

# Response to Vitamin D Supplementation during Antarctic Winter Is Related to BMI, and Supplementation Can Mitigate Epstein-Barr Virus Reactivation<sup>1–3</sup>

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## Abstract

Maintaining vitamin D status without sunlight exposure is difficult without supplementation. This study was designed to better understand interrelationships between periodic vitamin D supplementation and immune function in Antarctic workers. The effect of 2 oral dosing regimens of vitamin D supplementation on vitamin D status and markers of immune function was evaluated in people in Antarctica with no UV light exposure for 6 mo. Participants were given a 2000-IU (50  $\mu$ g) daily ( $n = 15$ ) or 10,000-IU (250  $\mu$ g) weekly ( $n = 14$ ) vitamin D supplement for 6 mo during a winter in Antarctica. Biological samples were collected at baseline and at 3 and 6 mo. Vitamin D intake, markers of vitamin D and bone metabolism, and latent virus reactivation were determined. After 6 mo, the serum 25-hydroxyvitamin D concentration (mean  $\pm$  SD) increased from  $56 \pm 17$  to  $79 \pm 16$  nmol/L and from  $52 \pm 10$  to  $69 \pm 9$  nmol/L in the 2000-IU/d and 10,000-IU/wk groups, respectively (main effect over time,  $P < 0.001$ ). Participants with a greater BMI (participant BMI range = 19–43 g/m<sup>2</sup>) had a smaller increase in 25-hydroxyvitamin D after 6-mo supplementation ( $P < 0.05$ ). Participants with high serum cortisol and higher serum 25-hydroxyvitamin D were less likely to shed Epstein-Barr virus in saliva ( $P < 0.05$ ). The doses given raised vitamin D status in participants not exposed to sunlight for 6 mo, and the efficacy was influenced by baseline vitamin D status and BMI. The data also provide evidence that vitamin D, interacting with stress, can reduce risk of latent virus reactivation during the winter in Antarctica. *J. Nutr.* 141: 692–697, 2011.

## Introduction

Several reports suggest, admittedly among a vigorous debate, that serum 25-hydroxyvitamin D above 75 or 80 nmol/L is optimal (1–7). At these concentrations, parathyroid hormone is maximally suppressed. Epidemiological studies suggest that relationships exist between vitamin D status and disease states at lower circulating vitamin D concentrations, often reported as  $< 50$  nmol/L. Evidence also exists that vitamin D may have a role in immune function. The active form of vitamin D (1,25-dihydroxycholecalciferol) is an important immune system regulator that can inhibit development of autoimmune diseases, including experimental inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes (8–11). The

2011 DRI report from the Institute of Medicine (12) stated that 50 nmol/L is optimal for serum 25-hydroxyvitamin D concentration to maximize benefits for bone; however, this number does not take into consideration the potential benefits for immune function, which may require higher serum concentrations.

Maintaining vitamin D stores without supplementation or rigorous attention to (and availability of) dietary sources is extremely difficult, if not impossible, in environments where UV light exposure is limited. One example of such an environment is spaceflight; astronauts lack UV light exposure because of spacecraft shielding, and the space food system contains limited natural sources of vitamin D (13). Astronauts on long-duration missions require supplemental vitamin D to maintain their vitamin D status. However, on International Space Station missions when crewmembers were provided with 400-IU (1 IU = 0.025  $\mu$ g) supplements, vitamin D status nevertheless consistently decreased during and after 4–6 mo of spaceflight (14–16).

Antarctica provides an excellent model to determine vitamin D metabolism in response to supplementation when there is virtually no UV light exposure for one-half of the year. In a recent study in Antarctica, 2000 IU/d was sufficient to raise participants' serum 25-hydroxyvitamin D concentration from

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43 ± 14 nmol/L to 71 ± 23 nmol/L (17). As is true over the rest of the globe, few dietary sources of vitamin D are available in the food supply for individuals spending winter months at McMurdo Station in Antarctica.

