

# Vitamin D Supplementation in Underway Submariners

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**Introduction:** We assessed the efficacy of vitamin D supplementation in maintaining normal calcium and bone homeostasis in underway submariners deprived of sunlight. **Methods:** Serum 25-hydroxycholecalciferol (25(OH)D), 1,25-dihydroxycholecalciferol (1,25(OH)<sub>2</sub>D), calcium, parathyroid hormone (PTH), phosphate, osteocalcin, bone specific alkaline phosphatase, and urinary levels of N-telopeptide were examined in 51 subjects aboard a submarine. These levels were obtained prior to a 76-d deployment, before and after a 6-d liberty period (deployment day 49 and 55), and on return to homeport. There were 26 subjects who received 400-IU of vitamin D daily supplementation, and 25 who received placebo. **Results:** Both groups exhibited significant reductions in 25(OH)D levels in the initial submergence (a decrease from  $28.3 \pm 15$  ng·ml<sup>-1</sup> to  $24.1 \pm 10$  ng·ml<sup>-1</sup> in the experimental group and  $26.3 \pm 10$  ng·ml<sup>-1</sup> to  $20.7 \pm 9$  ng·ml<sup>-1</sup> in the controls), an increase in 25(OH)D levels not significantly different from baseline during the liberty period, and decrements in 25(OH)D on repeat submergence ( $22.8 \pm 10$  ng·ml<sup>-1</sup> in experimental and  $21.4 \pm 10$  ng·ml<sup>-1</sup> in controls). Both groups exhibited an increase in post-liberty osteocalcin ( $20.4 \pm 6$  ng·ml<sup>-1</sup> to  $24.5 \pm 5$  ng·ml<sup>-1</sup> for experimental and  $18.3 \pm 6$  to  $23.5 \pm 7$  ng·ml<sup>-1</sup> for controls), and stable serum calcium levels throughout the patrol. **Conclusions:** 400-IU daily vitamin D supplementation was insufficient in maintaining serum vitamin D levels in underway submariners, engendering biochemical evidence of bone resorption and turnover. Six d sunlight exposure compensated for 49 d sunlight absence, supporting the enormous capacity of UV-B mediated vitamin D production.

**Keywords:** vitamin D supplementation, depression, submariners, ultraviolet light, vitamin D deficiency, risks for malignancy, risks for autoimmune disease.

THE SUBMARINE environment is unique, imposing numerous physiological and psychological stressors, including confinement, sunlight deprivation, circadian desynchronization, hypercarbia (crewmembers in U.S. Navy submarines breathe an artificial recirculated atmosphere with elevated carbon dioxide levels, precipitating a mild respiratory acidosis, and bone and renal buffering) (1), reduced access to perishable food, micronutrient alterations stemming from distilled water supplies, and inaccessibility to weight-bearing exercise. Significant decreases in serum 25(OH)D have been measured in deployed U.S. Navy submariners (2). Noting the seminal role that sunlight serves in maintaining normal vitamin D levels, this is not an unexpected result. Presently, neither conventional nor full spectrum lighting sources exist possessing the spectral wavelengths required in the ultraviolet region (290–320 nm) for clinically significant vitamin D production.

One must appreciate the central role exhibited by vitamin D in calcium and bone metabolism, and the pervasive hormonal activity of vitamin D (3–15), wherein a deficiency becomes a risk factor not only for fractures (13), osteoporosis, osteomalacia, and rickets, but numerous malignancies, autoimmune diseases, hypertension, depression, infectious diseases, and nonspecific musculoskeletal pain (3,5,13). Appreciating the potentially deleterious consequences of vitamin D deficiency, we investigated the efficacy of 400-IU daily vitamin D supplementation on vitamin D homeostasis in submariners serving on extended deployments, who are essentially deprived of all sunlight exposure. We hypothesized that this supplementation strategy was sufficient to maintain levels at the pre-deployment baseline throughout the underway.

