Received: 27 September 2018 | Revised: 7 May 2019 | Accepted: 12 May 2019

DOI: 10.1111/sms.13467

ORIGINAL ARTICLE

WILEY

# Effects of vitamin D supplementation on salivary immune responses during Marine Corps basic training

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**Funding information**
Defense Health Program, Military Operational Medicine Research Program (JPC-5), Grant/Award Number: HU0001-16-0018 to HGG

Vitamin D's role in regulating immune responses may increase during periods of elevated psychological and physiological stress. Due to the high demands placed on US Marine Corps recruits undergoing 12 weeks of basic military training, we hypothesized that vitamin D status would be related to markers of innate mucosal immunity, and daily vitamin D supplementation would augment immune responses during training. Males (n = 75) and females (n = 74) entering recruit basic training during the summer and winter volunteered to participate in a randomized, double-blind, placebo-controlled study. Subjects received either 1000 IU vitamin $D_3$ + 2000 mg calcium/d (n = 73) or placebo (n = 76) for 12 weeks. Saliva samples were collected pre-training, during (weeks 4 and 8), and post-training (week 12) in order to determine salivary SIgA and cathelicidin (indices of mucosal immunity) and α-amylase (indicator of stress). Initial (baseline) and post-training serum 25(OH)D levels were measured. Results were as follows: serum 25(OH)D levels were 37% higher in recruits entering training in summer compared with winter. A positive relationship was observed between baseline 25(OH)D levels and SIgA secretion rates (-SR). When stress levels were high during summer training, baseline 25(OH)D levels contributed to an increase in salivary secretory immunoglobulin A secretion rates (SIgA-SR) and cathelicidin-SR, the latter only in males. Vitamin D supplementation contributed to the changes in SIgA-SR and cathelicidin-SR, specifically SIgA-SR was higher in the treatment group. These data highlight the importance of vitamin D and mucosal immune responses during arduous basic military training when stress levels are increased.

**KEYWORDS**
antimicrobial peptides, cholecalciferol, exercise, immunology, military, mucosal immunity

## 1 | INTRODUCTION

Vitamin D is a lipid-soluble secosteroid that is primarily obtained via cutaneous synthesis upon exposure to ultraviolet B radiation and can also be obtained by limited dietary sources, for example fatty fish, egg yolks, liver, and fortified grains and dairy products. Vitamin D's main function is to maintain serum calcium levels within a very narrow range and to support normal bone turnover.[1] Vitamin D is also involved in regulating innate and adaptive immunity.[2] The immunomodulatory role of vitamin D may become increasingly important during periods of elevated stress, common during military training. In fact, US military recruits have a three- to fourfold higher risk of being hospitalized for acute respiratory illnesses compared with



  

age-matched civilians.[3] Suboptimal vitamin D 25(OH)D status has also been documented in US Army recruits[4-6] and Finnish military personnel,[7] with the latter group missing twice as many training days when 25(OH)D levels were <40 nmol $L^{-1}$.

Our current understanding of vitamin D and immunity during training stems from studies conducted in athletic populations. In endurance athletes undergoing winter training, upper respiratory tract illness (URTI) symptoms were more severe and extended in those with 25(OH)D levels <30 nmol $L^{-1}$.[8] In contrast, salivary secretory immunoglobulin A secretion rates (SIgA-SR) were higher in athletes with baseline 25(OH)D levels >120 nmol $L^{-1}$.[8] During winter training, endurance athletes supplemented with 5000 IU of vitamin $D_3$ daily had increased salivary SIgA-SR and cathelicidin-SR, an antimicrobial peptide, compared with placebo.[9] These data suggest suboptimal vitamin D status may contribute to compromised salivary immune responses in endurance athletes during periods of intense training. Remaining unknown is whether these responses similarly occur in recruits undergoing basic military training given the differences between military and athletic training programs.

Mucosal surfaces are the site for as many as 95% of all infections.[10] SIgA and cathelicidin serve as "the first line of defense" against pathogens at the mucosal surface.[11,12] SIgA is an indicator of mucosal immunity,[11] and vitamin D is involved in regulating salivary secretions.[13] Cathelicidin is also produced at mucosal surfaces, possessing a broad range of antimicrobial activity,[12] and its production is dependent upon sufficient vitamin D levels.[14] SIgA and cathelicidin are, therefore, useful indicators of mucosal immune responses, and this may be of particular interest to the military given the increased prevalence of respiratory illnesses during basic military training.[3]

US Marine Corps basic training is a challenging 12-week program that imposes a high level of physiological and psychological stress on the recruits, and vitamin D may beneficially enhance immune reactions to pathogens and reduce the incidence of illness. The objectives of this research were to examine the relationship between serum 25(OH)D and salivary SIgA and cathelicidin during training, and to determine whether vitamin $D_3$ supplementation (1000 IU $d^{-1}$) modifies salivary SIgA and cathelicidin responses to basic military training. Our hypothesis was that a positive relationship between 25(OH)D and salivary SIgA and cathelicidin would be observed during training, and vitamin D supplementation would augment these salivary indices of mucosal immunity. Since vitamin D status is influenced by season, we examined recruits entering training during summer and winter seasons.

