

The Efficacy of Vitamin D Supplementation During a Prolonged Submarine Patrol

Heath G. Gasier · Erin Gaffney-Stomberg ·
Colin R. Young · Douglas C. McAdams ·
Laura J. Lutz · James P. McClung

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Abstract Submariners spend prolonged periods submerged without sunlight exposure and may benefit from vitamin D supplementation to maintain vitamin D status. The primary objective of this study was to determine the efficacy of daily vitamin D supplementation on maintenance of 25-hydroxyvitamin D (25(OH)D) during a 3-month submarine patrol. Submariners were randomly divided into three groups: placebo ($n = 16$), 1,000 IU/day ($n = 20$), or 2,000 IU/day ($n = 17$). Anthropometrics, self-reported dietary calcium and vitamin D intake, serum markers of vitamin D and bone metabolism, and peripheral quantitative computed tomography (pQCT) parameters of the tibia were determined before and after the patrol. Prior to departure, 49 % of the subjects were vitamin D insufficient (<50 nmol/L). Following the patrol, 25(OH)D

increased in all groups ($p < 0.001$): 3.3 ± 13.1 (placebo), 4.6 ± 11.3 (1,000 IU/day), and 13 ± 14 nmol/L (2,000 IU/day). The changes in 25(OH)D levels were dependent upon the baseline concentration of 25(OH)D and body mass ($p < 0.001$). Osteocalcin increased by 38 % ($p < 0.01$), and pQCT analyses revealed small, yet significant increases in indices of tibial structure and strength ($p < 0.05$) that were independent of supplementation. These data suggest that vitamin D status was low prior to the patrol, and the subsequent changes in vitamin D status were dependent on the baseline 25(OH)D levels and body mass. Furthermore, short-term skeletal health does not appear to be negatively affected by 3 months of submergence in spite of a suboptimal response to vitamin D supplementation.

The authors declare no conflict of interest.

H. G. Gasier (✉) · C. R. Young
Department of Submarine Medicine & Survival Systems, Naval
Submarine Medical Research Laboratory, Groton, CT, USA
e-mail: heath.gasier@dm.duke.edu

C. R. Young
e-mail: colin.young@med.navy.mil

E. Gaffney-Stomberg · L. J. Lutz · J. P. McClung
Military Nutrition Division, United States Army Research
Institute of Environmental Medicine, Natick, MA, USA
e-mail: erin.g.stomberg.civ@mail.mil

L. J. Lutz
e-mail: laura.j.lutz2.civ@mail.mil

J. P. McClung
e-mail: james.p.mcclung8.civ@mail.mil

D. C. McAdams
Submarine Group Nine, Silverdale, WA, USA
e-mail: douglas.c.mcadams.mil@health.mil

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Introduction

In 1975, Preece et al. [1] first reported that a 2-month submarine patrol resulted in a 42 % decline in 25-hydroxyvitamin D (25(OH)D) and attributed this to a lack of sunlight, the major source of vitamin D₃ in humans [2]. Since then, researchers have reported decreases in vitamin D status from 15–47 % during patrols that ranged from ~ 1 to 2 months in duration [3–6]. The reasoning for the variable reported change in serum 25(OH)D may be attributed to the method of measurement, the season of departure and the duration of the patrol, and baseline vitamin D status [7]. For instance, Holy et al. [5] reported that the degree of decline in 25(OH)D that occurred over a 2-month submarine patrol was substantially greater when

the submarine departed following summer (-47% with a baseline of ~ 92 nmol/L) compared to a midwinter departure (-15% with a baseline ~ 44 nmol/L). Thus, a submarine patrol can result in a decline in 25(OH)D, primarily due to the lack of ultraviolet B light (UVB) radiation that is required for cutaneous synthesis of vitamin D₃ [2]; however, the biological consequences of this reduction remain unknown.

The principal function of vitamin D, when converted to its active hormone 1,25-dihydroxyvitamin D₃ in the kidney, is maintenance of circulating calcium and phosphorous within a narrow range, a process that is, in part, modulated by intestinal absorption [8–10]. When 1,25-dihydroxyvitamin D₃ is low, calcium absorption is reduced which leads to an increased production or release of parathyroid hormone (PTH) that serves to restore calcium balance by activating bone resorption, stimulating renal 1,25-dihydroxyvitamin D₃ production, and increasing renal calcium reabsorption [11]. A vitamin D deficiency can cause an acceleration in the loss of bone mineral and mass that can lead to osteoporosis and skeletal fractures [12–14]. As such, obtaining sufficient vitamin D either through sunlight and/or diet, and supplementation is essential for maintaining bone health in populations that may have poor vitamin D status.

While the Institute of Medicine has set a recommended dietary allowance (RDA) for vitamin D of 600 IU/day for adults [14], the RDA does not account for individuals whose occupation/residence is in an environment that is void of solar UVB radiation, e.g., spaceflight and prolonged periods of submergence [4, 7]. Although the US diet typically supplies ~ 300 IU/day of vitamin D [14], the mean dietary intake of submariners has been reported to be 159 ± 65 (SEM) IU/day before a patrol and 88 ± 44 IU/day during a patrol, which is likely due to the limited storage capacity for fresh dairy products onboard the submarine [15]. Due to insufficient vitamin D intake and the known effects of insufficient sunlight exposure, supplemental vitamin D may be warranted for the maintenance of skeletal health in submariners. Currently, however, the appropriate vitamin D supplemental dose is unknown for individuals that work/reside in sunlight absent environments.

