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Epidemiol. Infect.
doi:10.1017/S0950268807008308
(*First published online 12 March 2007*)

To The Editor:

The occurrence of the common cold and influenza shows clear seasonality. The cold and influenza season corresponds to the season of vitamin D insufficiency. This association was highlighted in the recent article in this journal by Cannell *et al.* [1], which proposed that the lack of vitamin D during the winter may be a 'seasonal stimulus' to the infectivity of the influenza virus.

Vitamin D is produced in the skin when sunlight is absorbed. Thus, vitamin D levels, or serum 25-hydroxyvitamin D (25-OHD), fluctuate seasonally. At latitudes above 37° N and below 37° S, sunlight is insufficient to induce cutaneous vitamin D synthesis during the winter months so 25-OHD levels are low [2]. New evidence has accumulated that vitamin D can have important functions in the immune system, specifically the innate immune system. Is our susceptibility to colds and influenza related to vitamin D insufficiency in the wintertime? Furthermore, would vitamin D supplementation boost our immune system and work as a preventive measure for colds and influenza?

We considered these questions after reviewing the adverse events of a vitamin D3 supplementation trial that we completed [3]. The design and primary results of the study have been published and are summarized here. We conducted a 3-year randomized controlled trial to test the hypothesis that vitamin D3 supplementation would prevent bone loss in calcium-replete, African-American post-menopausal women. A total of 208 women were randomized to receive vitamin D3 ($n=104$) or placebo ($n=104$). After 2 years, the vitamin D3 dose was increased to 50 μg/d (2000 IU) in the active group. None of the patients had a history of chronic obstructive pulmonary disease, congestive heart failure, or myocardial infarction. Few patients had a history of asthma and seasonal allergies with no significant difference between the vitamin D3 group and the placebo group.

The study was approved by the Institutional Review Board of Winthrop University Hospital. After randomization, patients were followed up every 6 months for 3 years. At each visit, information on URI symptoms was obtained by first asking the participants, 'Have you been well?' If the participant answered 'No', then she was asked, 'Have you had any colds or influenza?' A report of cold and influenza was recorded as an adverse event.

After 3 years, a total of 34 patients reported cold and influenza symptoms, eight in the vitamin D3 group *vs.* 26 in the placebo group ($P<0{\cdot}002$). When we examined the seasonality of the symptoms, we found that the placebo group had cold/influenza symptoms mostly in the winter. The vitamin D group had symptoms throughout the year while on 20 μg/d, whereas only one subject had a cold/influenza while on 50 μg/d (Fig.). None of the 34 patients with reported cold and influenza symptoms had significant comorbidities.

Vitamin D supplementation, particularly at higher doses, may protect against the 'typical' winter cold and influenza. A major flaw in this analysis is that the data on viral URI symptoms were collected in an insensitive and imprecise way. From a 3-year trial on 208 subjects, we would expect at least 139 colds, but we only collected 39 reports of viral URI symptoms,

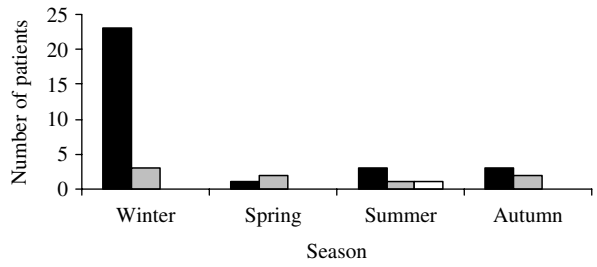

Fig. Incidence of reported cold/influenza symptoms according to season. The placebo group reported more cold/influenza symptoms in the winter. Only one subject had cold/influenza symptoms while taking high doses of vitamin D (50 μg/d). ■, Placebo; ▨, 20 μg/d vitamin D; □, 50 μg/d vitamin D.

indicating the incomplete capture of data. However, given the double-blinded, randomized nature of this trial, the responses should not have been biased.

The physiological basis of the protective effect of vitamin D lies in its ability to stimulate innate immunity and to moderate inflammation. The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25-OH_2D) stimulates the genetic expression of antimicrobial peptides (AMPs) in human monocytes, neutrophils, and epithelial cells [4]. Recognition of microbial particles by toll-like receptors (TLRs) induces expression of antimicrobial peptides, such as defensins and cathelicidins. These peptides have a broad range of actions against microorganisms, including bacteria, fungi and viruses. Liu *et al*. showed that stimulation of TLR 2/1 engages a vitamin D-dependent intracellular circuit that results in the expression of cathelicidin, enhancing the microbicidal capability of the monocyte [5]. Remarkably, the authors also observed that sera from African-American individuals, who are known to have substantially lower serum vitamin D levels than whites, were inefficient in inducing genetic expression of cathelicidin. When the authors supplemented the sera with 25-OHD, cathelicidin levels increased to levels observed in monocytes collected from whites. Wang *et al*. showed that 1,25-OH_2D induces expression of cathelicidin and defensin β2 genes [4]. Defensin β2 has been shown to have inhibitory effects on adenovirus and HIV-1 [6, 7]. Defensins can block viral infection by directly acting on the virion or by affecting the target cell and thereby indirectly interfering with viral infection [8]. One of the defensins called retrocyclin-2 inhibits influenza virus infection by blocking membrane fusion mediated by the viral hemagglutinin [9].