The role of vitamin D in the regulation of the immune system and its possible role in the prevention and treatment of cancer and immune-mediated diseases has been studied (18). Reactivation of latent viruses increases during spaceflight and shortly before and after flight (19–22). Increased viral reactivation has also been seen in association with Antarctic winters (23). Because latent viruses can cause clinical symptoms, it is important to know the cause of the reactivation so that countermeasures can be used to prevent it. The most likely causes of the increased viral reactivation in spaceflight are microgravity, increased radiation, and stress, and all may have an influence. Vitamin D status was not evaluated in these previous viral reactivation studies.

Providing vitamin D through food sources is optimal, but this is often difficult because there are so few foods that naturally contain vitamin D. Daily supplementation may be ideal to maintain a steady serum concentration of 25-hydroxyvitamin D, but compliance with a daily regimen can be a challenge for some.

In this investigation, we determined the efficacy of a once-weekly dose of vitamin D compared with a daily dose in maintaining 25-hydroxyvitamin D status. We also tested the hypothesis that vitamin D status can influence the immune response that allows increased viral reactivation among Antarctic expeditioners. The results of these studies, and our findings with regard to other factors affecting vitamin D status, provide evidence to fill documented gaps in vitamin D research (24).

## Participants and Methods

**Participants.** This study was conducted at McMurdo Station, Antarctica, during the winter months (February to September) of 2009. The protocol was approved by the Johnson Space Center (JSC)<sup>7</sup> Committee for the Protection of Human Subjects. The procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The participants provided written informed consent before they participated in the study.

Participants were recruited from the small population of individuals wintering over at McMurdo Station, Antarctica. There were no specific medical inclusion or exclusion criteria, but these individuals passed a physical examination as required for these deployments. Participants already taking vitamin D supplements in excess of 400 IU/d, or 500-mg/d calcium supplements, were excluded from the supplement aspect of the study but were invited to participate in a no-study-supplement group.

**Study design.** Thirty-five participants were recruited for the supplement study. Body weight, BMI, and age did not differ between groups (Table 1). Sixteen additional participants agreed to provide blood and saliva collections and to complete diet logs but took their own supplements. Three main sample and data collection sessions occurred throughout the winter.

After a baseline blood draw in February, the participants who agreed to take supplements were divided into 2 groups (alternating based on order of entry into the study, in a fashion not revealed to the participants) to take one 2000-IU vitamin D pill daily or one 10,000-IU vitamin D pill/wk (therefore the mean daily doses provided from supplements were 2000 and 1430 IU, respectively). The mid-winter blood draw was in early June and the post-winter blood draw was in September. The total duration of supplementation was 206 d (29 wk). Vitamin D intake was

estimated from the diet for 7 d before each blood draw (details provided below). Saliva samples were collected daily for 10 d before each blood draw.

Supplement doses were chosen conservatively to minimize any risks to the participants while they wintered over in Antarctica. The 2000-IU/d supplement dose was based on our earlier Antarctic study (17) and the dose for that study was largely based on the no-observable-adverse-effect level at the time of the study that was assigned by the U.S. Institute of Medicine for vitamin D in their 1997 report. The weekly 10,000-IU supplement dose was based on the view of many experts in the field that this level is a reasonable point for a safe upper limit (25).

Body weights were determined at each session using a standard scale (Health-O-Meter, Continental Scale) and heights were determined at the baseline session using a stadiometer.

**Supplements.** The vitamin D supplements (Tishcon) were prepared and provided to participants in blister-sealed cards of 30 pills (for the daily dose) or cards of 4 (for the weekly dose) and were collected at the end of the study to estimate compliance. Immediately after the first blood draw, the participants taking the daily and weekly doses were given enough pill cards to last for the entire winter.

Supplements were analyzed (Covance) to confirm content before and after they were returned from McMurdo. Data are reported on 3 separate analyses of 20 pills ground together. The 2000-IU pills (3 separate analyses) were measured to be 2230 ± 165 IU and 2520 ± 120 IU before and after the study, respectively. The 10,000-IU pills were measured to be 10,300 ± 361 IU and 11,000 ± 709 IU before and after the study, respectively.

