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Influence of Vitamin D Supplementation by Simulated Sunlight or Oral D_3 on Respiratory Infection during Military Training

Sophie E. Harrison[1], Samuel J. Oliver[1], Daniel S. Kashi[1,2], Alexander T. Carswell[1,3], Jason P. Edwards[1,2], Laurel M. Wentz[1,4], Ross Roberts[1], Jonathan C. Y. Tang[3], Rachel M. Izard[5,6], Sarah Jackson[7], Donald Allan[8], Lesley E. Rhodes[9], William D. Fraser[3, 10], Julie P. Greeves[3,7,11], Neil P. Walsh[2]

[1]College of Human Sciences, Bangor University, Bangor, Gwynedd, United Kingdom.
[2]Faculty of Science, Liverpool John Moores University, Liverpool, United Kingdom.
[3]Norwich Medical School, University of East Anglia, Norwich, Norfolk, United Kingdom
[4]Department of Nutrition and Health Care Management, Appalachian State University, Boone, North Carolina, United States of America.
[5]Headquarters Army Recruiting and Initial Training Command, Upavon, Wiltshire, United Kingdom.
[6]Defence Science and Technology, Porton Down, Wiltshire, United Kingdom.
[7]Army Health and Physical Performance, Army HQ, Andover, Hampshire, United Kingdom.
[8]Medical Physics Department, Salford Royal NHS Foundation Trust, and University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom.
[9]Faculty of Biology, Medicine and Health, University of Manchester, and Dermatology Centre, Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom.
[10]Departments of Endocrinology and Clinical Biochemistry, Norfolk and Norwich University Hospitals Trust, Norwich, United Kingdom.
[11]Division of Surgery and Interventional Science, UCL, London, United Kingdom.

Corresponding Author:

Dr. Samuel J. Oliver, College of Human Sciences, Bangor University, Bangor, LL57 2PZ, UK. Email: s.j.oliver@bangor.ac.uk Telephone: + 44 1248 383965

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ABSTRACT

Purpose: To determine the relationship between vitamin D status and upper respiratory tract infection (URTI) of physically active men and women across seasons (study 1). Then, to investigate the effects on URTI and mucosal immunity of achieving vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}) by a unique comparison of safe, simulated-sunlight or oral D_3 supplementation in winter (study 2). **Methods:** In study 1, 1,644 military recruits were observed across basic military training. In study 2, a randomized controlled trial, 250 men undertaking military training received either placebo, simulated-sunlight (1.3x standard erythemal dose, three-times-per-week for 4-weeks and then once-per-week for 8-weeks) or oral vitamin D_3 (1,000 IU·day^{-1} for 4-weeks and then 400 IU·day^{-1} for 8-weeks). URTI was diagnosed by physician (study 1) and Jackson common cold questionnaire (study 2). Serum 25(OH)D, salivary secretory immunoglobulin A (SIgA) and cathelicidin were assessed by LC-MS/MS and ELISA. **Results:** In study 1, only 21% of recruits were vitamin D sufficient during winter. Vitamin D sufficient recruits were 40% less likely to suffer URTI than recruits with 25(OH)D <50 nmol·L^{-1} (OR (95% CI) = 0.6 (0.4–0.9)); an association that remained after accounting for sex and smoking. Each URTI caused on average 3 missed training days. In study 2, vitamin D supplementation strategies were similarly effective to achieve vitamin D sufficiency in almost all (≥95%). Compared to placebo, vitamin D supplementation reduced the severity of peak URTI symptoms by 15% and days with URTI by 36% (P < 0.05). These reductions were similar with both vitamin D strategies (P > 0.05). Supplementation did not affect salivary SIgA or cathelicidin. **Conclusion:** Vitamin D sufficiency reduced the URTI burden during military training.

Keywords: cholecalciferol, 25-hydroxyvitamin D, exercise, UVB, immunity, virus.

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INTRODUCTION

Athletes and military personnel experience arduous training and nutritional inadequacy that may compromise host defense and increase their susceptibility to respiratory illness such as the common cold, particularly during the autumn-winter (1, 2). The immunomodulatory effects of vitamin D are considered to play a role in the seasonal stimulus for upper respiratory tract infection (URTI) (3, 4). This has fuelled considerable interest in potential prophylactic benefits of vitamin D supplementation on URTI. Vitamin D can be obtained from diet but is primarily synthesized by skin exposure to sunlight ultraviolet B (UVB) radiation. As dietary vitamin D intakes in the US and Europe (112–330 $IU \cdot day^{-1}$, (5-7)) are typically less than recommended (600 $IU \cdot day^{-1}$, (7, 8)) people who live at latitudes >35° or live indoors for the majority of sunlight hours and cover-up from the sun are at higher risk of vitamin D insufficiency. Indeed, epidemiological studies report vitamin D sufficiency (serum 25-hydroxyvitamin D (25(OH)D) ≥50 $nmol \cdot L^{-1}$) in only 40–65% of athletes and military personnel during the winter, when skin exposure to UVB radiation is negligible (9-11).