These reports provide a rationale for vitamin D supplementation in the prevention of colds and influenza. Since there is an epidemic of vitamin D insufficiency in the United States, the public health impact of this observation could be great. These findings should be confirmed by randomized, placebo-controlled trials.

Declaration of Interest

This research was supported by the National Institutes of Aging (RO1 AG15325), National Institutes of Health, Bethesda, MD.

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The authors reply:

We are obviously pleased with Drs Aloia and Li-Ng's *post-hoc* analysis, which found that supplemental cholecalciferol reduced the self-reported incidence of colds and influenza. Their work supports our theory that the seasonality of influenza is caused by the seasonality of 25-hydroxy-vitamin D [25(OH)D] levels [1] and that vitamin D is Hope-Simpson's long-lost 'seasonal stimulus' [2]. They lend crucial support to Hope-Simpson's 1992 prediction that 'understanding the mechanism of action (of the seasonal stimulus) may be of critical value in designing prophylaxis against the disease' [2, p. 235].

Aloia and Li-Ng found that the self-reported symptoms of colds and influenza were more than three times higher in the placebo group (30/104 patients) than in the vitamin D group (9/104 patients) over the entire 3 years of their study. We were somewhat surprised by such a robust effect, especially with the relatively low dose of 20 μg (800 IU)/d used during the first 2 years of their study.

As they point out, the next – and vitally important – step is a controlled interventional trial. In spite of the apparent effectiveness of 20 μg of cholecalciferol, and the more robust effect of 50 μg (2000 IU), we believe that trial should use enough cholecalciferol to raise 25(OH)D levels to those achieved by natural summertime sun exposure, about 50 ng/ml. At the end of the final year of their original trial, 50 μg/d still left many African-American women vitamin D-deficient; about 40% had 25(OH)D levels less than 32 ng/ml.

Furthermore, such a trial should use cholecalciferol, not ergocalciferol. Ergocalciferol is not vitamin D but a less potent vitamin D analogue that plays no role in normal human physiology [3]. Measuring 25(OH)D levels at baseline and periodically thereafter would assess compliance, the effect of the intervention, and avoid confounding factors which might otherwise go undetected. Serological tests done on baseline and convalescent serum could be used to confirm viral respiratory infections.

The lack of seasonality in their vitamin D group compared to their placebo group is intriguing. The vitamin D group reported a lower incidence of respiratory infections, but when they did get sick, they were just as likely to get sick in the summer as the winter. That is, the vitamin D abolished the seasonality of colds and influenza. We hypothesize their subjects continued to suffer rhinovirus infections.

Noah found that rhinovirus infections, which cause about 50% of common colds, have no significant seasonality [4]. Furthermore, the seasonality of the common cold [5] is quite different from the seasonality of epidemic influenza. Unlike epidemic influenza, common colds do not disappear during the summer, although the incidence is less in the warmer months.

Rhinoviruses are not enveloped with a lipoprotein coat and thus may be resistant to the mechanism of action of most antimicrobial peptides (AMPs) [6]. Although, to our knowledge, AMPs have not been shown to inactivate rhinoviruses, Duits *et al*. found AMPs may orchestrate a secondary innate immune response to the virus [7]. As such, we hypothesize that vitamin D will not prevent rhinovirus infections but it may attenuate their course.

Noah did find that influenza A and B, parainfluenza 1 and 2, and respiratory syncytial viruses are all more common in the winter. As all are enveloped viruses, they all may be sensitive to AMPs and potential targets of vitamin D. It is also intriguing that HIV, which is also enveloped, is inhibited by AMPs; there is evidence that vitamin D plays a role in HIV [8]. Invasive pneumococcal disease [9], meningococcal disease [10] and group A streptococcal disease [11] are highly seasonal and are sensitive to AMPs [12–14].

Drs Aloia and Li-Ng's work make it reasonable to believe that physiological doses of vitamin D prevent many viral respiratory infections. Furthermore, the dramatically increased production of AMPs by vitamin D and the broad spectrum of action of AMPs make it reasonable to hypothesize that other seasonal infections may be prevented as well. Using the same logic, it is also reasonable to postulate that pharmacological doses of vitamin D may be effective adjuvants in a breathtakingly large number of life-threatening infections.

Declaration of Interest

Dr Cannell heads the non-profit educational group, 'The Vitamin D Council'.

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