Three incidents were reported by participants to us during the course of the study. One participant reported a urinary tract infection “immediately after taking the supplement” and another participant felt “really off, extremely tired, and had a sore throat that was not clearing up” at the mid-season time point. Both of those participants voluntarily dropped out of the study. Finally, 1 participant reported a possible allergic reaction to the supplements and reported having an allergy to shellfish. It was confirmed that the supplements did not contain shellfish and the participant completed the study with no further issues.

Ten participants (2 from the daily supplement, 3 from the weekly supplement, and 4 from the no-study-supplement group) dropped out of the study before the final time point for unknown reasons other than the 2 listed above. Only data from participants who completed the study are included in this manuscript ( $n = 15, 14,$  and  $12$  for the 2000-IU/d, 10,000-IU/wk, and non-study-supplement groups, respectively).

**Blood collection and biochemical analyses.** Blood was collected into serum separator tubes (Becton Dickinson) after an 8-h fast, centrifuged, and stored upright at  $-80^{\circ}\text{C}$  until all samples were transferred to JSC in Houston in October 2009 after the study was completed. Within 3 wk of sample receipt at JSC, samples were thawed and the serum was separated into smaller aliquots for each test to be analyzed. For tests that are sensitive to freeze-thaws (such as parathyroid hormone), samples were analyzed the day the samples were thawed.

Serum was analyzed for 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, *N*-telopeptide (NTX), bone-specific alkaline phosphatase, vitamin D binding protein, phosphorus, and magnesium, as previously described (17). High-specificity C-reactive protein (hsCRP) was measured using a turbidimetric method (BN II System, Siemens). Serum cortisol was measured by RIA (DiaSorin). The JSC Nutritional Biochemistry Laboratory participates in the Danish External Quality Assessment Scheme program (26) for 1,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3 and also analyzes College of American Pathologists proficiency samples quarterly for quality assurance of serum 25-hydroxyvitamin D3, hsCRP, magnesium, NTX, cortisol, and phosphorus. We also participate in the recently initiated National Institute of Standards and Technology 25-hydroxyvitamin D3 standardization program.

**Saliva collection and latent virus reactivation analysis.** Saliva was collected into individual Salivettes (Sarstedt) as previously described (21,27) immediately in the morning before eating and drinking anything

<sup>7</sup> Abbreviations used: EBV, Epstein-Barr virus; hsCRP, high-specificity C-reactive protein; JSC, Johnson Space Center; NTX, *N*-telopeptide; VZV, varicella-zoster virus.

**TABLE 1** Gender, body weight, BMI, and age of participants taking a 2000-IU/d or 10,000-IU/wk vitamin D pill for ~6 mo during the winter in Antarctica<sup>1,2</sup>

Dose	n	Body weight, kg			BMI, <sup>3</sup> kg/m <sup>2</sup>	Age, y
		Feb	June	Sept		
2000 IU/d	9M/6F	85 ± 16	85 ± 18	87 ± 18*	28 ± 4	37 ± 10
10,000 IU/wk	12M/2F	94 ± 16	93 ± 18	97 ± 16*	29 ± 4	45 ± 10
No study supplements	4M/8F	79 ± 20	79 ± 21	79 ± 20*	27 ± 7	40 ± 11

<sup>1</sup> Values are means ± SD. \*Different from June,  $P < 0.05$ .

<sup>2</sup> Data are from all 41 participants.

<sup>3</sup> BMI was determined at the February (Feb) session.

each day for 10 d before each of the 3 blood draws. The Salivettes were stored at  $-80^{\circ}\text{C}$  until their contents were analyzed.

**Dietary vitamin D intake.** For 7 d before each blood draw, participants completed a modified dietary intake questionnaire listing foods available at McMurdo Station that were known to contain vitamin D (**Supplemental Material 1**) and were analyzed as previously described (17).

**Statistical analyses.** All data are reported as means ± SD. All statistical analyses were performed using Stata, IC software (v 11.1, StataCorp) and setting 2-tailed  $\alpha$  to reject the null hypothesis at 0.05.

