Vitamin D_3 and COVID-19: a Brief for Family Physicians in Patients

by

Dr. Kanji Nakatsu, Ph.D. and Dr. Niel Karrow, Ph.D.

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Canadian Covid Care Alliance
Alliance canadienne pour la prévention et prise-en-charge de la covid

https://www.canadiancovidcarealliance.or

This brief is intended for family doctors, especially those whose busy practices prevent them from reading extensively and who may graduated a few decades ago. It is broken into questions so that you can read thoroughly those that interest you the most. For questions specifically aimed at COVID-19 start at Section 5.

1. During medical school, we were taught that vitamin D (Vit D) was needed for bone health. As rickets is uncommon in Canada, why is Vit D mentioned here?

We now know Vit D does much more than maintain strong bones; it has actions throughout the human body, in virtually every type of cell. Vit D and its receptors (VDR) in cells associated with immunity and inflammation are relevant to COVID-19, but let's go back to review a few basics (see review (1)). Recall, that Vit D, like most vitamins, is not a single molecule, but any one of a number of similar molecules that serve the same essential function in body metabolism. The two most common forms of Vit D taken by Canadians are Vit D_2/ergocalciferol (from plants) and Vit D_3/cholecalciferol (from animals); the latter is also considered as the natural form for humans, because it is made in human skin from 7-dehydrocholesterol in the presence of UVB light. Vit D_3 *per se* is not active, but requires hydroxylation in the liver (via CYP2R1) to form 25OH-VD_3 (calcidiol, calcifediol) and another hydroxylation in the kidney (via CYP27B1) to form 1,25$(OH)_2$-VD_3 (calcitriol), which is the bioactive molecule. It is now known that other cells beyond the kidney can also perform the second hydroxylation. Circulating levels of 25OH-VD_3 are accepted internationally to represent the Vit D status of individuals, and are used widely in Canada to assess Vit D sufficiency. An international panel of Vit D researchers has promoted that concentrations of 100–150 nmol/l (40–60 ng/ml) are optimal (2). The Vitamin D recommended intake is at 400–800 IU/day or 10–20 micrograms, with increasing dosage with age. However, some studies indicate that a higher daily intake of 1,000–4,000 IU (25–100 micrograms) is needed to maintain optimal blood levels. Doses up to 10,000 IU per day have not been shown to be toxic, although the US National Institutes of Health recommends a safe upper limit of 4,000 IU per day.

2. Vit D is added to many foods in Canada, so isn't Vit D deficiency or insufficiency rare?

Schwalfenber *et al.* reviewed 16 studies and found that 70-97% of Canadians were Vit D insufficient, and 14-60% were clearly deficient (3); they defined insufficiency as <72-80 nmol/l and deficiency as <25-40 nmol/l. There are many factors contributing to Vit D insufficiency and deficiency, some of which are personal and others of which are environmental. With respect to synthesis of Vit D by exposure to sunlight, personal actions make a difference. A person must take the time to expose their skin to the sun, which sounds simple in theory, but is less simple in practice. Contemporary work often requires sitting in front of a computer indoors out of the sunlight. Moreover, application of sun blocking agents, promoted by cosmetic companies for prevention of wrinkles and by health promotion agencies to prevent skin cancer, interferes with Vit D synthesis. The Canadian environment contributes to lack of exposure to sunlight as our northern location results in inadequate solar radiation for much of the year, and almost any clothing blocks the sun's rays. Another relevant factor is skin colour; light skin facilitates Vit D synthesis, while darker skin reduces synthesis. Even within an individual, skin colour can make a difference, because tanning results in darker skin and less Vit D synthesis. Average individuals in much of Europe and North America synthesize only 500–600 IU/day during the summer and virtually none at all in the winter.