Previous studies determined that 400 IU/day of supplemental vitamin D was inadequate for maintaining 25(OH)D levels in U.S. astronauts conducting missions from ~ 4 to 6 months on the International Space Station [16], and in submariners deployed for 76 days [4]. Therefore, the primary objective of this randomized, double-blind, placebo-controlled trial was to determine the efficacy of higher doses of supplemental vitamin D (1,000 and 2,000 IU/day) on maintaining 25(OH)D levels in U.S. submariners during a 3-month patrol. Dietary intake of

calcium and vitamin D, serum markers of vitamin D and bone metabolism, and peripheral quantitative computed tomography (pQCT) parameters of the tibia were also examined. The data reported herein serve to advance our understanding of the effects of prolonged submergence on vitamin D metabolism and skeletal health.

Materials and Methods

Subjects

Male submariners from Kitsap Naval Base, Bangor, WA (latitude 47.7°N) assigned to a submerged ship ballistic nuclear (SSBN) submarine that was preparing to depart on a routine patrol were recruited to participate in this randomized, double-blind, placebo-controlled trial. Volunteers ($n = 67$) provided written informed consent prior to randomization into one of three groups: placebo, 1,000 IU, or 2,000 IU of vitamin D₃ per day.

Experimental Protocol

This study consisted of two data collection sessions (pre- and post-submarine patrol) that included a medical screening questionnaire (pre-submarine patrol only), a food frequency questionnaire, anthropometric measurements, venous blood samples, and a pQCT scan of the tibia. The pre-submarine patrol testing session occurred over the course of 4 days within 7 days of departure during the fall (October). The duration of the submarine patrol was 92 days, which included five underway replenishments and a transfer of personnel at sea. During this time, food rations are replenished and personnel may board or depart the boat. The location and time of day of the underway replenishments and transfer of personnel at sea are unknown due to the mission of submariners. The post-submarine patrol testing period occurred over 3 days within 6 days of returning from the patrol during the winter (February). The total elapsed time between the pre- and post-patrol testing sessions was 101 ± 1.4 days (mean \pm SD). Of the 67 submariners who provided informed consent, nine subjects were unable to attend both testing sessions due to scheduling conflicts and five did not complete the 92-day patrol; thus, a total of 53 subjects (placebo, $n = 16$; 1,000 IU/day, $n = 20$; and 2,000 IU/day, $n = 17$) completed pre- and post-patrol testing and were included in data analyses.

Vitamin D Supplementation

The placebo and vitamin D₃ supplements (Compounded Solutions in Pharmacy, LLC, Monroe, CT) were placed in

pill drams, labeled with the subject number and group identification code. Capsules were independently tested (Eagle Analytical Services, Houston, TX) to confirm vitamin D₃ content. The measured vitamin D₃ content of the capsules was 0 IU (placebo), 900 ± 185 IU (1,000 IU), and 1,895 ± 318 IU (2,000 IU). Subjects were instructed to take one capsule each day and return the pill container with any remaining pills to the investigators during the post-submarine patrol testing session for compliance determination.

Dietary Vitamin D and Calcium Intake

During the pre-submarine patrol testing session and the final 2-weeks of the patrol, subjects completed a validated food frequency questionnaire (FFQ; Fred Hutchinson Cancer Research Center, Seattle, WA) to determine dietary intake of calcium and vitamin D [17]. The FFQ, which was administered under the guidance of trained registered dietitians, instructs subjects to report the frequency of intake and portion size of ~125 food and beverage items. The pre-submarine patrol FFQ represented the subject's land-dwelling intake, whereas the FFQ administered near the end of the patrol corresponded to the submariner's sea-dwelling intake. Mean daily dietary intake of calcium and vitamin D from all food and beverage sources was calculated by the Nutrition Assessment Shared Resources (NASR; Fred Hutchinson Cancer Research Center) using Nutrition Data Systems for Research (NDSR) software (a US Department of Agriculture Nutrient Database). Subjects with implausible reported energy intakes (<800 or >5,000 kcal/day) were excluded from the analysis [18].

Anthropometrics

Standing height (0.1 cm) and weight (0.1 kg) were determined during both pre- and post-patrol data collection sessions using a portable stadiometer (Creative Health Products, Plymouth, MI), and a calibrated electronic scale attached to a BOD POD[®]Gold Standard system (COS-MED, Concord, CA). Fat and fat-free mass were measured by air-displacement plethysmography (BOD POD[®]) following the manufacturer's guidelines [19]. We determined a coefficient of variation for fat and fat-free mass to be 1.95 and 0.52 %, respectively.

Blood Collection and Biochemical Analyses

Subjects were instructed to fast for a minimum of 8-h prior to reporting to the testing sessions. Upon arrival, blood was collected from an antecubital vein, allowed to clot, and centrifuged at 3,600 rpm for 10-min to obtain serum. Prior to clotting, an aliquot of whole blood was utilized for the

assessment of ionized calcium using a handheld blood analyzer (iSTAT, Abbott Point of Care, Princeton, NJ). Serum was frozen and shipped at the end of each testing session to the Pennington Biomedical Research Center (PBRC, Baton Rouge, LA) and stored at -80 °C until both pre- and post-patrol samples were available for batch analysis. Serum 25(OH)D and osteocalcin were determined using radioimmunoassays (DiaSorin, Stillwater, MN). Serum intact PTH (Siemens 2000, Los Angeles, CA), bone-specific alkaline phosphatase (Osteia, Fountain Hills, AZ), carboxy-terminal cross-linking telopeptide (ImmunoDiagnostics Systems, Fountain Hills, AZ), and vitamin D-binding protein (Quantikine, Minneapolis, MN) were determined using immunoassays. Serum albumin, calcium, phosphorous, and magnesium were determined using a Beckman Coulter DXC 600 Pro system (Beckman Coulter, Fullerton, CA). The clinical chemistry lab at PBRC is accredited by the College of American Pathologists, and as such, regularly participates in inter-lab assay validation for several analytes including 25(OH)D.

