people vulnerable to infections such as
tuberculosis and can lead to problems like multiple sclerosis, type I diabetes and possibly even some virus-related cancers².

Vitamin D exists in two forms, D2 and D3. The D2 form is prevalent in most mushrooms and the D3 form is found in fish and is the form obtained from UVB rays from the sun³. When Vitamin D3 is ingested through dietary sources, it is converted into chylomicrons, which are droplets of fats and proteins that are transported through the blood. Vitamin D needs to be transported as chylomicrons because it is a fat-soluble vitamin. The chylomicrons then enter the lymphatic system through the lacteals packaged inside the villi of the small intestine. Once inside the lymphatic system, the chylomicrons eventually get deposited into the bloodstream. Absorption of Vitamin D3 through the skin, however, has a different process. Epithelial cells of the small intestine oxidize cholesterol, obtained from the diet, into 7-dehydrocholesterol and transport it to the epidermis where it is then isomerized into Vitamin D3 by UVB rays¹¹. Vitamin D3 is an inactive form and undergoes two metabolic conversions to become activated. Vitamin D3 is transported through the blood to the liver where it is converted into 25-hydroxyvitamin D, 25(OH)D, by the enzyme cholecalciferol 25-hydroxylase⁹. 25(OH)D then undergoes 1alpha-hydroxylation in the kidneys to be converted into the active secosteroid hormonal form 1,25-hydroxyvitamin D, 1,25(OH)D, via the enzyme 25(OH)D-1alpha-hydroxylase¹⁶. The CYP2R1 gene encodes the enzyme that catalyzes the first hydroxylation and the CYP27B1 gene encodes for the 1alpha-hydroxylation enzyme⁴. Both of these genes are members of a family of enzymes called cytochrome P450 enzymes. A genetic mutation in either one of these genes can also be a reason for Vitamin D deficiency.

Most of the body's cells, including leukocytes, contain VDR, or the vitamin D receptor. VDR is part of the nuclear receptor superfamily and when it is activated by the binding of 1,25(OH)D it forms a heterodimer with the retinoid-x-receptor⁸. 1,25(OH)D is
Vitamin D helps to catalyze the production of defensins, mainly beta defensin 2 and 3, and cathecidin LL-37. Vitamin D is able to do this by binding to the Vitamin D response element, VDRE, on the promoter for these genes. Cathecidin LL-37 is an amphipathic, alpha helical peptide that is 37 amino acids long and helps to destroy bacterial membranes, viral envelopes and even has some anti-fungal activity against Candida. Individuals who are vitamin D deficient may not be able to fully express cathecidin LL-37.

To show the antimicrobial affect of cathecidin, an experiment, “Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response,” was done where recombinant cathecidin peptide was incubated with Mycobacterium tuberculosis for three days. The viability of M. tuberculosis was measured using uracil uptake and colony forming units. Uracil uptake was significantly decreased as well as the number of colony forming units, indicating cathecidin’s antimicrobial affects.

In the same study, another experiment was done to observe the effects on Vitamin D and cathecidin when the toll-like receptors, or TLRs, were triggered. TLRs are signals on the surface of the white blood cells that are able to illicit immune responses because they are capable of distinguishing between bacterial cells and host cells. This experiment obtained human monocytes, macrophages, and dendritic cells from four donors. The cells either had their TLR 2/1 stimulated using a synthetic ligand from M. tuberculosis lipo-peptide or were treated with medium only. DNA microarrays were conducted and showed VDR up-regulation in monocytes but not in dendritic cells. Further investigation using quantitative PCR was done to determine if any other genes were up regulated. CYP27B1, the gene that encodes for the 1alpha-hydroxylation enzyme that catalyzes the conversion of 25(OH)D to 1,25(OH)D, was shown to have been increased significantly at twelve and twenty four hours after stimulation in monocytes and in macrophages, but again, not in
dendritic cells. This data confirms that stimulation of the 2/1 heterodimer toll like receptor induces the expression of the Vitamin D receptor and the enzyme that catalyzes the conversion of Vitamin D3 into the active, steroid form 1,25(OH)D.

1,25(OH)D was then added to primary human monocytes to support the hypothesis of cathecidin stimulation by Vitamin D. Quantitative PCR was again used to observe mRNA from cathecidin and Cyp 24, which is a gene that codes for 1,25-dihydroxyvitamin D3 24-hydroxylase, an enzyme that degrades 1,25(OH)D. Intracellular flow cytometry was used to detect cathecidin in monocytes and surface-enhanced laser desorption ionization-time of flight mass spectrometry was used to detect cathecidin-derived peptides. Analysis of monocytes pellets showed a peak at 4.5 kD which corresponds to the cathecidin peptide LL-37. This data provides evidence to show that when monocytes are treated with 1,25(OH)D, not only do they produce the gene for LL-37, but are able to convert it into its active form.