Normal dietary intake of Vit D in contemporary Western diets does not provide an abundance of Vit D as the daily dietary intake in central European adults is only about 80–120 IU. Some foods that contain significant quantities of Vit D include fatty fish (cod liver oil, salmon), liver and fat from certain aquatic mammals (seals and polar bears), and egg yolks. As our Indigenous peoples have adopted Western diets, they have consumed less Vit D from traditional foods, and the general population has been exhorted to limit consumption of egg

yolks for decades because the cholesterol content. Thus, our Canadian environment and dietary habits contribute to lower body levels of Vit D, and ingestion of Vit D supplements is necessary to obtain optimal levels of the sunshine vitamin. Vegetarians may choose to take Vit D_2.

3. How does Vit D exert it actions at the cellular level?

The nuclear receptor for Vit D_3 (VDR) was discovered in 1969, and subsequently has been found in many cells beyond those involved with the disposition of calcium. VDR are located in almost every cell in the human body where they mediate hundreds of different actions (4); calcitriol is now considered to be a hormone, being released from the kidney, and Vit D_3 is the precursor or pre-hormone. The VDR is present in cells within all major organ systems- intestine, kidney, cartilage, bone, pancreas, hair follicles, epithelium, endothelium, vascular smooth muscle, cardiomyocytes, and immune cells such as activated B and T lymphocytes, macrophages, dendritic cells. When calcitriol enters a cell, it binds to the VDR, which heterodimerizes with retinoid-X-receptor (RXR), and together they translocate into the nucleus. This VDR-RXR transcription factor complex is then able to bind to target genes expressing a specific nucleotide sequence, referred to as a vitamin D response element, after which the expression of various genes is either regulated upward or downward. Some examples of genes that are activated are those for CYP24A1 (prevents Vit D toxicity, see below), and immune-related genes such as CD14, and NOD2, as well as cathelicidin and β2 defensin that are peptides with antimicrobial and antiviral activities. Examples of inhibited genes are those for immune-related genes IFN-γ and hepcidin. The full breadth of all the genes affected by Vit D is beyond scope of this brief.

4. What is the scope of Vitamin D effects?

Just as all the genes controlled by Vit D cannot be covered herein, we cannot include all of the ultimate effects of Vit D. The purpose of this brief is to describe some of the effects of Vit D that are important in terms of COVID-19. Suffice it to say, the importance of Vit D can be appreciated by the number of disease states that are associated with deficiency of this vitamin as described in the reviews by Grober *et al*. (4) and Maretzke *et al.* (5); these include autoimmune diseases (multiple sclerosis, type 1 diabetes), inflammatory bowel disease (Crohn's disease), infections (such as respiratory tract), asthma, immune deficiency, cardiovascular diseases (hypertension, heart failure, sudden cardiac death), cancer (colon cancer, breast cancer, non-Hodgkin's lymphoma) and neurocognitive disorders (Alzheimer's disease).

In the context of COVID-19, the role of Vit D in viral infections and controlling the associated pathological inflammatory response is extremely relevant.

5. Does Vit D protect against the SARS CoV-2 virus? And how does it affect immune function?

The short answer is Yes. Vit D has a number of characteristics that oppose establishment of SARS CoV-2 infection. By virtue of supporting both innate and adaptive immune responses, Vit D is protective. Vit D affects the functions of macrophages, natural killer (NK) cells, dendritic cells (DC), T-cells and B-cells. In macrophages, Vit D can increase phagocytosis and production of antimicrobial/antiviral cathelicidins and defensins (6). Human cathelicidin has been reported to bind to the SARS CoV-2 spike protein with high affinity and also binds to ACE2. Similarly, defensins are reported to have direct actions on viruses and indirect effects on target host cells (7).