Statistical analyses were conducted in 2 phases. The first focused only on the 29 participants who agreed to be randomized to either of the 2 study treatment groups for vitamin D supplementation (2000 IU/d or 10,000 IU/wk), with the goal of determining whether the treatment arms differed in their ability to increase serum 25-hydroxyvitamin D concentration over time. Mixed-effects linear regression techniques were used to evaluate the fixed effect of treatment arm on repeated observations of 25-hydroxyvitamin D with a random intercept to account for within-subject clustering of the data and time-indicators comparing the early (February) data to middle (June) and end (September). Markers of vitamin D and bone metabolism in these groups were also analyzed using this model. Some of the analytes had to be log transformed to achieve normality before statistical analyses were performed (hsCRP, magnesium, NTX, phosphorus, and parathyroid hormone).

Data for the participants who did not take our study supplements are included in the tables in the Results section, but they were not included in this phase of the statistical analysis. This approach was chosen when it became clear that most of these participants took relatively large amounts of their own supplements, and we had no way to confirm compliance throughout the winter or to evaluate the actual dose by pill analysis as we did in the participants who took study supplements.

In the second phase of analysis, data from all 41 participants were analyzed, including those in the no-study-supplement group (4 participants did not provide any vitamin D intake data and were not included). The effect of vitamin D intake and BMI on repeated observations of 25-hydroxyvitamin D during the winter were determined, adjusting for baseline levels of 25-hydroxyvitamin D and participants' baseline BMI. We used mixed-effects linear regression modeling to assess the effects of participants' BMI, vitamin D intake measured 3 times during the study, and the BMI  $\times$  vitamin D intake interaction term on serum 25-hydroxyvitamin D concentration measured longitudinally, adjusting for participants' baseline 25-hydroxyvitamin D levels. This model included a random intercept to account for within-subject clustering of longitudinal data and dummy-coded indicators that compared participants' baseline data (February) to the measurements taken in June and September.

A mixed-effects logistic regression was used to assess the effects of participants' serum cortisol and 25-hydroxyvitamin D concentrations on the probability of detecting Epstein-Barr virus (EBV)<sup>7</sup> and varicella-zoster virus (VZV) in the blood (i.e. EBV or VZV copies  $> 0$ ). Because saliva samples were collected for 10 consecutive days leading up to each of the 3 blood collections (Feb, June, Sept), the model incorporated random intercepts to account for clustering within time period and participant.

## Results

Diet logs were received from all 15 of the 2000-IU/d participants, all 14 of the 10,000-IU/wk participants, and 8 of the 12 no-study-supplement participants. Vitamin D intakes (including any personal or study supplement) were calculated for the 7 d before each blood draw (**Table 2**). Among the 8 participants in the no-study-supplement group who turned in diet logs, it was evident that only 1 participant did not regularly take some sort of vitamin D supplement. The remaining 7 took 400–3000 IU/d vitamin D. Used blister-packed pill cards were returned from 14/15 participants in the 2000-IU/d group, with a mean compliance of  $92 \pm 11\%$ , and from 12/14 participants in the 10,000-IU/wk group, with a mean compliance of  $97 \pm 5\%$ .

Vitamin D insufficiency (defined as 25-hydroxyvitamin D of  $< 50$  nmol/L) was observed in 13 of the 41 participants at baseline. By the end of the winter, all of the participants taking the study supplements had vitamin D concentrations  $> 50$  nmol/L. In both the 2000-IU/d and 10,000-IU/wk supplement groups, 25-hydroxyvitamin D was higher than at baseline ( $P < 0.001$ ) after 3 and 6 mo of supplementation (**Table 3**). The group  $\times$  time interaction effect was not significant ( $P = 0.36$ ), suggesting that changes over time were not significantly affected by whether participants supplemented with smaller daily doses compared with a large weekly dose.

Of the 12 participants in the no-study-supplement group, 4 had circulating vitamin D concentrations of  $< 50$  nmol/L at baseline and after 6 mo, 2 were still considered to have insufficient blood concentrations of vitamin D.

**TABLE 2** Daily vitamin D intake of participants taking a 2000-IU/d or 10,000-IU/wk vitamin D pill for ~6 mo during the winter in Antarctica, for the 7 d before each blood draw<sup>1,2</sup>

Dose	n	Vitamin D intake, IU/d		
		Feb <sup>3</sup>	June	Sept
2000 IU/d	15	291 ± 285	2200 ± 242	2236 ± 293
10,000 IU/wk	14	333 ± 447	1720 ± 484	1780 ± 503
No study supplements <sup>4</sup>	8	1660 ± 1040	1780 ± 876	1400 ± 999

<sup>1</sup> Values are means ± SD.