pQCT Measurements

During both pre- and post-submarine patrol testing sessions, 52 out of the 53 subjects underwent a pQCT scan (XCT 3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) of the tibia [20]. Tibia length (cm) was measured on the non-dominant leg using a tape measure from the palpated medial malleolus to the tibial plateau prior to positioning the lower leg horizontally within the X-ray gantry. An initial scout view scan was performed using a scan speed of 40 mm/s to identify the distal aspect of the tibia and fibula and the talus prior to conducting scans at 4 % (distal epiphysis) and 66 % (diaphysis) of the approximated segment length proximal to the distal end-plate of the tibia. Slice thickness was 2 mm and voxel size was set at 0.4 mm with a scanning speed of 20 mm/s. Detection thresholds for total and trabecular, and cortical bone properties were 169–650 mg/cm² and 710 mg/cm³, respectively. The parameters assessed at the 4 %-site included total, cortical-subcortical (ctsub), and trabecular BMC, BMD, and area. Bone parameters assessed at the 66 %-site included cortical BMC, BMD, area, and the section modulus across the anterior–posterior (AP) and medial–lateral (ML) axes. The bone strength index (BSI) was used to estimate the epiphysis axial compression strength, and calculated as the product of the total bone cross-sectional area and the total density squared [21]. The stress strain index (SSI) was used to estimate the diaphysis bending and torsional strength, and calculated for the anterior–posterior (SSI-AP) and medial–lateral (SSI-ML) axes as previously reported [21, 22]. For each volunteer, the reference line for the post-scan was placed

automatically by the XCT3000 software (Stratec, version 6.2) using the pre-scan image. All measurements were conducted by the same technician. Coefficients of variation were determined for total area and BMD (0.25 and 0.29 %), cortical area and BMD (0.32 and 0.04 %), and trabecular area and BMD (0.28 and 0.31 %) using the manufacturer's phantom.

Statistical Analysis

Statistical analyses were performed using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA) and SPSS version 20.0 (IBM Corp., Armonk, NY). A one-way ANOVA was used to determine pre-submarine patrol group differences. A two-way repeated-measures ANOVA was used to determine differences between groups over the course of the 3-month submarine patrol. When a significant main effect was observed, post hoc comparisons were made using a Bonferroni correction. For serum osteocalcin, six data points were below the lower limit of detection, and for serum vitamin D-binding protein there were four data points above the upper limit of detection. In these instances, the lower or upper limits of detection value were used in the analyses. Backward stepwise regression was used to determine independent associations between changes in 25(OH)D levels and pre-patrol 25(OH)D levels, supplemental vitamin D consumed (calculated as product of the quantity of supplemental vitamin D and % of pills consumed), time spent topside, dietary vitamin D intake, and changes in weight. Pearson's correlation coefficients were calculated to assess the associations for some of the variables. Values of $p < 0.05$ were considered statistically significant. All data are presented as mean \pm SD.

Results

Pre-submarine Patrol

Submariner characteristics and serum levels of vitamin D and biomarkers of bone metabolism before a 3-month patrol are displayed in Table 1. There were five replenishments and a transfer of personnel at sea during the 3-month patrol with 43.8 % of the placebo, 40 % of the 1,000 IU/day and 29.4 % of the 2,000 IU/day groups reporting that they spent a mean time of 0.9 ± 1.9 h topside during the patrol ($p > 0.05$ between groups). The majority (~ 89 %) of the subjects returned the pill containers to the post-testing session, and the remainder (placebo, $n = 2$; 1,000 IU/day; $n = 3$, and 2,000 IU/day, $n = 1$) provided an estimated pill count. Mean compliance during the study was similar between each group, 80 ± 22 % (placebo), 83 ± 17 % (1,000 IU/day), and

Table 1 Submariner characteristics and serum levels of vitamin D and biomarkers of bone metabolism before a 3-mo patrol

	Placebo ($n = 16$)	1,000 IU/day ($n = 20$)	2,000 IU/day ($n = 17$)
<i>Submariner characteristics</i>			
Age (year)	28.3 ± 4.7	29.4 ± 5.0	28.1 ± 5.4
Race			
Non-Hispanic white (n)	14	16	16
Other (n)	2	4	1
Time spent topside during patrol (h)	0.5 ± 0.8	1.3 ± 2.6	0.7 ± 1.6
Pill compliance (%)	80.4	83.5	79.6
Height (cm)	179.3 ± 6.9	176.3 ± 7.2	178.2 ± 5.6
Weight (kg)	83.9 ± 11.5	85.2 ± 13.8	95.5 ± 15.7
BMI (kg/m^2)	26.2 ± 3.9^a	27.4 ± 3.8^{ab}	30.0 ± 4.2^b
Fat mass (kg)	20.9 ± 8.5^a	23.9 ± 8.9^a	28.6 ± 9.2^b
Fat-free mass (kg)	63.0 ± 6.0	61.3 ± 6.5	66.9 ± 8.2
<i>Serum levels of vitamin D and biomarkers of bone metabolism</i>			
25(OH)D (nmol/L)	49.92 ± 17.56	52.39 ± 14.01	53.44 ± 16.86
DBP ($\mu\text{mol}/\text{L}$)	4.04 ± 2.35	3.96 ± 1.63	4.23 ± 1.53
Albumin (g/dL)	4.54 ± 0.22	4.50 ± 0.28	4.47 ± 0.28
Free 25(OH)D (pmol/L)	18.91 ± 9.99	18.71 ± 7.37	16.70 ± 4.41
Bio-25(OH)D (nmol/L)	7.75 ± 4.18	7.56 ± 2.93	6.74 ± 1.97
PTH (pg/mL)	41.33 ± 21.80	38.84 ± 20.28	32.56 ± 11.68
Calcium (mmol/L)	2.31 ± 0.06	2.30 ± 0.06	2.34 ± 0.06
Ionized calcium (mmol/L)	1.23 ± 0.04	1.23 ± 0.03	1.23 ± 0.03
Phosphorous (mmol/L)	1.35 ± 0.25	1.25 ± 0.17	1.40 ± 0.22
BAP ($\mu\text{g}/\text{L}$)	15.99 ± 6.59	16.05 ± 6.49	19.87 ± 8.26
CTX ($\mu\text{g}/\text{L}$)	0.80 ± 0.48	0.66 ± 0.37	0.76 ± 0.41
Osteocalcin ($\mu\text{g}/\text{L}$)	6.37 ± 3.76	4.68 ± 2.36	6.56 ± 2.82