## METHODS

Human testing was approved by ethical review boards for the protection of human subjects at the Naval Submarine Medical Research Laboratory, Naval Health Research Center, and the Bureau of Medicine and Surgery. Each subject provided written informed consent before participating. We recruited 51 subjects who were randomized into an experimental group (n = 26) receiving 400-IU daily vitamin D supplementation, and a control group (n = 25), who were given an identically appearing placebo tablet (vitamin B<sub>6</sub>, Nature's Bounty, Inc., Bohemia, NY). Subjects were randomly assigned a randomized code prepared using computer-generated random numbers, and the study was conducted in a double-blind fashion.

At the time this study was conceived, the investigators believed that the convenient, inexpensive, and readily accessible 400-IU vitamin D<sub>3</sub> supplementation was a reasonable dose to maintain 25(OH)D levels well within the normal range. This assertion was predicated

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on the fact that prior investigations noted that while submariners experienced a decrease in serum 25(OH)D levels after a 68-d deployment, their levels remained within the normal range (2), and the acknowledgment that dairy products were supplemented with vitamin D and available for consumption underway (fortified foodstuffs: ice cream, yogurt, powdered milk).

We used 50 mg · d<sup>-1</sup> (U.S. RDA) of vitamin B<sub>6</sub> as the placebo in this study because we believed this vitamin had no influence on bone and calcium metabolism and pathology (3). The selection was motivated by convenience, as the tablets appeared similar to vitamin D, were readily procured by the same company furnishing the vitamin D tablets, and circumvented acquisition of a more expensive placebo. Some recent evidence in an animal model suggests that vitamin B<sub>6</sub> could function as a co-factor to build up collagen cross-links; however, there is little evidence to date to support this in humans (14). Based on the existing literature, we do not believe use of vitamin B<sub>6</sub> as the placebo affected our study's results or conclusions, but we cannot make this claim with complete certainty.

Baseline sampling of serum 25(OH)D, 1,25(OH)<sub>2</sub>D, calcium, phosphorus, parathyroid hormone, alkaline phosphatase activity, isolated urinary N-telopeptide (N-Tx), and osteocalcin was performed within 1 wk prior to deployment. This was subsequently repeated on day 49, prior to the submarine's port visit (Hawaii), at the end of the 6-d port visit (day 55), and on return to the homeport 21 d later (day 76), for a total of four collections.

The setting of this study was a U.S. fleet ballistic missile submarine (homeport: Bangor, WA; latitude: 47.7°N) scheduled for a patrol of 76 d from January to April 2003. The schedule included an initial 49-d submerged underway, followed by a 6-d port call in Pearl Harbor, HI (latitude 21.3°N), concluding with another 21-d submerged underway. The latitudes noted are significant, in that they appreciably influence the exposure levels to UV-B, and thus to vitamin D homeostasis. As will be discussed, the brief exposure to the sunny Hawaiian latitude significantly influenced vitamin D status.

The study participants were 51 sailors (all men), assigned to the submarine. Two men were African American, the rest Caucasian. The mean age of the study participants was 28 yr with a range of 26 yr (minimum age 20 yr, maximum age 46 yr). The mean number of patrols for the study participants was 5 with a range of 17 (minimum 1 patrol, maximum 18 patrols).

Collected samples were immediately placed in closed containers, with care taken to minimize light exposure, and packed in dry ice in preparation for shipment to the laboratory testing facilities. All laboratory analyses were performed by Quest Diagnostics (Los Angeles, CA). Parathyroid hormone (PTH) was analyzed via an immunochemiluminometric assay; calcium and phosphate via spectrophotometry; 25(OH)D via radioimmunoassay; 1,25(OH)<sub>2</sub>D via extraction chromatography and radioreceptor assay; bone specific alkaline phosphatase via immunoenzymatic assay; random NTx via

enhanced chemiluminescence; and osteocalcin via immunoradiometric assay.