Dendritic cell function is to initiate and refine adaptive immune responses. Vit D can inhibit dendritic cell maturation, differentiation and survival as well as decrease their migration and inflammatory cytokine production. This may be the basis of the immunomodulatory role of Vit D discussed below. On the one

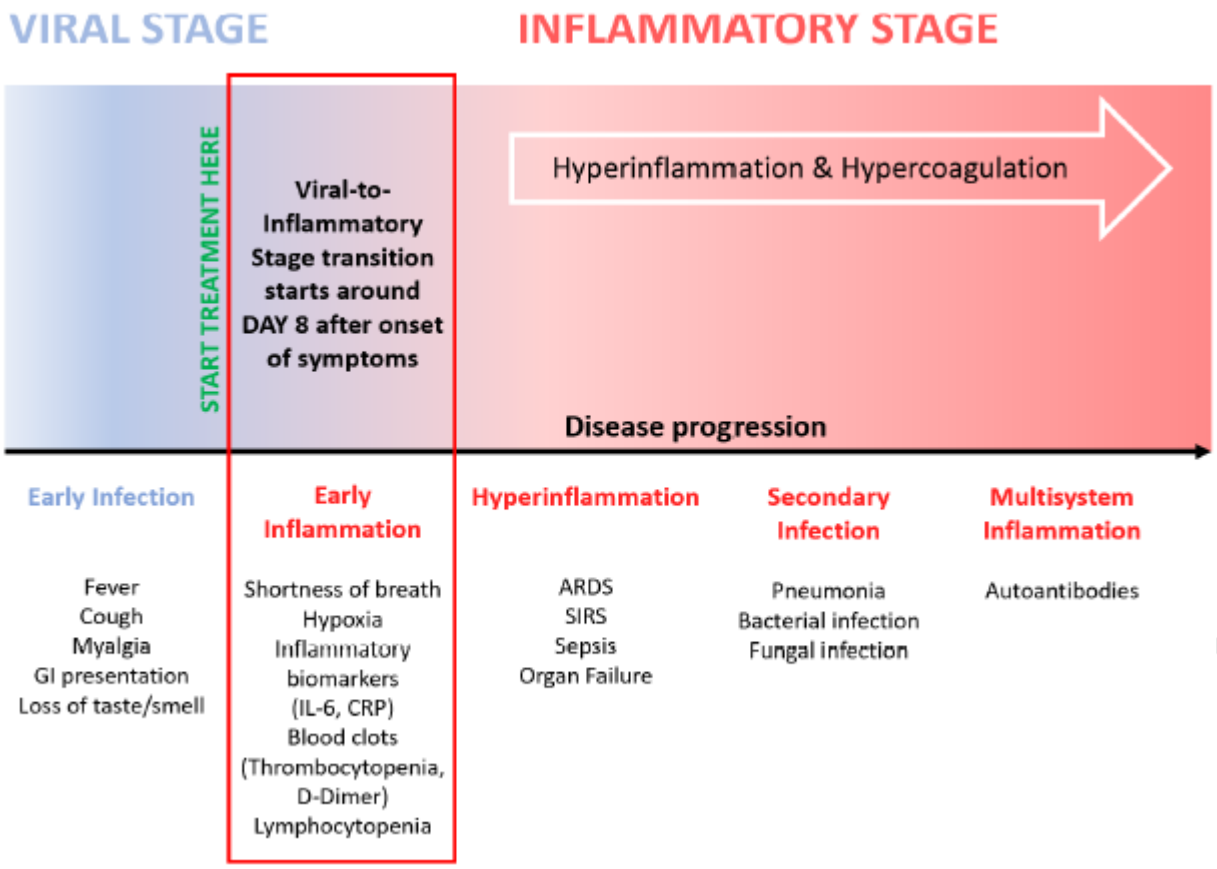
hand, Vit D is also known to reduce dendritic cell ability to present antigen to T-cells. Resting T-cells do not respond to Vit D, because they lack Vit D receptors. On the other hand, chronically activated T-cells can be driven to differentiation into anti-inflammatory T-cell subpopulations (Treg and Th2), which may contribute to reduction of the inflammatory response during the hyper-inflammatory phase of COVID-19. The ability of Vit D to increase the number of memory T-cells may help with subsequent exposure to the virus. As a key component of the adaptive immune system, the B-cell is traditionally considered to be the precursor of antibody-producing plasma cells. Vit D suppresses B-cell proliferation, differentiation into plasma cells and immunoglobulin production, promotes apoptosis/programmed death of activated B-cells and inhibits memory B-cell formation (8). Antiviral Natural Killer cell activity is also supported by Vit D (9).