Vitamin D is widely accepted to influence both innate and adaptive immunity with implications for host defense (12, 13). 25(OH)D is converted in the kidney to the biologically active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which enhances the innate immune response by the induction of antimicrobial proteins like cathelicidin (13). Antimicrobial proteins help to prevent URTI as part of the first line of defense. The actions of vitamin D on adaptive immunity may also be anti-inflammatory or 'tolerogenic' (3). Immune tolerance has been described as the ability to dampen defense yet control infection at a non-damaging level (14); prompting the search for tolerogenic nutritional supplements to reduce URTI burden (3). URTI burden can be assessed by URTI prevalence, or the duration or severity of URTI. As such, maintaining or achieving vitamin D sufficiency may reduce URTI burden by

preventing URTI symptoms but also by reducing the duration and/or severity of URTI (3, 9, 11)).

Large cross-sectional and randomized, placebo-controlled supplementation studies in the general population highlight that vitamin D reduces the burden of URTI (4, 15, 16). However, cross-sectional studies in young healthy and athletic populations present conflicting findings (17-19), which might be explained by small samples with few URTI, a limited range of vitamin D concentrations due to single-season data collections, and a lack of control for factors known to independently influence URTI (e.g. sex and smoking). Randomized, controlled trials investigating the effect of vitamin D supplementation on URTI and immunity in military recruits and athletes are extremely limited and present a mixed picture (20-23). These studies show reduced URTI symptoms (22), improved mucosal immunity (i.e. salivary cathelicidin and immunoglobulin A (IgA)) (21, 23) and fewer missed training days due to URTI (20), as well as, no effect on URTI symptoms (20) or mucosal immunity (22, 23). The significant heterogeneity reported in these trials may stem from variations in participant baseline vitamin D status and dosing regimens; these factors are considered to modify the effect of vitamin D on immunity to respiratory pathogens (15). The participants in these studies were vitamin D sufficient at baseline (20, 21), which likely limited the need and potential benefit of vitamin D supplementation (11). Also participants were administered higher oral vitamin D doses than recommended by the Institute of Medicine (IOM) and European Food Safety Authority (EFSA) (21, 22) increasing the risk of adverse outcomes (tolerable upper intake 4000 $IU \cdot day^{-1}$) (7, 8). Although vitamin D is derived from skin exposure to sunlight the effect of safe skin sunlight exposure on URTI burden and mucosal immunity has yet to be studied. Ultraviolet (UV) radiation has a range of vitamin D-dependent and -independent effects on immunity (24); however, whether there are additional benefits of safe sunlight exposure, compared to oral vitamin D supplementation, is

unknown. Given the negative impact of URTI on training and performance it is important to determine whether vitamin D supplementation has measurable and meaningful effects on URTI in physically active populations (2, 9, 11).

First the relationship between vitamin D status and URTI prevalence was determined in a large, prospective cohort study of young men and women commencing military training across all seasons (study 1). It was hypothesized that vitamin D sufficient recruits would be less likely to suffer URTI, compared to those who had serum 25(OH)D <50 nmol·L^{-1}. Then, in a randomized, placebo-controlled trial (study 2), the effects on overall URTI burden (prevalence, duration and severity) and mucosal immunity of achieving vitamin D sufficiency by either simulated sunlight, following recommendations on safe, low-level sunlight exposure (25), or oral D_3 supplementation, in wintertime was investigated. Vitamin D sufficiency was targeted because maintaining serum 25(OH)D concentration ≥50 nmol·L^{-1} has been recommended for health by the IOM and EFSA and is achievable using safe doses of oral vitamin D_3 and simulated sunlight (7, 8). It was hypothesized that achieving vitamin D sufficiency during winter by vitamin D supplementation would reduce URTI burden, and improve mucosal immunity, compared to placebo supplementation.

METHODS

British Army recruits voluntarily participated in study 1 and study 2 after providing fully informed written consent and passing a clinician-screened medical assessment, which excludes for a number of medical conditions, including chronic lung diseases, and asthma symptoms or treatment in the last year. Men (study 1 and study 2) were located at Infantry Training Centre Catterick, UK (latitude 54°N), and women (study 1) were located at Army Training Centre Pirbright, UK (latitude 51°N). All volunteers were studied during 12 weeks

of Basic Military Training that follows a syllabus of basic military skills including physical training, weapon handling, map reading, and fieldcraft. The progressive, structured, physical training program included: endurance training, circuit training, agility-based gymnasium work, assault course practice, and marching with a load. The studies received ethical approval from the UK Ministry of Defence Research Ethics Committee and were conducted in accordance with the Declaration of Helsinki (2013) (study registration references at www.clinicaltrials.org [NCT02416895, NCT03132103]).