## 2 | METHODS

### 2.1 | Subjects

This study was approved by the Institutional Review Boards at the US Army Research Institute of Environmental Medicine and Medical Research and Material Command. The study was conducted at the Marine Corps Recruit Depot, Parris Island, SC, USA (32.4°N latitude). Potential subjects included males and females aged ≥17 years who entered Marine Corps basic training in July 2015 and February 2016. Subjects participated in this study after providing voluntary written informed consent. Data presented in this manuscript are from a randomized, double-blind, placebo-controlled study designed to evaluate the effect of a calcium and vitamin D-fortified food product on bone health during training.[15]

Subjects were excluded from the study if they reported being pregnant or were breastfeeding, or had a history of kidney stones, kidney disease, endocrine disorders (eg, diabetes, hypoparathyroidism, or hyperparathyroidism), bone-modifying disorders (eg, osteogenesis imperfecta, osteoporosis, or rickets), or if they were allergic to any ingredients in the fortified food product. A total of 149 subjects (75 men and 74 women) completed the study. Anthropometric, demographic, and biochemical data were collected within 1 week of arriving at basic training (pre-) and again 12 weeks later just prior to graduation (post-). In addition to pre- and post-training, saliva samples were collected at weeks 4 and 8.

### 2.2 | Training

US Marine Corps basic training is a 12-week program divided into four phases. During phase one (entry through week 4), recruits begin physical training, didactic military instruction, and military exercises. Physical training includes running, marching, obstacle courses, swimming, and strength training. During phase two (weeks 5-7), recruits focus on marksmanship and live fire exercises. During phase three (weeks 8-10), recruits learn and apply basic combat survival skills, including marksmanship, land navigation, and maneuvering under enemy fire, while concurrently completing various academic and physical examinations. During phase four (weeks 11-12), recruits prepare for graduation and their next phase of training. Throughout training, recruits consume three self-served cafeteria-style meals daily. No foods are permitted outside the mess hall, and consumption of dietary supplements is strictly prohibited.

### 2.3 | Intervention

Subjects were block randomized by race (Caucasian, African American, or other) and sex to either placebo or treatment (calcium + vitamin D). The doses of calcium (2000 mg $d^{-1}$) and vitamin D (1000 IU $d^{-1}$) used in the current study were selected based on the efficacy of this combination on bone health during Army and Navy basic training.[5,16] To our knowledge, calcium does not have an immunomodulatory role, and thus, our interest was specific to vitamin D and immunity. Vitamin D was provided as a fortified food bar that was

manufactured and labeled by the US Department of Defense Combat Feeding Directorate using a three-letter group identification code. Independent analysis by Covance Laboratories determined each treatment bar contained 494 IU of vitamin $D_3$, 1035 mg calcium, 130-140 kcal, 23-25 g carbohydrate, 5 g fat, and 1-2 g protein. Placebo bars were identical in taste, appearance, kcal, macronutrient distribution, and contained ≤20 mg of incidental calcium and <1.4 IU vitamin $D_3$. Food bars were individually packaged and labeled with study number, and subjects were instructed to consume two bars per day in-between meals. Bars were disseminated on a weekly basis (14 bars) with the subjects first providing the wrappers and/or uneaten bars from the previous week prior to being issued another batch. Compliance was >95% throughout the study and did not differ between groups.

## 2.4 | Determination of 25(OH)D

Pre- and post-training and following an overnight fast, blood was collected from the antecubital vein into vacuum tubes, centrifuged at 4°C for serum separation, and frozen at −20°C overnight prior to shipping on dry ice to the Pennington Biomedical Research Center (PBRC). PBRC is accredited by the College of American Pathologists and routinely participates in inter-laboratory standardization testing. Serum 25(OH)D was determined using a radioimmunoassay (DiaSorin Inc). The inter-assay coefficient of variation was 6.7%.