Values are mean \pm SD (n for race and % for pill compliance). The data were analyzed using a one-way ANOVA. Values in a row not sharing similar superscript letters are significantly different, $p < 0.05$ (post hoc Bonferroni t test)

25(OH)D 25-hydroxyvitamin D, BAP bone-specific alkaline phosphatase, Bio-25(OH)D bioavailable 25(OH)D, CTX carboxy-terminal cross-linking telopeptide, DBP vitamin D-binding protein, PTH parathyroid hormone

80 ± 19 % (2,000 IU/day). Of all the baseline measures, significant differences were only observed for body weight ($p = 0.034$, no post hoc significance), BMI (2,000 IU $>$ placebo, $p = 0.027$) and fat mass (2,000 IU $>$ placebo, $p = 0.046$). Interestingly, 49.1 % of the subjects had

Table 2 Changes in submariner anthropometrics and serum levels of vitamin D and biomarkers of bone metabolism following a 3-month patrol

	Placebo (n = 16)	1,000 IU/day (n = 20)	2,000 IU/day (n = 17)	Time (p value)
<i>Changes in anthropometrics</i>				
Weight (kg)	-3.1 ± 2.6	-2.4 ± 3.8	-4.4 ± 4.7	<0.001
BMI (kg/m ²)	-1.0 ± 0.8	-0.8 ± 1.2	-1.3 ± 1.4	<0.001
Fat mass (kg)	-3.1 ± 2.5	-2.9 ± 3.1	-1.3 ± 1.4	<0.001
Fat-free mass (kg)	0.1 ± 1.3	0.5 ± 1.4	0.4 ± 1.8	
<i>Changes in serum levels of vitamin D and biomarkers of bone metabolism</i>				
25(OH)D (nmol/L)	3.3 ± 13.1	4.6 ± 11.3	13.0 ± 14.0	<0.001
DBP (μmol/L)	-0.2 ± 2.4	-0.3 ± 1.4	-0.1 ± 2.4	
Albumin (g/dL)	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.3	<0.001
Free 25(OH)D (pmol/L)	-1.3 ± 11.1	2.9 ± 6.6	7.2 ± 12.7	<0.01
Bio-25(OH)D (nmol/L)	0.9 ± 4.1	1.4 ± 2.6	3.2 ± 5.5	<0.01
PTH (pg/mL)	-4.1 ± 22.4	0.6 ± 16.5	-5.9 ± 10.4	
Calcium (mmol/L)	0.05 ± 0.04	0.02 ± 0.06	0.03 ± 0.05	<0.001
Ionized calcium (mmol/L)	0.04 ± 0.04	0.04 ± 0.04	0.05 ± 0.03	<0.001
Phosphorous (mmol/L)	0.1 ± 0.3	-0.03 ± 0.2	-0.1 ± 0.2	
BAP (μg/L)	-0.6 ± 7.0	-0.2 ± 7.0	-1.0 ± 8.0	
CTX (μg/L)	-0.1 ± 0.3	0.02 ± 0.3	-0.02 ± 0.3	
Osteocalcin (μg/L)	0.5 ± 2.6	1.2 ± 1.6	0.9 ± 1.8	<0.01

Values are absolute mean changes ± SD. The data were analyzed using a two-way repeated-measures ANOVA

25(OH)D 25-hydroxyvitamin D, BAP bone-specific alkaline phosphatase, Bio-25(OH)D bioavailable 25(OH)D, CTX carboxy-terminal cross-linking telopeptide, DBP vitamin D-binding protein, PTH parathyroid hormone

25(OH)D levels <50 nmol/L (insufficiency) and 5.7 % had levels <30 nmol/L (deficiency) prior to the patrol [14].

Post-submarine Patrol

Changes in submariner anthropometrics and serum levels of vitamin D and biomarkers of bone metabolism following a 3-month patrol are displayed in Table 2. Significant decreases in weight (3.2 ± 3.8 kg), BMI (1.1 ± 1.2 units) and fat mass (2.4 ± 2.6 kg) were observed following the patrol that were independent of group ($p < 0.001$). Serum 25(OH)D levels increased by 6.9 ± 13.2 nmol/L upon returning to port ($p < 0.001$), yet 26.4 and 3.8 % still had 25(OH)D levels <50 and 30 nmol/L, respectively (Fig. 1). Although there was no group x time interaction ($p = 0.06$), only 11.8 % of the subjects supplemented with 2,000 IU/day had 25(OH)D levels <50 nmol/L following the patrol compared to 35 and 31.3 % in the 1,000 IU/day and placebo groups, respectively. The calculated bioavailable and free 25(OH)D, which were calculated from measured 25(OH)D, vitamin D-binding protein and albumin [23], followed a similar pattern as 25(OH)D, increasing over the course of the patrol ($p < 0.01$) with no group x time interaction.