A useful source of information for your patients may be the video "Does Vitamin D Protect Against COVID-19?" by Dr. John Campbell (10).

6. What should we look for in studies of Vit D effectiveness in COVID-19?

When did Vit D intervention take place?

The stages of COVID-19 infection are shown in the figure below (11). During the early infection stage, the virus is multiplying and herein antiviral support of Vit D is most effective. During the next, early inflammation stage (boxed) other properties to control the inflammatory response become relevant.



What form of Vit D was used?

Above, we mentioned that Vit D_3 is inactive and must undergo two hydroxylations to become the active molecular calcitriol/1,25$(OH)_2$-VitD. This can take several days, especially the first step to calcifediol/25OH-Vit D. If the intended treatment is expected to occur immediately, calcitriol or calcifediol should be used. In some clinical trials involving Vit D_3, the necessity for metabolic activation was overlooked. This consideration also applies if you recommend Vit D to your patients.

What is the quality of the Vit D clinical trials?

This is a question that applies to virtually all trials on nutrients and natural health products as well as traditional medicines and older off-patent drugs. The "gold-standard" of evidence-based medicine is the large, placebo-controlled, randomized, double-blind clinical trial. This is possible in the world of novel chemical entities wherein the major pharmaceutical company sponsor has sufficient resources to mount such trials and the patient populations can be made available. Thus, a novel lipid-lowering drug can be tested in a population in that elevated blood lipids have never been treated with any drug, because drug-free cohorts are available for the trial. This is problematic for substances such as vitamins, because it is impossible to find populations that are free of the vitamin in question. This dictates studies comparing low versus high quantities of the vitamin, and more subtle distinctions require larger samples sizes and costs for adequate statistical analysis. When the vitamin in question cannot be protected by a patent and generate substantial profits, the gold-standard trial becomes unrealistic. Many studies on Vit D in COVID-19 are population-based association studies in which an outcome is associated with various levels of Vit D sufficiency. Despite these challenges, there are data supporting the beneficial role of Vit D regarding COVID-19 (12).

7. Is Vit D prophylactic?

The short answer to this is a qualified Yes; qualified by acknowledging the limitations of the studies attempting to address the question. Accordingly, there can be no Vit D-free placebo groups for comparison to those supplemented with Vit D. Many of the studies compare groups of subjects based on levels of Vit D sufficiency; unfortunately, the circulating levels of Vit D used to define sufficiency varies among the research groups.

A running meta-analysis of Vit D for COVID-19 prophylaxis is maintained in the website dedicated to COVID-19 early treatment (13). The overall suggestion after reviewing multiple studies on Vit D, is that ensuring sufficiency in Vit D results in more than a 50% reduction in the probability of contracting COVID-19. An example of such a report is that of Abdollahi *et al*. (12), who conducted a case-control study with 201 patients and 201 matched controls in Iran and showed that Vit D sufficiency was associated a 53.9% decrease in cases ($p=0.001$). Another study conducted in England, involving 80,670 participants, median serum 25(OH)D in non-hospitalized participants with COVID-19 was 50.0 nmol/L versus 35.0 nmol/L in those admitted with COVID-19 ($P < 0.005$). Hospital admissions were 2.3- to 2.4-times higher among those with serum 25(OH)D <50 nmol/L (14).

Borshe *et al.* (15) in their recent review addressed the question of whether low Vit D is caused by COVID-19 infection, or whether Vit D deficiency predisposes to the disease. Accordingly, they included studies in which patients' pre-infection Vit D levels were documented, and also some in which Vit D levels up to the day after hospitalization were recorded. Their analysis revealed a significant ($p=0.0194$) negative correlation between high Vit D pre-infection concentrations and mortality. Extrapolation of the regression line to the X-axis showed that the risk of death virtually disappears at 50 ng/ml or 125 nmol/l. Using Vit D in this manner, may offer prophylaxis rivalling the mRNA and adenovirus vaccine injections whose efficacy is known to wane in a few months. This also indicates that our numerical standard of Vit D sufficiency should be increased.