## 2.5 | Saliva collection

Subjects were instructed to fast and avoid fluids 2 hours prior to providing saliva samples for 3 minutes using the passive drool collection method. The samples were immediately cooled on ice, re-weighed, stored at −20°C overnight, and shipped on dry ice to the Uniformed Services University of the Health Sciences where they were stored at −80°C until batch analysis.

## 2.6 | Salivary SIgA, α-amylase, and cathelicidin

Salivary α-amylase (an indicator of physiological and psychological stress), SIgA (antibody involved in mucosal immunity), and cathelicidin (antimicrobial peptide involved in mucosal immunity) were determined using commercially available ELISA kits: α-amylase, kinetic enzyme assay (Salimetrics LLC); SIgA, indirect competitive immunoassay (Salimetrics LLC); and cathelicidin, a competitive enzyme-linked immunosorbent assay (Lifespan Biosciences Inc). The coefficients of variations for all assays were <10%.

Due to inter-subject variability in saliva production, secretion rates (SR) were calculated to account for differences in volume: α-amylase, SIgA, and cathelicidin concentration × volume of saliva collected over 3 minutes.

## 2.7 | Statistical analysis

Analyses were performed using SigmaPlot version 13.0 (Systat Software) and SAS software version 9.4, Proc Mixed (SAS Institute Inc). A *t* test was used to determine whether there were seasonal (winter vs summer) differences in recruits entering training and whether vitamin D supplementation increased 25(OH)D levels. A one-way repeated measures ANOVA was used to assess the effects of training on α-amylase and α-amylase-SR. Pearson's product-moment correlations between SIgA-SR and cathelicidin-SR with baseline 25(OH)D and α-amylase-SR were examined prior to performing longitudinal mixed modeling to explore whether vitamin D supplementation influenced SIgA-SR and cathelicidin-SR. All of the salivary measures were first log transformed due to the data being positively skewed. Model fit was first used to determine the best option for modeling time, that is covariance structure, inclusion of random intercepts, and inclusion of variables and interactions. Phase of training (baseline, weeks 4, 8, and 12), sex, season, baseline 25(OH)D, and α-amylase were used to predict SIgA-SR and cathelicidin-SR. For the best fit-models, SIgA-SR was modeled with compound symmetry and no random intercepts; cathelicidin-SR with compound symmetry and heterogeneous variances and random intercepts. Restricted maximum likelihood estimation was used for the final models. Final mixed models were based off the exploratory models with changes implemented to adjust for baseline differences in SIgA-SR and cathelicidin-SR across treatment groups: α-amylase-SR was removed, only three time points were considered (weeks 4, 8, and 12), and baseline SIgA-SR and cathelicidin-SR were included as covariates. SIgA-SR and cathelicidin-SR responses to vitamin D supplementation were determined if the effect of treatment and treatment by time interaction significantly improved model fit.[17] Maximum likelihood estimation was used to compare models. Values are presented as means ± SD (for simple descriptives), marginal means ± SEM (for ANOVAs), or coefficients ± SE (for other longitudinal models). We accepted a $P < 0.05$ as being statistically significant.

# 3 | RESULTS

Baseline characteristics, serum 25(OH)D, and salivary α-amylase, SIgA, and cathelicidin are displayed in Table 1. Significant differences in age, gender distribution, body mass index, and body fat % were found in recruits entering training in winter compared with summer. Recruits entering training in winter had lower levels of 25(OH)D compared with summer (59 ± 23 vs 81 ± 21 nmol $L^{-1}$, $P < 0.05$). Salivary

**TABLE 1** Baseline characteristics and biological indicators of vitamin D status, stress, and immune function in Marine Corps recruits entering 12 wk of basic training