The changes in 25(OH)D levels following the 92 day submarine patrol were negatively correlated with pre-patrol 25(OH)D levels ($r = -0.40$, $p = 0.003$), and the change in weight from baseline ($r = -0.42$, $p = 0.002$) (Fig. 2). Out of the variables included in the regression analysis, vitamin D supplementation ($p = 0.046$), pre-patrol 25(OH)D levels

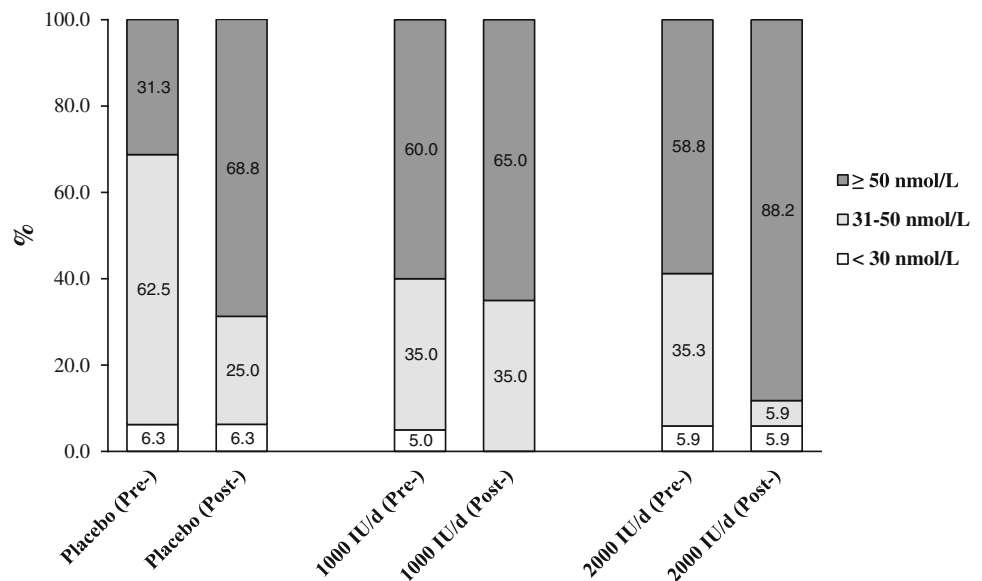
($p = 0.010$), and the change in weight from baseline ($p = 0.014$) were independently associated with the changes in 25(OH)D levels (adj. $R^2 = 0.31$, $p < 0.001$).

PTH was unaffected by vitamin D supplementation or time, yet there was a negative correlation between PTH and 25(OH) levels ($r = -0.37$, $p < 0.001$). Serum total and ionized calcium both increased during the submarine patrol ($p < 0.001$), but did not differ between groups, and were within normal clinical limits at both time points. A negative association between PTH and serum calcium ($r = -0.36$, $p < 0.001$) was also observed. Serum phosphorous did not change following the patrol, yet was significantly lower in the 1,000 IU/day group compared to placebo ($p = 0.019$). Of the bone turnover markers (bone-specific alkaline phosphatase, carboxy-terminal cross-linking telopeptide and osteocalcin), only osteocalcin changed following the patrol, $+38 \pm 71.4$ % ($p = 0.017$), a change that was independent of group.

Dietary Vitamin D and Calcium Intake

There were no changes in dietary vitamin D (D₂ and D₃) or calcium intake between groups prior to or at the end of the patrol (Fig. 3). The submariner diet met 44 ± 28 % (land-dwelling) and 53 ± 37 % (sea-dwelling) of the RDA for vitamin D, with vitamin D₃ accounting for ~98 % of the total dietary vitamin D intake before and 100 % during the patrol ($p > 0.05$). Regarding dietary calcium intake, subjects consumed 107 ± 51 % (land-dwelling) and 94 ± 51 % (sea-dwelling) of the RDA for calcium.

Fig. 1 Submariners classified by serum 25(OH)D levels before and after a 3-month submarine patrol for placebo ($n = 16$), 1,000 IU/day ($n = 20$), and 2,000 IU/day ($n = 17$) groups. Serum 25(OH)D cutoffs were ≥ 50 , 31–50 and < 30 nmol/L [14]



pQCT Parameters

Vitamin D supplementation did not influence changes in bone mineralization, geometry, or strength (Table 3). At the epiphysis (4 %-site), which is mainly comprised of trabecular bone (~ 65 % in the present investigation), there were increases in total BMD (0.6 ± 2.1 %, $p < 0.05$), ctsub BMC (1.7 ± 4.4 %, $p = 0.013$), ctsub area (0.5 ± 1.6 %, $p = 0.032$) and ctsub BMD (1.1 ± 3.9 %, $p = 0.045$), and BSI (0.5 ± 1.7 %, $p = 0.027$) following the patrol. At the diaphysis (66 %-site), which is primarily comprised of cortical bone, cortical area increased by 0.4 ± 1.3 % ($p = 0.024$), while there were no detectable changes in BMC or BMD. There were significant decreases in the section modulus across the ML axis (-2.4 ± 5.5 %, $p = 0.003$) and SSI-ML (-2.2 ± 5.7 %, $p = 0.004$), while the section modulus across the AP axis (1.6 ± 3.4 %, $p = 0.001$) and SSI-AP (1.1 ± 3.1 %, $p = 0.008$) both increased following the submarine patrol. Although these changes were small, they were above previously reported short-term errors associated with repeated pQCT measurements [24, 25].

Discussion

The primary objective of this research was to determine whether supplementing vitamin D₃ in the amount of 1,000 or 2,000 IU/day maintains serum 25(OH)D levels during a 3-month submarine patrol. A secondary objective was to determine whether vitamin D supplementation affected indices of bone turnover, and bone mineralization, geometry, and strength during the patrol. The main findings

reported herein are as follows: (1) nearly half of the submariners studied had 25(OH)D levels < 50 nmol/L prior to the patrol, (2) 25(OH)D increased during the patrol, and the changes were dependent upon baseline 25(OH)D levels and weight loss, and (3) short-term skeletal health does not appear to be negatively affected during 3 month's of submergence.