<sup>2</sup> Data include all vitamin D from foods, study supplements, and personal supplements, and include only the 37 participants who returned diet logs.

<sup>3</sup> The baseline blood draw was in February and participants started taking study supplements (either 2,000 IU/d or 10,000 IU/wk) after that time point.

<sup>4</sup> Participants in the no-study-supplements group took their own supplements, before and throughout the study. Many of these participants had been excluded from the supplement study because of their supplement intake (see Methods section for details).

**TABLE 3** Markers of vitamin D, mineral, and stress status of participants receiving a 2000-IU/d or 10,000-IU/wk vitamin D pill at baseline and after 3 and 6 mo during an Antarctic winter<sup>1,2</sup>

Dose	Feb	June	Sept
25-hydroxyvitamin D, <sup>a</sup> nmol/L			
2000 IU/d	56 ± 17	73 ± 17 <sup>c</sup>	79 ± 16 <sup>c</sup>
10,000 IU/wk	52 ± 10	64 ± 7 <sup>c</sup>	69 ± 9 <sup>c</sup>
No study supplement	59 ± 16	65 ± 17	72 ± 24
1,25 dihydroxyvitamin D, <sup>a,b</sup> pmol/L			
2000 IU/d	92 ± 49	133 ± 99 <sup>e</sup>	139 ± 98 <sup>c</sup>
10,000 IU/wk	66 ± 32	69 ± 22 <sup>c</sup>	82 ± 32 <sup>c</sup>
No study supplement	98 ± 38	91 ± 25	100 ± 50
Parathyroid hormone, ng/L			
2000 IU/d	35 ± 16	29 ± 13	30 ± 14
10,000 IU/wk	28 ± 8	31 ± 15	27 ± 12
No study supplement	34 ± 15	35 ± 20	33 ± 17
Vitamin D binding protein, <sup>a</sup> mg/L			
2000 IU/d	266 ± 52	274 ± 77	320 ± 55 <sup>c</sup>
10,000 IU/wk	273 ± 51	281 ± 58	289 ± 57 <sup>c</sup>
No study supplement	250 ± 24	272 ± 40	270 ± 53
Bone-specific alkaline phosphatase, U/L			
2000 IU/d	23 ± 6	22 ± 7	25 ± 8
10,000 IU/wk	23 ± 8	22 ± 11	22 ± 10
No study supplement	19 ± 4	19 ± 4	20 ± 4
NTX, nmol/L			
2000 IU/d	17 ± 4	16 ± 4	19 ± 4
10,000 IU/wk	17 ± 4	17 ± 6	19 ± 8
No study supplement	15 ± 4	15 ± 4	14 ± 4
Magnesium, mmol/L			
2000 IU/d	0.84 ± 0.16	0.77 ± 0.17	0.88 ± 0.13
10,000 IU/wk	0.83 ± 0.16	0.82 ± 0.16	0.82 ± 0.10
No study supplement	0.79 ± 0.06	0.85 ± 0.09	0.79 ± 0.07
Phosphorus, mmol/L			
2000 IU/d	1.08 ± 0.19	1.08 ± 0.26	1.20 ± 0.21
10,000 IU/wk	1.03 ± 0.25	1.05 ± 0.19	1.01 ± 0.24
No study supplement	1.05 ± 0.10	1.11 ± 0.12	1.09 ± 0.11
hsCRP, mg/L			
2000 IU/d	1.35 ± 0.74	1.11 ± 0.85	1.49 ± 1.42
10,000 IU/wk	2.26 ± 2.16	2.04 ± 1.91	2.45 ± 2.35
No study supplement	1.66 ± 3.47	2.00 ± 4.19	2.28 ± 3.77
Cortisol, nmol/L			
2000 IU/d	458 ± 186	531 ± 199	627 ± 309
10,000 IU/wk	409 ± 143	459 ± 144	454 ± 177
No study supplement	473 ± 153	570 ± 205	486 ± 220

<sup>1</sup> Values are means ± SD. <sup>a</sup>Main effect of time,  $P < 0.05$ ; <sup>b</sup>main effect of group,  $P < 0.05$ ; <sup>c</sup>different from Feb,  $P < 0.05$ .