All analyses were conducted using SigmaStat® 2.03 software by SPSS, Inc. (Chicago, IL). The number enrolled (51 subjects) yields a power of 0.83 to detect a conservative difference in 25(OH)D levels of 9 ng · ml<sup>-1</sup> in a distribution possessing a standard deviation of 12 ng · ml<sup>-1</sup> at a significance level of 0.05 for a one-tailed test. For each variable, a one-way repeated measures analysis of variance of the drug and placebo groups was performed to determine statistically significant changes over the study period. The Tukey test was used for all pair-wise multiple comparisons. The treatment group was compared with the placebo group at baseline and at each of the follow-up collection dates using a *t*-test when the data passed the test of normality. When the data failed the test of normality, the comparison was performed using a Mann-Whitney rank sum test. Differences were considered statistically significant at *p* < 0.05.

## RESULTS

Results are significant for an anomalous increase in calcium in the placebo group at the beginning of the liberty period. There was a significant decrease in phosphate experienced by both groups at the end of the liberty period, and a significant increase experienced by both groups, exceeding all prior measurement points, on return to homeport.

During both phases of the patrol in both groups, 25(OH)D decreased significantly, and exhibited an increase not significantly different from baseline during the liberty period. Notably, the decrease experienced on return to homeport was significantly below both baseline and end of liberty values. During the first phase of patrol and through the liberty period, 1,25(OH)<sub>2</sub>D decreased significantly in both groups while increasing significantly on return to homeport.

Alkaline phosphatase exhibited a significant increase in the first phase of deployment in both groups, but otherwise was not significant from baseline. Osteocalcin exhibited a significant increase from baseline and from the end of the liberty period on return to homeport in both groups. N-Tx levels in both groups exhibited a non-significant decreasing trend after the 49-d submergence, an increasing trend at 55 d, and a decreasing trend on return to homeport. There was no significant difference between the two groups at any of the time-points.

## DISCUSSION

Our results corroborate prior investigations identifying significant decrements in serum vitamin D [25(OH)D] levels in underway submariners due to sunlight deprivation. The critical bioassay reflecting overall vitamin D status is 25(OH)D and not 1,25(OH)<sub>2</sub>D (representing the instantaneous hormonal response to calcium and phosphate status) or calcium levels. The body has an enormous capacity to maintain normal calcium and 1,25(OH)<sub>2</sub>D levels despite acute vitamin D deficiency, drawing on the PTH and skeletal systems at the

expense of the bone reservoir of sequestered calcium (3). Our averaged pooled experimental and control data yielded a decrement from  $27 \text{ ng} \cdot \text{ml}^{-1}$  to  $22 \text{ ng} \cdot \text{ml}^{-1}$  or 19% in 49 d. Prior investigations have yielded a 39% decrement in 68 d (2). The relative normalization of serum 25(OH)D levels to the pre-deployment baseline after a mere 6-d exposure to sunlight, coupled with the repeatable decrements observed after each submergence, substantiates the prominent, expedient, and robust mechanism of UV-B mediated vitamin D production.

The calcium and phosphate levels exhibited expected profiles given the vitamin D decrements observed. Normal calcium homeostasis can be maintained via the PTH-mediated increase in bone resorption, conversion of 25(OH)D to  $1,25(\text{OH})_2\text{D}$  mediating increased intestinal absorption, and renal retention of calcium coupled to phosphorus excretion (3). The phosphate decrease observed during the initial submergence reflects and supports this mechanism. The elevation in phosphate noted at the end of the study may suggest escape from this mechanism after replenishment of the vitamin D stores after the liberty call not appreciated at day 55 due to a delay in the vitamin D mediated suppression of PTH elaboration. The isolated increase in calcium experienced by the placebo group at the beginning of the liberty period may stem from the cyclical deposition and release of carbon dioxide and calcium from the bone buffering system experienced in chronic low-level hypercapnia.