Controversy exists as to what the appropriate threshold value for serum 25(OH)D is when defining insufficiency as it relates to general health, i.e., 50 or 75 nmol/L [14, 26, 27]. A preponderance of clinical evidence suggests that sustained levels < 50 nmol/L are suboptimal for skeletal health. In the current study, the mean pre-patrol 25(OH)D levels were 52 ± 16 nmol/L with 63 % of the placebo and 35 % of the treatment groups having baseline values < 50 nmol/L. This observation was somewhat unexpected as previous research conducted at the same location during the winter determined the mean pre-submarine patrol 25(OH)D levels to be ~ 68 nmol/L [4]. Interestingly, only 11 % of the submariners that participated in the present study exceeded this concentration prior to the patrol, and only 4 % exceeded 75 nmol/L. Moreover, only 32 % of the subjects were determined to have values ≥ 58 nmol/L, the mean concentration of U.S. males between the ages of 19–50 [14]. The low baseline serum 25(OH)D levels observed in this study are, however, consistent with reports from other investigations conducted at northern latitudes ($> 40^\circ\text{N}$) during late fall and winter [5, 14, 28]. The low levels can likely be attributed to the region and season, both of which influence the available UVB radiation that is required to stimulate the cutaneous production of vitamin D₃ [29]. In addition to the environment, pre-patrol dietary vitamin D intake was low

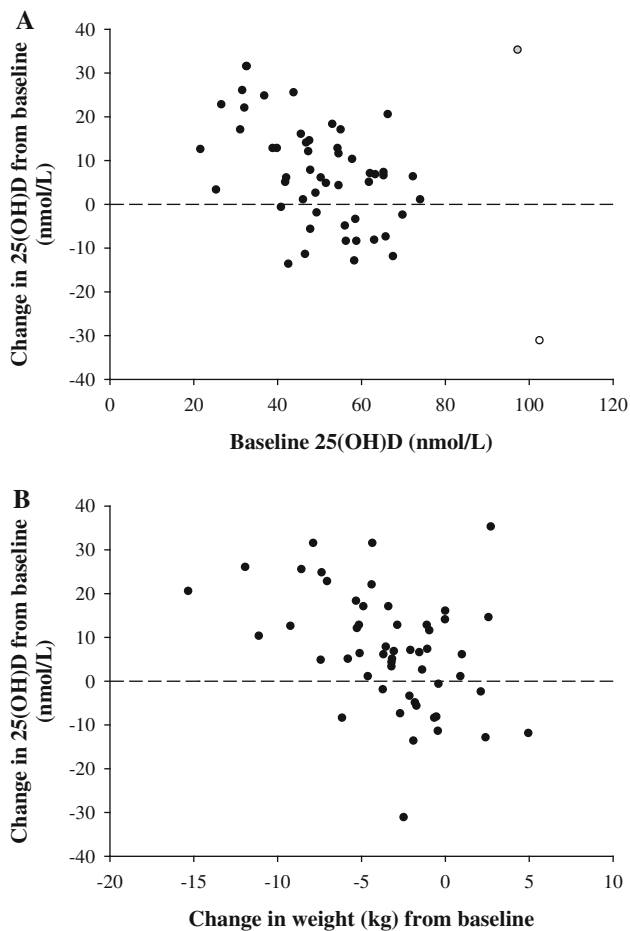


Fig. 2 Correlation between the change in serum 25(OH)D following a 3-month submarine patrol and baseline (pre-submarine patrol) serum 25(OH)D (**a**), and the change in weight from baseline (**b**); $n = 53$. The changes in 25(OH)D were negatively correlated with baseline 25(OH)D levels ($r = -0.40$, $p < 0.01$) and the change in weight from baseline ($r = -0.42$, $p < 0.01$). In **a**, gray colored point (+35.2 %) represents subject number 15 (2,000 IU/day) and white colored point (-31.2 %) represents subject number six (placebo)

(256 ± 157 IU/day), less than half of the RDA for this age group [14]. Thus, it would appear that the geography, season, and insufficient dietary vitamin D intake were responsible for the low baseline serum 25(OH)D levels, an observation that may be of concern for submariners preparing to submerge for a prolonged period of time.

Despite low baseline 25(OH)D, the submariners were able to maintain and increase their 25(OH)D levels over the 3-month patrol. The doses of vitamin D examined in the current study provided 1.7 (1,000 IU/day) and 3.3 (2,000 IU/day) times the RDA. Although the 2,000 IU/day group exhibited the greatest change in 25(OH)D following the patrol, +13 nmol/L with only two subjects having levels <50 nmol/L, this was not statistically significant. The mean change in 25(OH)D for every 100 IU of vitamin D₃ supplemented daily has been reported to be ~ 1 nmol/L