<sup>2</sup> The data from the no-study-supplement group are reported in this table but were not included in statistical analyses.

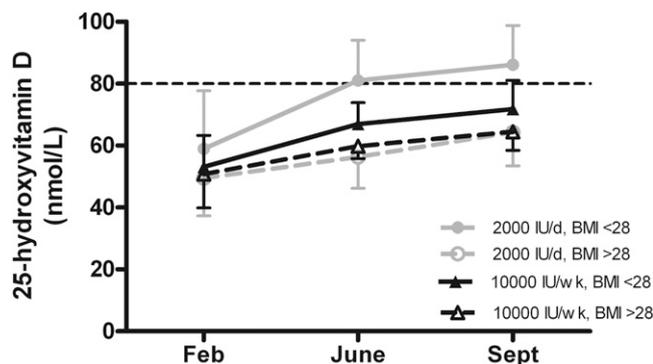
Baseline-adjusted serum 25-hydroxyvitamin D also revealed increases in its status over time ( $P < 0.001$ ) and a positive association with vitamin D intake ( $P < 0.03$ ), but these changes were qualified by time by (baseline) BMI interaction effects. The change in serum 25-hydroxyvitamin D after 6 mo of supplementation was greater for participants with lower BMI relative to higher BMI ( $P < 0.03$ ). This effect was weaker and not significant after only 3 mo of supplementation ( $P = 0.19$ ). While these statistical analyses were performed on the continuum of data, the results for 2 subgroups (BMI  $<$  or  $>$  28 kg/m<sup>2</sup>) are shown (Fig. 1). The change in 25-hydroxyvitamin D after 6 mo

of supplementation was also related to baseline 25-hydroxyvitamin D status ( $P < 0.05$ ) (Fig. 2).

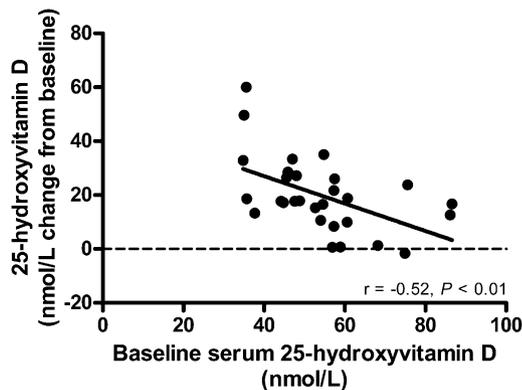
Serum 1,25-dihydroxyvitamin D increased in both supplemented groups after 3 and 6 mo of supplementation ( $P < 0.001$ ), but the increase remained within the normal clinical range for 1,25-dihydroxyvitamin D. The change in 1,25-dihydroxyvitamin D was not correlated with the change in serum 25-hydroxyvitamin D (data not shown). Vitamin D binding protein was higher after 6 mo of supplementation compared with baseline ( $P < 0.01$ ).

There were no time, group, or interaction effects on serum parathyroid hormone, hsCRP, cortisol, NTX, magnesium, or phosphorus (Table 3).