We noted a ~20% decrease in  $1,25(\text{OH})_2\text{D}$  levels after 49 d underway, which corroborates underway vitamin D deficiency. This was unexpected, however, given that prior clinical and operational investigations have noted stable  $1,25(\text{OH})_2\text{D}$  and calcium levels. This observation underscores the assertion that the 25(OH)D level is the biomarker representative of overall nutritional status, not the  $1,25(\text{OH})_2\text{D}$  or calcium levels. However, the anticipated  $1,25(\text{OH})_2\text{D}$  stability coupled to increased PTH levels was not observed in our study (2). It is implausible for the  $1,25(\text{OH})_2\text{D}$  levels to decrease in this period, and we ascribe this observation to the sample variation and potential specimen degradation in transport elaborated on later.

Although there was no statistical change in PTH, we did observe a trend for stability in the treated group with a decrease in the controls after sunlight exposure during liberty. The controls may have maintained an elevated PTH level, compensating for a more marked vitamin D deficiency which was otherwise mitigated in our treatment group, which had uniform PTH levels. Alternatively, an underlying physiological process could have reduced parathyroid gland sensitivity to declining calcium levels in the unique submarine environment. Such speculation requires further investigation.

During bone resorption, the amino- and carboxyl-terminal extension peptides of procollagen are released into the circulation. These peptides may be captured by the N-Tx and carboxy-telopeptide assays. Urinary N-Tx is a relatively specific marker of bone resorption and exhibited no significant change in this study, although

decreasing trends during the initial deployment, and on return to homeport after the liberty call, was observed. These results are puzzling, as one would anticipate the opposite trends in bone resorption given the decrements observed in serum 25(OH)D levels. One palatable explanation stems from the observation that due to the logistics of conducting an operational study, the ideal 24-h urine collection could not be procured. A first morning void is acceptable in most research; however, not in those undergoing circadian variations, as experienced in our subjects. We accepted a random urine sampling, recognizing the deficiency of this approach while hoping to identify potentially significant changes. Future investigations can circumvent this difficulty by employing a serum carboxy-telopeptide level, which is more amenable to an operational study. A superior solution, however, may be to aggressively monitor 24-h urine sample collections for biomarkers of bone turnover exhibiting significant circadian variation (4,8). A spot random sample makes interpretation untenable due to the superimposed circadian variation in timing of sample collection (4,8).

Osteocalcin and bone-specific alkaline phosphatase are secreted by osteoblasts, and are relatively specific biomarkers for skeletal formation and accelerated bone turnover. Osteocalcin decreased during the initial 49-d underway, consistent with the observed decrements in vitamin D levels mandating bone resorption for maintenance of serum calcium. Elevation of this biomarker at the end of deployment represents increased bone formation after replenishment of 25(OH)D stores following sunlight exposure during port call. Bone-specific alkaline phosphatase, in contradistinction, increased in both groups after the 49-d underway, opposite the decrease experienced in osteocalcin, an inconsistent result. This may reflect a generalized state of bone remodeling activity experienced due to the observed decrease in serum 25(OH)D levels. The inconsistency may also be attributed to the circadian variation in sample concentration, an underappreciated concept in the inception of this study, but with obvious ramifications in our population, who were experiencing circadian desynchronization. An additional factor is artifact.

We could not control for the timing of lab collections, contingent on both the imposed operational constraints and the crew circadian desynchronization experienced underway. Since all the biomarkers exhibit circadian variation, exhibiting their highest concentrations in early morning, and with a wide range in accepted normal concentrations (see **Table I**), the intra-individual variation can be extreme, particularly for urinary measurements (4).

Additionally, specimen processing, storage, and transport, coupled to inter-laboratory and inter-assay variations (both of which may approach 40% in some labs), are technical issues which influence the measurements (8). Thermodegradation and photolysis are specific concerns with bone metabolic assays (8). Instability in sample collections may occur in 1 h if serum samples are maintained at room temperature due to enzymatic cleavage or proteolysis (i.e., osteocalcin). This mandates

TABLE I. BIOMARKERS OF CALCIUM AND BONE METABOLISM (MEAN ± SD).