| | | Season | |
|---|---|---|---|
| | **Total (n = 149)** | **Summer (n = 76)** | **Winter (n = 73)** |
| Characteristic | | | |
| Treatment[a], n (%) | 73 (49) | 39 (51) | 34 (47) |
| Age[b], y | 19 ± 2 | 18 ± 1 | 19 ± 2[e] |
| Male[a], n (%) | 75 (50) | 45 (59) | 30 (41)[e] |
| Body mass index[b], kg $m^{-2}$ | 23.9 ± 2.6 | 23.4 ± 2.5 | 24.3 ± 2.7[e] |
| Height[b], cm | 169 ± 8 | 170 ± 8 | 168 ± 8 |
| Weight[b], kg | 68.5 ± 11.0 | 68.4 ± 10.6 | 68.5 ± 11.4 |
| Fat-free mass[b], kg | 56.7 ± 10.4 | 57.8 ± 10.3 | 55.5 ± 10.4 |
| Body fat[b] % | 17.2 ± 6.8 | 15.5 ± 6.7 | 19.0 ± 6.5[e] |
| Race[a], n (%) | | | |
| White | 95 (64) | 50 (66) | 45 (62) |
| African American | 23 (15) | 9 (12) | 14 (19) |
| Other | 31 (21) | 17 (22) | 14 (19) |
| Biological indicators[d] | | | |
| Serum[b] | | | |
| 25(OH)D, nmol $L^{-1}$ | 70 ± 23 | 81 ± 21 | 59 ± 23[e] |
| Salivary[c] | | | |
| α-amylase, U $mL^{-1}$ | 13 (6-20) | 12 (7-20) | 12 (4-21) |
| α-amylase-SR, U $min^{-1}$ | 4 (1-10) | 8 (4-16) | 1.3 (0.4-3.5)[f] |
| SIgA, μg $mL^{-1}$ | 121 (79-183) | 102 (65-152) | 163 (102-303)[f] |
| SIgA-SR, μg $min^{-1}$ | 44 (25-86) | 69 (32-96) | 26 (15-44)[f] |
| Cathelicidin, ng $mL^{-1}$ | 1.2 (0.6-2.8) | 1.9 (1.1-3.5) | 0.7 (0.3-1.1)[f] |
| Cathelicidin-SR, ng $min^{-1}$ | 3.0 (1.4-5.6) | 3.0 (1.6-5.6) | 2.8 (1.2-6.4) |

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; SIgA, salivary secretory immunoglobulin A; -SR, secretion rates (calculated as the product of concentration and salivary flow rates, mL collected over 3 min).
Values are presented as
[a]n (%),
[b]Means ± SD, or
[c]Medians with interquartile ranges (25th-75th).
[d]Actual sample sizes for biological indicators range from 115-142. A *t* test was used to compare seasons for characteristics and 25(OH)D. The data were non-normally distributed for salivary biological indicators of mucosal immunity, thus a Mann-Whitney rank sum test was used to compare seasons.
[e]$P < 0.05$.
[f]$P < 0.001$ vs summer.

markers of SIgA, SIgA-SR, α-amylase-SR, and cathelicidin also showed seasonal variation.

Levels of α-amylase and α-amylase-SR increased during training, peaking at week 8 (Figure 1).

Vitamin D supplementation only increased serum 25(OH)D levels during summer training (Figure 2).

Baseline SIgA-SR were positively associated with baseline 25(OH)D ($r = 0.29$, $P < 0.01$) and baseline α-amylase-SR ($r = 0.58$, $P < 0.01$). Baseline cathelicidin-SR was negatively associated with baseline α-amylase-SR ($r = -0.23$, $P < 0.05$; Figure 3).

## 3.1 | Predictors associated with salivary SIgA-SR and cathelicidin-SR

Salivary secretory immunoglobulin A secretion rates decreased during training (weeks 4 and 8) and then increased above baseline post-training (Table 2). SIgA-SR were higher during summer training. Baseline 25(OH)D levels did not influence SIgA-SR when other predictors were included in the model; however, there was an interaction between baseline 25(OH)D levels and season, that is the association between baseline 25(OH)D and SIgA-SR was stronger during summer training.

Since salivary α-amylase-SR was increased during training, this predictor was included in the model and determined to be associated with an increase in SIgA-SR during training.

Time-dependent changes in cathelicidin-SR were also documented, decreasing at week 4 and post-training. Baseline 25(OH)D interacted with sex, contributing to increased cathelicidin-SR in male recruits. Dissimilar to the association between α-amylase-SR and SIgA-SR, α-amylase-SR was associated with a decrease in cathelicidin-SR during training.

### 3.2 | Vitamin D supplementation and SIgA-SR and cathelicidin-SR responses

In order to assess the effects of vitamin D supplementation on SIgA-SR, we started with a similar model as above (Table 3). First, baseline SIgA-SR was included as a covariate and α-amylase-SR was removed. Next, treatment and treatment by time were entered into the model and significantly improved model fit ($\chi^2 = 7.5$, $df = 2$, $P = 0.02$), indicating vitamin D supplementation influenced SIgA-SR over the course of training. Specifically, SIgA-SR was significantly higher in the treatment group at week 4 ($P < 0.01$) and trended for an increase at week 8 ($P = 0.08$).