The mixed-effects logistic regression model evaluating the effects of participants' serum cortisol and 25-hydroxyvitamin D concentrations on the probability of EBV shedding over time revealed a serum cortisol by 25-hydroxyvitamin D concentration interaction effect ( $P < 0.03$ ). Both cortisol and 25-hydroxyvitamin D were measured on a continuous scale and interaction effect among continuous variables is best illustrated by a figure depicting the marginal means estimated from the data and model. In our data, the 25th and 75th percentiles for cortisol levels were 131 and 225  $\mu$ g/L, with 25-hydroxyvitamin D ranging from 20 to ~104 nmol/L. The interaction effect revealed in the data was illustrated by modeling the probability of EBV shedding for participants with cortisol levels at low (25th percentile) and high (75th percentile) levels across the range of observed 25-hydroxyvitamin D concentrations (Fig. 3). For individuals with a high serum cortisol concentration, increasing 25-hydroxyvitamin D tended to reduce the likelihood of EBV shedding relative to individuals with low serum cortisol concentrations. Vitamin D status had no effect on VZV shedding in these participants. Six participants in the no-study-supplement group had detectable EBV copies in saliva at baseline and 4 participants in the 2000-IU/d and 1 in the 10,000-IU/wk groups had EBV copies in saliva at baseline (Supplemental Fig. 1). In June, the number of participants with detectable EBV copies was 6, 2, and 2 for the no-study-supplement, 2000-IU/d, and 10,000-IU/wk groups, respectively. In September, there were 6, 3, and 3 participants with detectable EBV copies in the 3 groups,



**FIGURE 1** Serum 25-hydroxyvitamin D at baseline and 3 and 6 mo after supplementation began for participants with BMI  $<$  28 kg/m<sup>2</sup> or  $>$  28 kg/m<sup>2</sup> in groups taking vitamin D in a once-daily dose of 2000 IU or a once-weekly dose of 10,000 IU. Values are means ± SD,  $n = 10$  (daily, BMI  $<$  28), 5 (daily, BMI  $>$  28), 8 (weekly, BMI  $<$  28), or 6 (weekly, BMI  $>$  28). Data were statistically analyzed over the continuum of BMI but were split into subgroups to visually present the relationship here. The change in serum 25-hydroxyvitamin D after 6 mo of supplementation was greater for participants with lower BMI than for those with higher BMI ( $P < 0.03$ ).



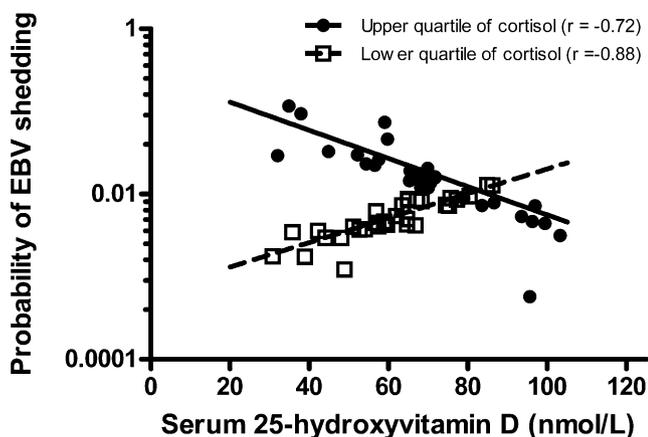
**FIGURE 2** Serum 25-hydroxyvitamin D of participants expressed as the change from baseline after 6 mo of supplementation plotted against baseline serum 25-hydroxyvitamin D. The points represent values for individual participants in all groups (no study supplement, 2000 IU/d, 10,000 IU/wk;  $n = 29$ ).

respectively. VZV was shed more frequently in the month of June than at baseline (Feb) or in September ( $P < 0.05$ ), but the number of copies shed did not differ between groups. Similar responses occurred with EBV (Supplemental Fig. 2).

## Discussion

These data show that a once-daily 2000-IU and a once-weekly 10,000-IU vitamin D supplement were equally effective in increasing vitamin D status in participants not exposed to sunlight for 6 mo. The response to supplementation depended on both BMI and baseline vitamin D concentration. Furthermore, the roles of vitamin D status and stress in immune responsiveness to viral reactivation interacted. These findings represent valuable information about vitamin D and provide evidence targeted at gaps that were identified earlier in our understanding of vitamin D (24).