	Group	Baseline	Day 49: First Day of Liberty	Day 55: Last Day of Liberty	Day 76: End of Deployment
Biomarkers of Calcium Homeostasis**					
Calcium (mg/dL) (8.5–10.4)	Experimental	9.3 (0.5)	9.4 (0.3)	9.5 (0.5)	9.3 (0.3)
	Placebo	9.1 (0.5)	9.6 (0.3)*	9.4 (0.6)	9.3 (0.3)
Phosphate (mg/dL) (2.5–4.5)	Experimental	3.6 (0.5)	3.8 (0.4)	3.4 (0.5)*/+++	4.2 (0.4)*/++/§
	Placebo	3.5 (0.4)	3.8 (0.6)	3.1 (0.5)*/+++	4.3 (0.6)*/++/§
25(OH)D (ng/ml) (10–68)	Experimental	28.3 (15)	24.1 (10)*	27.5 (11)++	22.8 (10)*/§
	Placebo	26.3 (10)	20.7 (9)*	23.5 (8)++	21.4 (10)*/§
1,25(OH) <sub>2</sub> D (pg/ml) (15–60)	Experimental	41.0 (11)	34.4 (7)*	32.6 (11)*	46.0 (8)++/§
	Placebo	44.0 (9)	33.0 (8)*	31.2 (10)*	46.5 (12)++/§
PTH (pg/ml) (10–65)	Experimental	43.5 (21)	44.6 (20)	46.0 (27)	45.0 (17)
	Placebo	52.4 (18)	51.9 (26)	44.8 (22)	51.7 (22)
Biomarkers of Bone Turnover					
Alkaline Phosphatase (μg/L) (5.9–22.9)	Experimental	13.8 (4)	16.5 (5)*	13.1 (7)++	11.4 (6)++
	Placebo	14.1 (7)	17.9 (8)*	13.8 (3)++	13.4 (5)++
N-telopeptide (nmol/mmol creatinine) (> 85)	Experimental	40.8 (19)	37.8 (12)	48.8 (20)	40 (13)
	Placebo	38.2 (16)	34.1 (10)	41.4 (14)	35 (12)
Osteocalcin (ng/ml) (11.3–35.4)	Experimental	20.4 (6)	19.4 (5.4)	20.7 (5.9)	24.5 (5)*/++/§
	Placebo	18.3 (6)	17.2 (5.4)	18.3 (5.8)	23.5 (7)*/++/§

\* denotes significance from baseline; ++ denotes significance from beginning of liberty; § denotes significance from end of liberty.

\*\* Normal values listed below each biomarker; values obtained from Quest Diagnostics, Los Angeles, CA.

immediate specimen refrigeration and freezing if analysis is delayed by more than 3 d.

The logistics of collecting blood samples underway, with appropriate preparation, storage, and transport of specimens, is more laborious than in the clinical setting. Although we strove to maintain consistency by enlisting one lab facility, employing the same assays for analysis, and expedited shipment of lab collections, the transport of specimens collected in the pre- and post-liberty periods from Hawaii arrived at the lab facilities 5 and 7 d after collection, respectively, whereas the collections procured in Bangor at baseline and on trial completion arrived only 1 and 2 d after collection. Sub-optimal sample refrigeration during transport, or excessive exposure to sunlight, may have produced specimen degradation resulting in inconsistent and lower than anticipated levels of PTH, alkaline phosphatase, and 1,25(OH)<sub>2</sub>D observed pre- and post-liberty. However, these assertions are only speculative, and would apply to the handling and transport by the contracted courier service. The collected samples were immediately placed in closed containers with dry ice after collection, which would suggest that vagaries in the logistics of sample collection were probably not a significant threat to validity.