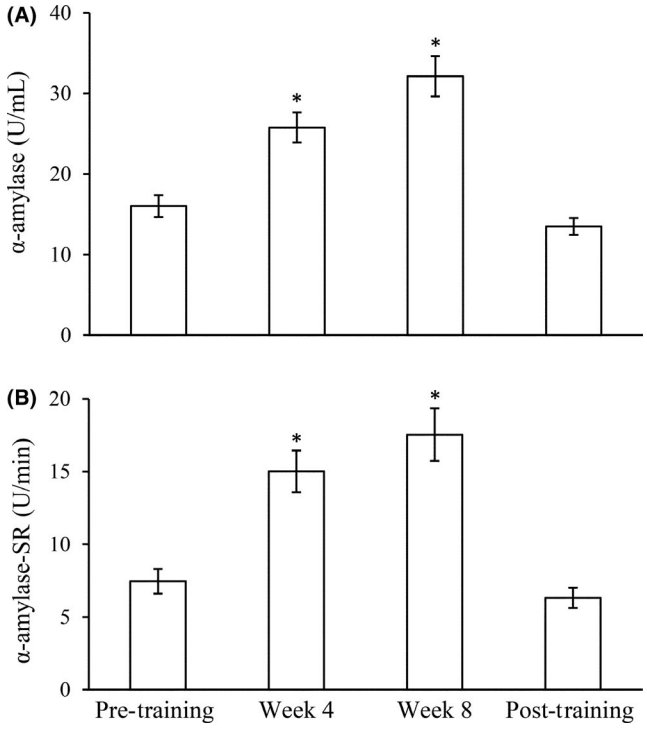


**FIGURE 1** Effects of 12 wk of Marine Corps basic training on salivary α-amylase and α-amylase-SR (A) Salivary α-amylase levels and (B) salivary α-amylase-SR were determined at baseline (pre-training), weeks 4, 8, and post-training (week 12). Values are presented as means ± SEM. A one-way repeated measures ANOVA was used to determine the effect of time. $^*P < 0.001$ vs baseline

Vitamin D supplementation also contributed to the changes in cathelicidin-SR ($\chi^2 = 6.2$, $df = 2$, $P = 0.04$); however, there were no treatment or treatment by time between group differences.

## 4 | DISCUSSION

US Marine Corps recruits undergoing basic training experience a high degree of stress during training. The goal of this research was to determine whether vitamin D status contributed to salivary SIgA and cathelicidin responses in recruits performing 12 weeks of arduous basic military training and whether vitamin D supplementation (1000 IU $d^{-1}$) modified mucosal immune markers. There are four novel findings from this research: one, basic military training, primarily in the early phase, negatively impacted SIgA-SR, and cathelicidin-SR, suggesting training stressors challenged immune responses. Two, SIgA-SR was positively associated with 25(OH)D, but 25(OH)D only contributed to an increase

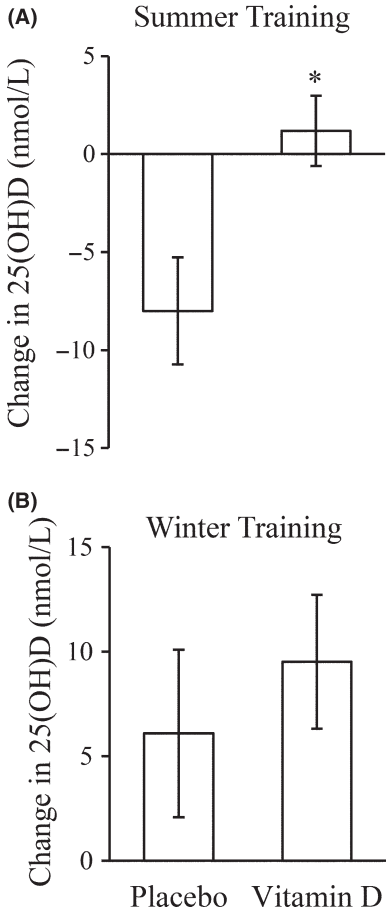


**FIGURE 2** Changes (pre- to post-training) in 25-hydroxy vitamin D (25(OH)D) levels in Marine Corps recruits. 25(OH)D levels were determined at baseline and post-training (week 12) for summer (A) and winter (B) cohorts. Values are presented means ± SEM. A *t* test was used to compare groups. $^*P < 0.01$ vs placebo