In this study, 80 nmol/L was the target for serum 25-hydroxyvitamin D concentration, because this was considered the optimal level for vitamin D status for many health outcomes at the time the study was designed [reviewed in (7)]; however, the recently released dietary guidelines (12) have highlighted both



**FIGURE 3** The interaction of serum cortisol with the relationship between study participants' 25-hydroxyvitamin D status and the probability of EBV shedding. Data from all 41 participants were included in this graph. Data were statistically analyzed over the continuum of cortisol data but were split into subgroups to visually present the relationship here.

positive and negative nonskeletal health outcomes at higher vitamin D concentrations. After ~6 mo of supplementation, 8 of 15 participants in the 2000-IU/d group achieved serum 25-hydroxyvitamin D  $> 80$  nmol/L and 1 of 14 participants in the 10,000-IU/wk group had serum 25-hydroxyvitamin D  $> 80$  nmol/L at the end of the study. Those participants who ended the winter with serum 25-hydroxyvitamin D  $> 80$  nmol/L had a lower BMI ( $25 \pm 3$  kg/m<sup>2</sup>) than those who never reached 80 nmol/L ( $29 \pm 5$  kg/m<sup>2</sup>), regardless of their supplementation group. When we reevaluated data from our earlier vitamin D supplementation study in Antarctica (17), we found similar relationships. That is, individuals with lower BMI were more responsive to vitamin D supplementation (data not presented). Similar to our previous study, the mean change per 100 IU over the course of the winter was  $1.0 \pm 0.7$  and  $1.0 \pm 0.8$  for the 2000-IU/d and 10,000-IU/wk groups, respectively. For participants with a BMI  $> 28$  kg/m<sup>2</sup>, the change per 100 IU was  $0.7 \pm 0.5$  nmol/L and  $0.8 \pm 0.8$  nmol/L for the 2000-IU/d and 10,000-IU/wk groups, respectively. Participants with a BMI  $< 28$  kg/m<sup>2</sup> had a mean change of  $1.3 \pm 0.7$  nmol/L and  $1.5 \pm 0.4$  nmol/L for the 2 groups, respectively.

Another factor that affected the change in 25-hydroxyvitamin D status was the baseline 25-hydroxyvitamin D concentration. Participants with lower baseline 25-hydroxyvitamin D not only did not need more vitamin D to achieve a serum concentration of 80 nmol/L, but they actually responded to a similar dose with a greater change in serum 25-hydroxyvitamin D than participants with higher baseline concentrations (Fig. 2).

In this study, we also investigated the effect of vitamin D status on a functional test of immune function by analyzing reactivation of 2 latent viruses in saliva. Vitamin D status had an effect on the predicted level of viral shedding only when the serum cortisol concentration was high. Those individuals with a higher serum 25-hydroxyvitamin D were less likely to have viral (EBV) shedding in saliva. Environments or occupations associated with high stress, such as spaceflight or polar workers, have an increased risk for immunosuppression (19,23). Attenuating the probability of viral shedding in persons whose immune function is compromised has the potential to have a positive impact on many people on Earth in high-stress environments as well as crewmembers during spaceflight. These data are the first to suggest that a higher vitamin D status may help protect against reactivation of latent viruses, which is associated with impairment of immune function.

Compliance was an issue in a previous supplementation study we performed at McMurdo Station with daily dosing (compliance ranged from 77 to 90%) (17). In the current study, compliance in the 2 groups was similar and was higher than in our previous study. Increased compliance may help explain why serum 25-hydroxyvitamin D concentrations were higher at the end of this study than at the end of our previous study. Compliance may have been improved in this study by provision of the pills in blister packs, which might have been easier to use (each blister card had a date for when the pill was to be taken). In the earlier study (17), supplement dose was masked, and participants were provided 2 bottles and were asked to take a pill from each bottle per day to achieve planned dosages. Masking was not possible given the design of the current study.

In conclusion, in the absence of UV light and with modest dietary intake of vitamin D, supplementation with either 2000 IU/d or 10,000 IU/wk can provide adequate levels of vitamin D. The response to supplementation was related inversely to BMI and was also related to the baseline concentration of circulating vitamin D. Finally, this study provides the first evidence to our knowledge that

a higher vitamin D status (serum 25-hydroxyvitamin D concentration toward the upper end of a range from ~30 to 105 nmol/L) has the potential to mitigate immunosuppression in environments where stress hormones are elevated, regardless of supplementation dosage or baseline vitamin D status. These conclusions have broad implications not only for other research studies but also for clinical settings, where vitamin D evaluation and supplementation have become almost routine.

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