Several important confounders may have influenced calcium and bone metabolism, namely: 1) compliance; 2) diet; 3) hypercarbia; 4) activity; and 5) circadian desynchronization. Low subject compliance in our study may have led to decreased vitamin D levels in the treatment group, and lack of differences between treatment and placebo. Some subjects may have occasionally missed a supplement dose, just as some subjects occasionally missed a blood or urine sampling session. We ended the study with 3% of data missing. It is unlikely that a similar rate of missed supplement doses would influence our results. All supplement containers were returned empty. Therefore, although we did not quantify compliance, we have no reason to believe low compliance

was pervasive enough to explain low serum vitamin D levels in treated subjects, or to otherwise affect our results systematically. Future investigations will necessarily mandate direct observed therapy to circumvent this potential confounder.

Vitamin D is fat-soluble, and fat malabsorption may occur in numerous clinical disorders including biliary, pancreatic, and intestinal disease. There was no evidence to support this condition in this cohort of healthy sailors who are screened for any significant disease prior to deployment, a prerequisite in submariners with diminished access to emergency care facilities. Ostensibly, no member suffered from any liver or kidney disease, which could influence metabolism.

Investigations have documented normal vitamin D absorption in underway submariners, eliminating this potential confounder (7). Finally, a study performed across submarine platforms, comparing underway vs. shore-based nutrition, failed to demonstrate any significant deviation in dietary practices or in biomarkers of lipid metabolism (9). Ship's stores of fresh fruits, vegetables, and dairy products are typically exhausted within 1–2 wk of an underway, followed by ad-lib consumption of powdered products (which are vitamin D supplemented). Unfortunately, dietary logs were not maintained in this study. It would be difficult to attempt to estimate, retrospectively, individual vitamin D consumption underway, given the independent access to dairy products (milk, ice cream, etc.). However, a rudimentary dietary survey conducted underway revealed that portions of meat, ice cream, yogurt, and cheese consumption remained constant, while milk consumption decreased by 8 oz, attributed to the depletion of fresh milk stores, usually within the first 2 wk of patrol (2). Thus, one could estimate a daily decrease of 100 IU in the dietary intake of vitamin D. This may equate to a decrease in 25(OH)D levels of approximately 1 ng · ml<sup>-1</sup> after several weeks, an appreciable effect, but accounting for only a small portion of the variance

identified. Dietary logs will need to be distributed in future investigations, permitting stratification of results based on dietary composition.

Submariners experience low-level chronic hypercapnia underway, which influences vitamin D, calcium, bone, and renal metabolism (1). Bone buffers the increased acidic load imposed by either metabolic or respiratory acidosis via unique mechanisms, which function by exchanging hydrogens for calcium, sodium, and potassium. While the decreased bicarbonate content of metabolic acidosis fosters physicochemical dissolution and reduced bone mineral formation, the increased partial pressure of carbon dioxide and bicarbonate encountered in chronic low-level hypercapnia (respiratory acidosis) favors the deposition of carbonated apatite, which subsequently reduces plasma calcium. Chronic low-level hypercapnia, lower than the acid load required to trigger renal compensation, subsequently engenders a cyclical deposition and release of carbon dioxide and calcium from the bone buffering system. This is mediated by the bone's relatively stable, slowly exchangeable carbonate ( $\text{CO}_3$ ) and bicarbonate ( $\text{HCO}_3^-$ ) in the hydration shell of the hydroxyapatite crystals. Once the buffering system becomes saturated, the  $\text{CO}_2$  levels exceed the renal threshold for compensation, promoting an increased acid ( $\text{H}^+$ ) excretion. Once the acidic load decreases below threshold, and some bone buffering capacity is replenished, the cycle repeats itself. Calcium follows  $\text{CO}_2$  passively in this cyclical acid-base regulatory mechanism. It is absorbed in bone during the buffering stages, and released from bone and excreted by the kidneys during the renal compensatory phases (7). Respiratory acidosis does not influence bone osteoclastic, osteoblastic, and surface ion (sodium and potassium) activity. However, the phenomena outlined suggest whole-body calcium retention, engendering a potentially increased risk for tissue calcifications and renal stones (1).