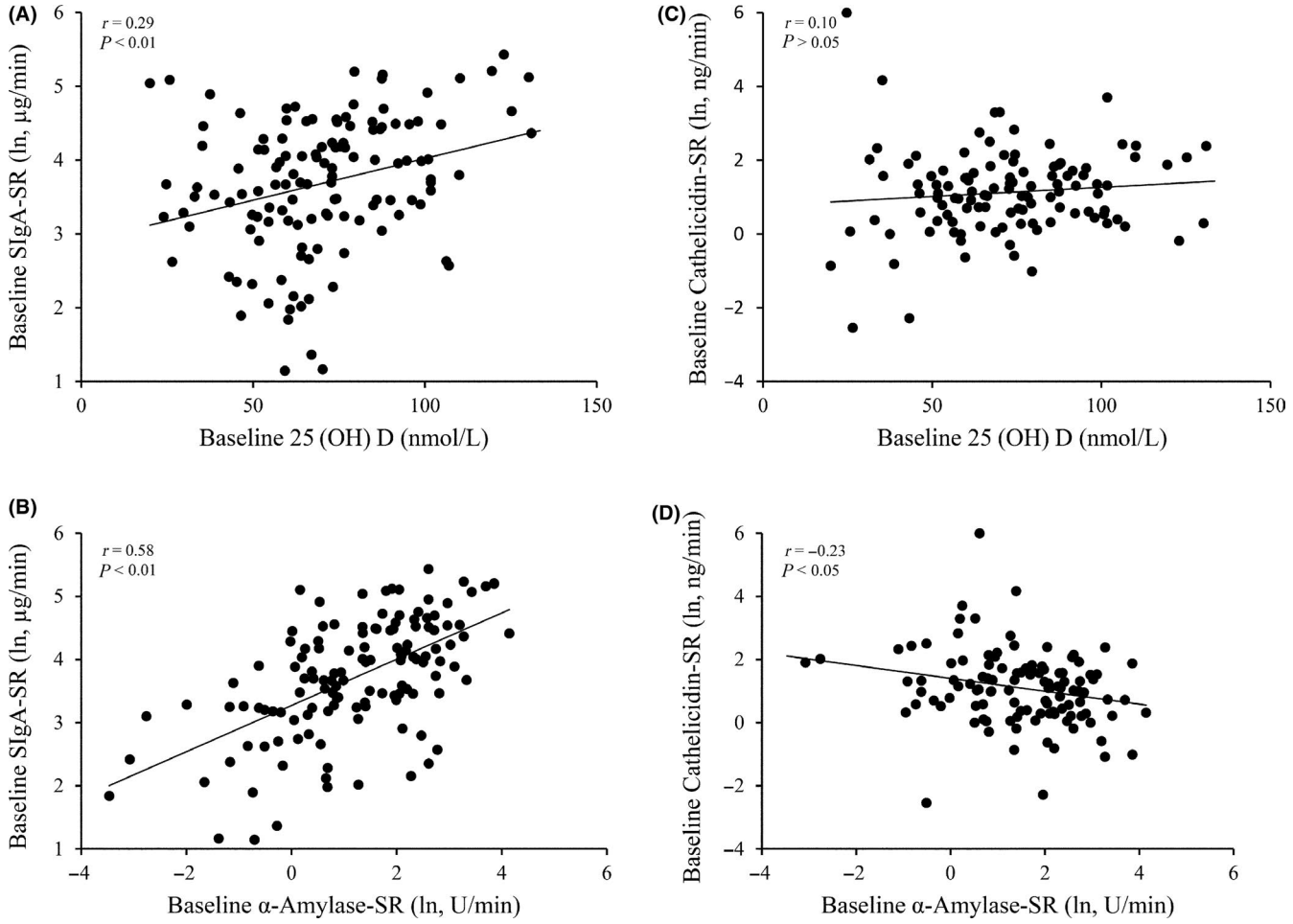


**FIGURE 3** The relationship between baseline salivary secretory immunoglobulin A secretion rates (SIgA-SR; n = 128) and cathelicidin-SR (n = 114) with baseline 25(OH)D (n = 142) and α-amylase-SR (n = 130) in Marine Corps recruits. Pearson's product-moment correlation coefficients were determined for (A) baseline SIgA-SR and baseline 25(OH)D, (B) baseline SIgA-SR and baseline α-amylase-SR, (C) baseline cathelicidin-SR and baseline 25(OH)D, and (D) baseline cathelicidin-SR and baseline α-amylase-SR

in SIgA-SR in the summer, perhaps due to lower baseline 25(OH)D levels in the winter. Three, elevated stress was associated with an increase in SIgA-SR, but a reduction in cathelicidin-SR during training, underscoring the complexity of immune responses to elevated stress. Four, daily vitamin $D_3$ supplementation contributes to an increase in SIgA-SR in the early phases of training when the stress level is high. These data highlight vitamin D's potential for improving mucosal immune function in military recruits undergoing intense training.