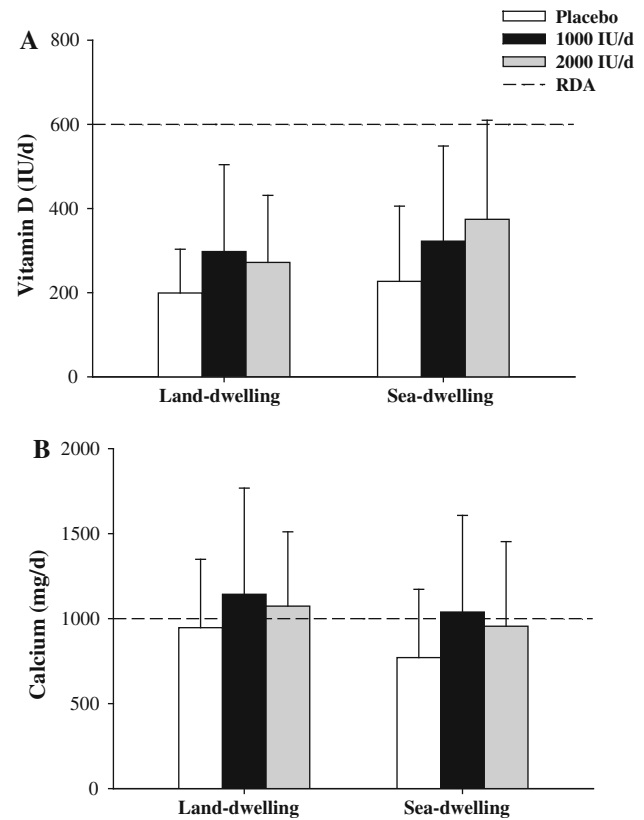


Fig. 3 Dietary vitamin D (vitamin D₂ and D₃) (**a**) and calcium (**b**) intake reported before (land-dwelling) and during (sea-dwelling) a 3-month submarine patrol. Values are mean \pm SD. $n = 12$ (placebo), 18 (1,000 IU/day) and 17 (2,000 IU/day). The dashed line represents the RDA for males between the ages of 19–50 years [14]

[30–32]. In the present study, the mean changes in 25(OH)D per 100 IU of supplemented vitamin D were lower, 0.5 ± 1.3 and 0.7 ± 0.7 nmol/L for the 1,000 and 2,000 IU/day groups, respectively. This was unanticipated considering that diet and time spent topside exposed to the external environment during the replenishments and personnel transfer were similar between groups during the patrol. The blunted responses may, in part, be attributed to the baseline concentration of 25(OH)D and/or the high body mass and subsequent weight loss that occurred during the patrol.

Previous studies have demonstrated that baseline 25(OH)D levels and body mass can modulate the response to supplemental vitamin D [7, 31–36]. Individuals with a low baseline concentration of 25(OH)D tend to display a greater response to a given dose of vitamin D compared to individuals that have higher baseline levels [32, 36]. Body mass also influences the change in 25(OH)D in response to supplementation, such that overweight and obese individuals achieve lower serum 25(OH)D levels compared to normal weight individuals [7, 32, 34, 35]. Adipose tissue serves as a major reservoir for vitamin D₃ and 25(OH)D

Table 3 pQCT parameters in submariners before and after a 3-month patrol

pOCT-parameter	Placebo		1,000 IU/day		2,000 IU/day		Time (<i>p</i> value)
	Pre	Post	Pre	Post	Pre	Post	
<i>4 % site</i>							
Total BMC (mg/mm)	425 ± 40	425 ± 41	410 ± 58	409 ± 56	431 ± 54	430 ± 55	
Total area (mm ²)	1236 ± 122	1232 ± 120	1206 ± 127	1197 ± 134	1208 ± 99	1193 ± 105	
Total BMD (mg/cm ³)	345 ± 29	346 ± 31	342 ± 54	345 ± 56	357 ± 37	361 ± 37	<0.05
Ctsub BMC (mg/mm)	143 ± 11	144 ± 12	148 ± 26	150 ± 27	148 ± 30	152 ± 30	<0.05
Ctsub area (mm ²)	247 ± 16	249 ± 15	250 ± 25	250 ± 24	249 ± 31	251 ± 30	<0.05
Ctsub BMD (mg/cm ³)	577 ± 32	580 ± 31	588 ± 53	594 ± 58	588 ± 56	600 ± 52	<0.05
Trabecular BMC (mg/mm)	282 ± 32	283 ± 34	262 ± 39	259 ± 38	283 ± 34	278 ± 37	
Trabecular area (mm ²)	989 ± 111	983 ± 110	956 ± 122	946 ± 132	958 ± 89	942 ± 98	
Trabecular BMD (mg/cm ³)	286 ± 25	286 ± 27	277 ± 48	277 ± 48	296 ± 28	296 ± 29	
Total BSI (mg/mm ⁴)	147 ± 21	147 ± 22	143 ± 42	143 ± 42	156 ± 33	157 ± 33	<0.05
<i>66 % site</i>							
Cortical BMC (mg/mm)	408 ± 46	411 ± 46	412 ± 49	412 ± 49	414 ± 57	414 ± 55	
Total area (mm ²)	365 ± 38	367 ± 40	365 ± 42	366 ± 43	370 ± 49	371 ± 48	<0.05
Cortical BMD (mg/cm ³)	1122 ± 20	1120 ± 19	1126 ± 18	1124 ± 19	1117 ± 20	1117 ± 17	
Section modulus AP (mm ³)	2399 ± 344	2412 ± 374	2385 ± 346	2423 ± 377	2494 ± 454	2561 ± 456	<0.01
Section modulus ML (mm ³)	1851 ± 307	1805 ± 282	1824 ± 303	1790 ± 299	1929 ± 374	1862 ± 361	<0.01
SSI-AP (mm ³)	2144 ± 324	2148 ± 346	2124 ± 308	2156 ± 345	2218 ± 412	2259 ± 410	<0.01
SSI-ML (mm ³)	1661 ± 288	1642 ± 250	1661 ± 272	1618 ± 255	1727 ± 343	1676 ± 320	<0.01

Values at baseline are mean ± SD. The data were analyzed using a two-way repeated-measures ANOVA

Ctsub Cortical subcortical, *AP* anterior posterior, *ML* medial lateral

[37], with subsequent 25(OH)D release following weight loss [38]. In the present investigation, 44, 70, and 82 % of the placebo, 1,000, and 2,000 IU/day groups, respectively, were either overweight or obese by BMI classification, with the 2,000 IU/day demonstrating a significantly higher body mass than the other two groups. Unexpectedly, the submariners lost a significant amount of weight and fat-mass independent of the dietary intervention during the patrol. This change in body mass, as well as the low baseline 25(OH)D levels, were associated with the observed changes in 25(OH)D. Thus, the increased 25(OH)D levels observed in the placebo group following the patrol may be attributed to weight loss. Taken together, these data are consistent with others [7, 32], supporting the notion that overweight and obesity are related to low serum 25(OH)D and affect the overall response to vitamin D supplementation.