Thus, the initial days of deployment are accompanied by complex interrelated metabolic alterations imposed on the bones and kidneys due to acid-base alterations. These stem from chronic low-level hypercarbia, yielding lowered plasma calcium and reduced renal calcium excretion.  $\text{CO}_2$  levels were not measured underway; however, recent advances in atmospheric control equipment have resulted in progressively lowered ambient  $\text{CO}_2$  levels ranging from 0.22–0.43% on submarines similar to ours. This is decreased from values ranging from 0.5–1% in earlier investigations (2). This observation would seem to mitigate the influence of  $\text{CO}_2$  on calcium levels. As a result, deviations in calcium homeostasis are more likely due to vitamin D deficiency (7).

Activity and exercise affect bone metabolism and density via inertial and gravitational impact loading, muscle forces acting on bones, and circulatory shear stresses within bone tissue. Reduced physical activity is known to increase plasma calcium due to reduced bone formation, as has been well documented in patients undergoing prolonged bed rest and subjects enduring spaceflight. Submarine deployment substantially decreases crewmember activity and exercise from anecdotal

evidence and prior research. Specifically, submariners spend a proportionally increased time in recumbent, semi-recumbent, or sitting positions, resulting in an overall reduction in the amount of stress applied to the skeleton (1). We did not regulate or quantify subject activity or exercise before or during deployment or liberty periods.

Serum and urinary biomarkers of bone metabolism all exhibit circadian variation, which may influence interpretation of lab results reflecting bone turnover, particularly those biomarkers which sustain acute fluctuations. This necessitates consistent timing of sample collections, optimally in the morning when concentrations are highest (4). This is in all aspects similar to the rationale for collecting an a.m. cortisol level.

U.S. submarines operate on an 18-h watchstanding schedule (18-h "day"), fostering free running circadian rhythms, which undermine any attempt to standardize timing of data collection. This undoubtedly can potentially cause undesirable variability, mandating 24-h urine collections in future investigations.

The lack of significance may be attributed in part to the large standard deviations accompanying the measurements, suggesting wide inter-individual differences. This may have stemmed in part to an inadequate control over dietary intake and liberty sunlight exposure. In total absence of sunlight, a minimum of 1000 IU  $\cdot \text{d}^{-1}$  vitamin D supplementation may be required (6,13). Support corroborating this assertion may be provided by the fact that 400 IU did not prevent wintertime insufficiency in northern latitudes (11,13).

Numerous sources continue to quote normal minimums of 25(OH)D down to 8 ng  $\cdot \text{ml}^{-1}$  (2). Recent investigators, however, have asserted a much higher value, between 30–40 ng  $\cdot \text{ml}^{-1}$  for optimal health. This is based on bone density measurements, fracture prevention studies in the elderly, arrest of osteoarthritis progression, and from levels proven to suppress elevated PTH-mediated bone osteoclastic activity (6,13). Achieving this level of vitamin D may require a higher daily supplementation than presently prescribed for young adults, an adequate intake of 200 IU (11).

A notable secondary observation from our study is the low baseline level of 25(OH)D, corroborated by prior investigations on underway submariners noting levels  $< 20$  ng  $\cdot \text{ml}^{-1}$  prior to an underway. This suggests a chronic, pervasive, and universal deficiency in light of newly championed guidelines. These observations are not confined to submariners, but are germane to an appreciable percentage of our population residing at northern latitudes (6,7,13).

Sunlight is less available in winter with the sun's rays passing at a more oblique angle, reducing available UV-B due to absorption and light scattering. In fact, above about 35° latitude, the angle of the sun is so oblique during the winter months that most, if not all, of the UV-B photons below 315 nm are absorbed by the ozone layer, thereby either reducing or completely preventing the production of vitamin D in the skin. Inverse correlations between residence at increased latitudes,

decreased wintertime vitamin D levels, and decreased bone density have been observed. For example, residents in Boston (42°N), Edmonton (52°N), and Bergen, Norway (61°N), cannot produce sufficient quantities of vitamin D in their skin for 4, 5, and 6 mo, respectively (6).