Salivary SIgA-SR were lowest at week 4, but increased above baseline thereafter. These observations support the notion that SIgA concentrations and SIgA-SR decrease after repeated bouts of intense training.[18-22] Decreased levels of SIgA have previously been reported to be associated with an increased risk for URTI in most,[18-20] but not all studies.[21,22] The decrease in SIgA-SR observed at week 4 suggests compromised mucosal immunity, while an increase at weeks 8 and 12 suggests mucosal immune responses are augmented. Cathelicidin-SR was also lowest at week 4, and although levels gradually increased during training, this antimicrobial peptide did not return to baseline values following training. Several pathogens, including bacterial and viral, can decrease cathelicidin production.[23,24] A decrease in cathelicidin-SR may indicate that the pathogenic load was exceedingly high, although this is merely speculative and cannot be confirmed from the current study.

Differences in baseline 25(OH)D may have contributed to the summer increase in SIgA-SR. Elevated SIgA-SR can, in-part, be explained by higher salivary flow rates, previously reported to be positively associated with 25(OH)D concentrations.[8] Interestingly, summer recruits had higher 25(OH)D concentrations and a twofold to 2.5-fold higher salivary flow rate at each time point throughout training. Alternatively, salivary flow rates can vary based on sex, such that females have lower unstimulated salivary flow rates due to smaller salivary glands.[25] In the summer, ~40% of recruits were female compared with 60% in winter. Taken

**TABLE 2** Predictors associated with salivary secretory immunoglobulin A secretion rates (SIgA-SR) and cathelicidin-SR

| Predictor variable | SIgA-SR | Cathelicidin-SR |
|---|---|---|
| Baseline | 2.94 (0.08) | 1.51 (0.15) |
| Week 4 | −0.49 (0.06)[b] | −0.63 (0.12)[b] |
| Week 8 | −0.30 (0.06)[b] | −0.17 (0.10) |
| Post-training | 0.16 (0.06)[b] | −0.55 (0.11)[b] |
| Baseline 25(OH)D | −0.005 (0.003) | −0.007 (0.005) |
| Summer | 0.32 (0.11)[b] | – |
| Summer × baseline 25(OH)D | 0.011 (0.004)[a] | – |
| Male | – | −0.03 (0.17) |
| Male × baseline 25(OH)D | – | 0.020 (0.007)[b] |
| α-amylase-SR | 0.40 (0.03)[b] | −0.24 (0.05)[b] |

*Note*: Values are B coefficients (SE). SIgA-SR, cathelicidin-SR and α-amylase-SR were log transformed prior to analysis.
[a]$P < 0.05$.
[b]$P < 0.01$.

**TABLE 3** Effects of vitamin D supplementation on salivary secretory immunoglobulin A secretion rates (SIgA-SR) and cathelicidin-SR responses

| Predictor variable | SIgA-SR | Cathelicidin-SR |
|---|---|---|
| Week 4 | 3.14 (0.12)[b] | −0.13 (0.16) |
| Week 8 | 3.43 (0.12)[b] | 0.56 (0.12)[b] |
| Post-training | 3.55 (0.12)[b] | 0.40 (0.16)[a] |
| Vitamin D supplementation | 0.26 (0.13)[a] | 0.30 (0.21) |
| Vitamin D supplementation × week 8 | −0.04 (0.13) | −0.45 (0.19)[a] |
| Vitamin D supplementation × week 12 | −0.29 (0.14)[a] | −0.36 (0.26) |
| Baseline SIgA-SR | 0.49 (0.06)[b] | – |
| Summer | 0.49 (0.13)[b] | – |
| Baseline vitamin D (summer) | 0.005 (0.003) | – |
| Baseline vitamin D (winter) | −0.003 (0.004) | – |
| Baseline cathelicidin-SR | – | 0.54 (0.06)[b] |
| Male | – | 0.38 (0.13)[b] |
| Baseline vitamin D | – | 0.006 (0.004) |
| Baseline vitamin D × male | – | −0.010 (0.006) |

*Note*: Values are B coefficients (SE). SIgA-SR and cathelicidin-SR were log transformed prior to analysis.
[a]$P < 0.05$.
[b]$P < 0.01$.

together, higher baseline levels of 25(OH)D and a greater proportion of males in the summer cohort may have contributed to elevated salivary flow rates, and subsequently, increased SIgA-SR.