Compliance with the intervention is another factor that may have affected the outcomes, as observed in previous studies conducted in austere environments [4, 7]. The preferred approach would have been to directly observe oral pill consumption, yet this was impractical during a submarine patrol. In an effort to circumvent poor compliance, a crewmember (non-investigator) offered weekly reminders to study participants. The compliance reported

herein is, however, similar to a 5-month Antarctica study, where there were significant group differences in 25(OH)D levels between the placebo and 2,000 IU vitamin D groups at the end of the intervention; however, BMI was similar between groups and weight loss was not reported [7]. Even so, compliance cannot be excluded as causative or contributing to suboptimal changes in 25(OH)D.

The efficacy of vitamin D supplementation may be gauged by changes in mineral homeostasis and bone turnover markers. Although 1,25(OH)₂D₃ was not assessed, other investigators reported a similar, but less robust change in 1,25(OH)₂D₃ compared to the concentration of 25(OH)D [4, 7, 32]. The increased serum calcium levels observed herein, and by others, have historically been ascribed to increased atmospheric CO₂ and an ensuing mixed acidosis that is initially compensated by the buffering capacity of bone, a process that causes cyclical changes in serum and urinary calcium and phosphorous [5, 39–42]. In these studies, atmospheric CO₂ was ≥0.7 % with the most pronounced effects occurring when CO₂ was ≥1.0 %. Currently, the atmospheric CO₂ of U.S. ballistic missile submarines (to include the submarine utilized in the present study) is substantially lower, 0.29 ± 0.12 % (mean daily). This concentration is likely insufficient to have caused the increased serum calcium observed

following the patrol. An alternative explanation may be that bone turnover was occurring and osteocalcin was elevated to support the mineralization phase by stimulating calcium release, i.e., osteocalcin was not related to weight and fat loss, insulin and adiponectin (data not shown), discounting the possibility that osteocalcin was released to support insulin release/sensitivity [43]. Consistent with our data, Smith et al. [7] reported an increase in serum calcium and bone-specific alkaline phosphatase with vitamin D supplementation in a sunlight absent environment, excluding CO₂ as causative for the elevation in serum calcium.

Although we acknowledge the slow turnover properties of the skeleton [44], and the fact that a 3-month patrol may have been too short to detect measurable changes in the tibia, the effects of residence in a confined space (i.e., exercise limitations) without sunlight in a normal gravitational (1g) environment on the skeleton have not been explored. The increases in total vBMD, and ctsub vBMD and BMC at the epiphysis suggest increased mineralization, consistent with the increased osteocalcin levels. At the diaphysis, there were contrasting changes in the section modulus and SSI across the AP axis (increased) and across the ML axis (decreased), suggesting changes in bone geometry. Researchers that examined the effects of an 8-week exercise program on tibial osteogenesis reported regional effects on the medial aspect of the tibial diaphysis despite no changes in periosteal circumference or cortical area [45]. These changes in bone geometry may have been reflective of early exercise adaptation, preceding periosteal expansion. Similarly in the current study, there were increases in the section modulus and SSI across the AP axis. In addition, there was a subtle increase in cortical area at the 66 %-site suggestive of periosteal expansion. Although an objective measurement of physical activity was not performed in this study, the observed significant weight and fat loss with maintenance of fat-free mass suggests that the submariners at least maintained and possibly increased their physical activity during the patrol. Thus, the changes in area and geometry at the 66 %-site may reflect the appropriate initial adaptation to physical activity, which should result in improved bone strength over an extended period of time.

In addition to supplement compliance, there are other limitations that may affect the interpretation of the study data. First, based on initial sample size power estimates, 24 subjects were needed in each group, a calculation based off of a 20 % higher baseline 25(OH)D level in a previous study conducted at the same location [4]. Unfortunately, the sample size in the present study was limited by the size of the submarine crew. Yet, our low pre-patrol 25(OH)D levels and sample size were similar to two previous studies conducted in Antarctica with reported changes in serum

25(OH)D levels of ≥ 1 nmol per 100 IU of daily supplemental vitamin D, and a significant benefit over the non-supplemented group [7, 32]. Another limitation is that the study was conducted on a sample of a submarine crew assigned to the NW region of the U.S. and may not reflect vitamin D and bone status in submariners assigned to areas where sunlight is plentiful. This, however, was done intentionally to examine the most extreme conditions, i.e., submariners that reside in a region that has little UVB radiation half of the year and undergo prolonged periods of submergence. In light of this, one may consider this to be a study strength.

Conclusion

The results from this randomized trial suggest that baseline 25(OH)D levels were suboptimal for skeletal health in submariners preparing to depart on a 3-month patrol. Although the greatest changes in mean serum 25(OH)D levels were observed in submariners supplemented with 2,000 IU/day of vitamin D₃, a significant advantage for the supplemented (1,000 or 2,000 IU/day) over the non-supplemented was not evident. The responses to the intervention appeared to be affected by the baseline serum 25(OH)D, the high BMI, and the significant reduction in weight (fat mass) that occurred over the patrol. While the changes in bone quality assessed in both trabecular and cortical bone were small, they do suggest that short-term skeletal health does not appear to be negatively affected during prolonged submergence. These findings will not only expand our limited understanding of vitamin D metabolism and skeletal health in an extreme environment, but also serve as guidance for future research aimed at optimizing vitamin D status in those that obtain little or no exposure to sunlight.

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