The relation between vitamin D deficiency, rickets in children, and osteomalacia in adults is well appreciated (6,11). Successful reduction in elderly rates of bone fractures and osteoporosis with vitamin D supplementation has confirmed the often-underappreciated role of vitamin D deficiency in these serious clinical diseases (11). Increasingly recognized, population subgroups such as the elderly and infirm are vitamin D deficient, including over half of an internal medicine inpatient population surveyed, according to a conservative definition of  $25(\text{OH})\text{D} < 15 \text{ ng} \cdot \text{ml}^{-1}$  (10).

The Navy has conformed to recommended guidelines, requiring the fortification of foodstuffs (powdered milk, ice cream) and providing more than the recommended adequate intake. All prior investigations have revealed  $25(\text{OH})\text{D}$  levels exceeding the minimum recommended thresholds. One may reasonably surmise that vitamin D deficiency is not intrinsic to submarine life, but may reflect a more pervasive universal public health issue affecting an appreciable percentage of the northern population.

Recent research has unveiled the "hormonal" action of vitamin D, the ubiquitous presence of cellular vitamin D receptors, and the pervasive role in mediating cellular differentiation and proliferation in varied tissues. Impressive epidemiological and circumstantial evidence implicates vitamin D deficiency (or its "surrogate," lack of UV-B exposure) as a risk factor for malignancies (breast, ovarian, prostate, colorectal, lung, bladder, renal, pancreatic, stomach, and lymphomatous), hypertension, fibromyalgia, non-specific musculoskeletal pain, depression, and varied autoimmune diseases, notably multiple sclerosis, type I diabetes, and rheumatoid arthritis (5,6,11,13).

While chronic vitamin D deficiency is being aggressively investigated, the effects of repeated cyclical vitamin D deficient states on healthy adult men are unknown. Seasonal variations of PTH are associated with osteomalacia in elderly patients (2). Nuclear submarine operations have been exposing sailors to sunlight deprivation for nearly 50 yr and, to date, no anecdotal evidence suggests that career submariners have a higher incidence of bone fractures or other skeletal problems than their non-submariner peers. However, an epidemiologic investigation surveying submariner bone density, fracture incidence, renal stone incidence, and rates of autoimmune diseases and malignancies vs. matched controls appear warranted to address these questions.

## CONCLUSIONS

Our results: 1) support the assertion that submariners experience decrements in vitamin D levels underway, presumably attributed to sunlight absence; 2) suggests that submariners residing at this northern location possess low  $25(\text{OH})\text{D}$  levels, as promulgated by many au-

thors (5,6,12), prior to their underway; 3) demonstrate that 400 IU of daily vitamin D supplementation failed to arrest the observed vitamin D decrements sustained underway; and 4) reveal cyclical bony metabolism resulting from the vitamin D decrements experienced underway, with unknown long-term consequences. Therefore, contrary to our stated hypothesis, this supplementation strategy did not maintain markers of vitamin D metabolism at the pre-deployment baseline throughout the underway.

We believe there is compelling data supporting an increase in the accepted normal range of  $25(\text{OH})\text{D}$ . Accepting this position, we would recommend investigations into the physiological, metabolic, and biochemical impact of escalated vitamin D supplementation on the concurrent metabolic pathways of calcium, bone, renal, and acid-base homeostasis in underway submariners immersed in an artificial atmosphere of increased carbon dioxide, absent sunlight, and reduced activity. Additionally, future investigations may investigate vitamin D supplementation in non-deployed sailors, exhibiting at best, relatively low vitamin D levels.

A voluminous array of efficacious dosing strategies may be employed, reconciling renal, acid-base, and bone physiology, compliance, cost, safety, operational practicality, endocrine physiology, and the burden imposed with fortification. We recommend a comparative trial investigating the efficacy of 1000 IU and 2000 IU daily vitamin  $\text{D}_3$  supplementation underway ( $2000 \text{ IU} \cdot \text{d}^{-1}$  is the present limit posted by the Institute of Medicine).

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