The relationship between stress and indices of immune function is complex. The positive correlation between baseline α-amylase and baseline SIgA-SR suggests stress may enhance mucosal immunity, whereas the opposite may hold true for cathelicidin-SR. Interestingly, once recruits begun training we observed a decrease in SIgA-SR and an increase in α-amylase at week 4. Repeated bouts of strenuous training are associated with a decrease in SIgA-SR and may persist for ≥7 days.[26] Although α-amylase remained elevated at week 8, SIgA-SR values increased above baseline, suggesting the recruits were adapting to the intense training. The relationship between baseline α-amylase-SR and cathelicidin-SR is less clear. Our data suggests that stress and cathelicidin have a negative correlation, which is in contrast to our original hypothesis. One explanation is that exposure to antigens at baseline was insufficient to elicit an immune response. Alternatively, vitamin D is known to regulate cathelicidin expression independent of α-amylase, and vitamin D levels may have been insufficient to fully activate the cathelicidin microbial response. These observations emphasize the complexity of immune activation during periods of increased stress where risk of infection is elevated.

Although SIgA-SR responses to training were augmented with vitamin D supplementation, the magnitude of change may have been insufficient to counteract the high degree of stress during training, and higher doses of vitamin D may be required. In support of this, He et al[9] reported a 48% increase in SIgA-SR at week 7 and 20% increase at week 14 when supplementing athletes with 5000 IU D3 $d^{-1}$ for 14 weeks during winter training. In the present study, we observed modest changes in SIgA-SR at weeks 4 (−2%), 8 (19%), and 12 (7%). While these data suggest supplementation with 5000 IU daily may have led to more favorable SIgA-SR responses, we cannot be certain because research volunteers, environment, and geography differed between studies.

Remaining unanswered is whether the changes in salivary SIgA-SR with vitamin D supplementation prevent URTI, as we did not collect nasopharyngeal swabs to confirm illness. Although a self-reported UTRI symptoms questionnaire was administered to recruits before saliva collection (data not shown), the validity of such questionnaires are questionable because they are unable to discern whether self-reported symptoms are due to opportunistic infection, allergies or additional causes.[27] In the present study nearly all recruits (97%) presented with at least one URTI symptom at some point during training. Others have hypothesized that the immune-enhancing benefits of vitamin D supplementation only occurs in vitamin D deficient (ie, <30 nmol $L^{-1}$) individuals[28]; however, there is insufficient evidence to support an absolute cutoff. In the present study, only six subjects had baseline 25(OH)D levels <30 nmol $L^{-1}$, three in each group. Thus, the study was not

adequately powered to draw conclusions based on the classification of vitamin D status. Regardless, our data demonstrate that vitamin D does contribute to salivary SIgA-SR responses during the first 8 weeks of training. Last, this study was a secondary aim of a larger clinical trial that focused on calcium and vitamin D supplementation and bone health. Although we cannot exclude the possibility of calcium contributing to the present results, we feel this is rather unlikely since, to our knowledge, calcium supplementation and immune benefits have not been reported.

This is the first study that has explored the effects of vitamin D on indices of mucosal immunity in Marine Corps recruits undergoing 12 weeks of basic military training. Baseline 25(OH)D levels were positively correlated with SIgA-SR, and daily supplementation with 1000 IU of vitamin $D_3$ improved SIgA-SR responses during the most stressful period of training. Vitamin D supplementation may augment immune function during high-stress military training, and future studies should explore whether supplementation decreases the incidence of illness.

## 5 | PERSPECTIVES

The results presented herein highlight the impact of arduous basic military training on immunity and the potential benefits of vitamin D in augmenting indicators of mucosal immunity. These data imply that the early phase of basic military training challenges immune function by suppressing SIgA-SR and cathelicidin-SR, however, providing recruits with 1000 IU $d^{-1}$ of vitamin $D_3$ daily increases SIgA-SR during training. Although the SIgA-SR responses to training were augmented with vitamin D supplementation, the magnitude of change may have been insufficient to counteract the high degree of training induced stress, and higher doses of vitamin D may be required for improved outcomes. Additional research is required to determine the optimal dosage of vitamin D needed to support mucosal immunity during basic military training and whether supplementation leads to decreased illness.

## ACKNOWLEDGEMENTS

The authors extend their gratitude to all of the United State Marine Corps recruits that voluntarily participated in this research. The authors have no conflicts of interest to report. The views expressed are those of the authors and do not reflect the official position of the Uniformed Services University, US Navy, Army, or Department of Defense.

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**How to cite this article:** Scott JM, Kazman JB, Palmer J, McClung JP, Gaffney-Stomberg E, Gasier HG. Effects of vitamin D supplementation on salivary immune responses during Marine Corps basic training. *Scand J Med Sci Sports*. 2019;29:1322–1330. https://doi.org/10.1111/sms.13467