Some of the monocytes in this experiment were infected with Mycobacterium bovis, the bacteria that causes tuberculosis in cattle, and Bacille Calmette-Guerin-expressing green fluorescent protein. These monocytes were then stimulated with 1,25(OH)D and labeled with a red monoclonal antibody to detect cathecidin. Fluorescent microscopy shows overlap where cathecidin was contained in bacteria vacuoles in the monocytes treated with Vitamin D, but not in the control monocytes. This also aids in the support of Vitamin D enhancing antimicrobial activity. The monocytes that were not stimulated with Vitamin D did not synthesize LL-37, and therefore cathecidin was not seen in those bacteria vacuoles.

Vitamin D not only helps to synthesize cathecidin, but it can aid in activating macrophages as well. Macrophages are activated by interferon gamma, produced by T cells or natural killer cells, or by macrophage activating factor. Activated macrophages act as a boost to antimicrobial defense mechanisms. They can fuse phagosomes with lysosomes to
produce reactive oxygen and nitrogen species that are used to combat bacteria, viruses, and parasites\textsuperscript{15}. Even though activated macrophages can be beneficial, they require a lot of energy to maintain, and they can also be destructive to nearby tissues because of their toxicity when producing reactive species. It is because of this, that activated macrophages are only initiated when they are needed. A study done by Nobuto Yamamoto and Sadamu Homma titled, "Vitamin D3 Binding Protein (Group-Specific Component) Is a Precursor for the Macrophage-Activating Signal Factor from Lysophosphatidylcholine-Treated Lymphocytes," used chromatographic fractionation of human serum and an antibody against Gc protein to show how Vitamin D can activate macrophages. There were three parts to this experiment and all are equally important to providing evidence for macrophage activation by Vitamin D. Mouse peritoneal cells were obtained, lymphocytes and macrophages and treated with 1-20 micro grams of lysophosphatidylcholine, or lyso-PC. Lyso-PC treated B cells were cultured for two hours with various concentrations of Gc protein. Gc protein is the human Vitamin D3 binding protein and was obtained from blood blank plasma. The B cell culture was then added to untreated T cells and they were cultured for two more hours. Analysis also showed that the B cells were able to modify the Gc protein and convert it to a pro-activating factor, which was measured using intracellular transmission signaling. The T cells then modified the pro-activating factor into macrophage-activating factor and activated nearby macrophages\textsuperscript{13}. Since this conversion happened so rapidly, the same treatment was done on B and T ghost cells, which are cells without a nucleus, in order to determine where the signaling was taking place. Treatment of B ghost cells and T ghost cells increased macrophage activation significantly. This shows that activation is due to membranous enzymes in B cells and T cells. To test the activation of macrophages \textit{in vivo}, mice were injected with Gc protein intramuscularly. Eighteen hours after being injected, peritoneal cells were obtained and incubated for thirty minutes. Results
showed that only 10pg of Gc protein mixture had a seven-fold increase in macrophage phagocytic activity in peritoneal cells\textsuperscript{13}.

Most research has been on how Vitamin D enhances immune response, but a journal article titled, “1,25-dihydroxyvitamin D3 is a Potent Suppressor of Interferon Gamma-Mediated Macrophage Activation,” studied how Vitamin D can suppress certain immune responses. In one part of the experiment, the researches obtained bone marrow derived macrophages and treated some with 1,25(OH)\textsubscript{D}, some with interferon-gamma, and some with both. The macrophages were then infected with the intracellular pathogen \textit{Listeria monocytogenes}. One and three hours after infection, the listericidal activity was quantified by staining and plating the macrophages and counting the intracellular bacteria that were still alive\textsuperscript{16}. Interferon-gamma alone led to decreased amounts of the intracellular bacteria, however, 1,25(OH)\textsubscript{D} and interferon-gamma lead to suppression of IFN-gamma induced listericidal activity\textsuperscript{16}. This suppression was even more severe in the 48 hour treatment than the 24 hour treatment. The researches for this experiment were aware of the enhancing ability of Vitamin D on developing macrophages and suggest that this response is a negative feedback mechanism in which Vitamin D helps to regulate IFN-gamma responses in mature macrophages. Since activated macrophages need to be tightly controlled due to their toxicity, this mechanism may be a way to control their activity. Once 1,25(OH)\textsubscript{D} levels reach a concentration that is too high, vdr expression is induced and the vdr protein is allowed to enter the nucleus where it is able to suppress IFN-gamma-induced genes\textsuperscript{16}.

Without vitamin D, white blood cells cannot efficiently produce antimicrobial peptides or activate macrophages as well and this greatly reduces the first line of defense against invading pathogens. As more research is being done, the importance of Vitamin D is coming to light. Vitamin D plays a role in innate immunity and those who are deficient are more susceptible to bacterial infections, viruses, such as the flu, and fungal diseases.
Vitamin D deficient individuals can also be predisposed to respiratory infections\(^3\). These articles show how stimulating TLR can lead to the conversion of the inactive form of Vitamin D to its active form and express genes to synthesize defensins and cathelicidin. They also show how Vitamin D has the ability to regulate macrophage listericidal activity. These are all very important aspects of innate immunity that are needed to protect the body against different types of bacteria. Daily supplementation can increase serum Vitamin D levels and benefit those who are deficient and can increase immunity for everyone during winter months.
Works Cited