8. Does Vit D improve treatment of COVID-19?

Two studies conducted in Spain illustrated the effectiveness of treating infected patients with the Vit D metabolite, calcifediol. In a retrospective study, 79 patients treated with calcifidiol had a lower risk of death, as 4 of the 79 (5.1%) Vit D treated subjects died compared with 90 of 458 (19.7%) untreated subjects, which indicates that calcifediol treatment appeared to prevent ~80% of deaths in this study (16).

In an earlier randomized clinical study, Castillo *et al.* (17) found that calcifediol treatment in a smaller cohort resulted in no COVID-19 deaths in 50 Vit D treated subjects, while there were 2 deaths in the 26 untreated controls from COVID-19. Moreover, only 1 out of the 26 Vit D treated patients were admitted to ICU compared with 13 of 26 the control patients.

9. Vit D is a fat-soluble vitamin, so isn't it quite toxic?

Theoretically, one would expect to observe significant toxicity, because Vit D could accumulate and become toxic. In contrast, observed Vit D toxicity is rare and readily treatable by stopping supplementation. With respect to single doses of Vit D, a systemic review published in 2014 indicated that over 300,000 IU (was this injected im?) was acceptable and that doses of 500,000 IU should be used with care (18). With respect to chronic Vit D supplementation, Malihi *et al.* (19) identified the major risks to be hypercalcemia and hypercalciuria; the risk of kidney stones was not elevated. Some context to the question of Vit D toxicity is gained by considering that ~20,000 IU is formed in the skin of an untanned Caucasian person in about 20 minutes of extensive body exposure to full sunshine. Greater exposures may result in sun burn, but not Vit D toxicity. According to Grober *et al.* (4), "To prevent recurrence of vitamin D deficiency, 50 000 IUs of vitamin D once every 2 weeks (equivalent to 3300 IUs daily), forever, is effective in maintaining a healthy vitamin D status without causing toxicity." Consider also that calcitriol/1,25(OH)2 Vit D levels are controlled by a number of processes in the kidney and elsewhere. For example, a mitochondrial P450 enzyme (CYP24A1) can hydroxylate substrates at the 24-position converting 25OH Vit D to $24,25(OH)_2$ Vit D, and $1,25(OH)_2$ Vit D to $1,24,25(OH)_3$ Vit D; both reactions result in lower concentrations of the active molecule. As mentioned above, Vit D increases CYP24A1 expression to create a negative feedback mechanism for controlling serum Vit D levels.

Another aspect of the fat solubility of Vit D is its distribution to adipose tissue. Accordingly, obese individuals may require higher doses of Vit D, especially during the administration of loading doses. This is complicated by the existence of Vit D in the body as Vit D_3, calcifediol and calcitriol, all with different distributions between body fat and water. If there are concerns about the Vit D status of specific patients, it may be prudent to simply order a lab test for 25OH Vit D. As mentioned above, deaths due to COVID-19 could be almost eliminated by increasing the serum 25OH Vit D in the population to ~125 nmol/l (15).

10. Summary.

Vit D sufficiency is very important in the context of COVID-19 as well as the Flu. Sufficiency of this vitamin can be ensured by adequate dietary supplementation, especially during the winter months in Canada. Vit D protects people from various respiratory tract infections like SARS CoV-2 infection; judicious use of Vit D might even rival vaccinations. Once infected, treatment with activated Vit D can enhance recovery and reduce the likelihood of death.

Canadians could supplement themselves with Vit D_3 (2000 IU/day or more, but less than 10,000 IU/day), the cost would probably be only cents/day, while the savings to healthcare budgets could be substantial. With

increased Vit D intake, we must be also be mindful of Vit K2 intake to ensure the appropriate distribution of calcium (20).

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