Study one

Participants and study design. 1,644 men and women (n = 1,220 men: 95% white ethnicity, age 21 ± 3 years; body mass 75.3 ± 9.9 kg, height 1.77 ± 0.06 m, body mass index (BMI) 24.0 ± 2.7 kg·m^{-2}, 38% smokers; n = 424 women: 95% white ethnicity, age 22 ± 3 years, body mass 64.8 ± 8.2 kg, height 1.65 ± 0.06 m, BMI 23.7 ± 2.4 kg·m^{-2}, 24% smokers) participated in this prospective cohort study between January 2014 and September 2015. Participants were included if they gave baseline blood samples and URTI data was available during the entire 12 weeks of military training.

Experimental procedures. Baseline measures were collected from each participant during the initial medical assessment; including a venous blood sample for determination of serum 25(OH)D; height and body mass; ethnicity and smoking history by self-reported questionnaire (Figure 1). Medical records were accessed to obtain physician-diagnosed URTI and lost training days due to URTI. The URTI were diagnosed by a single general practice-trained physician. A lost training day was recorded when a recruit was unavailable for normal military training.

Study two

Participants and study design. 250 men (age 22 ± 7 years, body mass 76.3 ± 10.8 kg, height 1.77 ± 0.06 m, BMI 24.2 ± 3.0 $kg \cdot m^{-2}$) participated in this double-blind, randomized, placebo-controlled trial (Figure 1). Participants were recruited at the start of 12 weeks of Basic Military Training during January and February of 2016 and 2017; when ambient UVB is negligible at UK latitudes (50–60°N), and serum 25(OH)D is at its annual nadir. Participants were eligible to participate if they had sun-reactive skin type of I to IV on the Fitzpatrick Skin Type Scale (26), were not consuming supplements containing vitamin D, and had not used a sunbed or traveled to a sunny climate in the 3 months before the study.

Experimental procedures. Participants were randomized within their platoons to one of four intervention groups: 1) oral vitamin D_3 supplementation (ORAL); 2) oral placebo supplementation (ORAL-P); 3) solar simulated radiation (SSR); or, 4) solar simulated radiation placebo (SSR-P). Block randomization was used (www.randomiser.org) to achieve an equal distribution of intervention groups within each platoon so any differences in training conditions between platoons did not influence the outcomes of the study. The intervention strategy for the SSR and ORAL groups was to restore and then maintain IOM and EFSA recommended vitamin D sufficiency (serum 25(OH)D ≥50 $nmol \cdot L^{-1}$). Participants completed a 4-week restoration phase, necessary because serum 25(OH)D was at its annual wintertime nadir, followed by an 8-week maintenance phase.

At baseline, during the routine initial medical assessment, height and body mass were measured, a venous blood sample was collected for the determination of serum 25(OH)D, and a lifestyle questionnaire was completed to determine smoking and alcohol use. Additional blood samples were obtained at week 5, and week 12. At baseline, week 5, and week 12 saliva samples were collected in the evening, between 18:00 and 21:30 h, at least 15 minutes postprandial. Participants were excluded from analysis if they did not achieve ≥80%

compliance with the intervention. Compliance with the interventions was calculated from researcher weekly counts of oral capsules remaining in recruit pill boxes and SSR cabinet visit records. Vitamin D from the diet was estimated in week 12 using a food frequency questionnaire, and solar UVR exposure was measured in weeks 4 and 11 using polysulphone badges, worn on the upper chest/anterior shoulder region on the outer clothes, as described (10, 27). The change in absorbance of the badges due to exposure was measured using a spectrophotometer and related to the erythemal effective UVR (sunburning) through a standard polynomial relationship; data are expressed as standard erythemal dose per day (27). Participant dietary vitamin D intake was calculated excluding the oral D_3 supplement participants received in the ORAL group. On completion of the study, to confirm participant blinding, participants were asked to guess the intervention they had received.

Simulated sunlight intervention. Simulated sunlight was provided following guidelines on safe, low-level sunlight exposure for vitamin D synthesis (6); described previously to achieve serum 25(OH)D $\geq$50 nmol·L^{-1} in the majority of individuals with sun-reactive skin type of I to IV (28). Those assigned to the SSR intervention were exposed three-times-a-week during the restoration phase and once-per week during the maintenance phase to an experimenter-controlled constant UVR dose using a whole body irradiation cabinet (Hapro Jade, Kapelle, The Netherlands) fitted with Arimed B fluorescent tubes (Cosmedico, Stuttgart, Germany). The fluorescent tubes emitted a UVR spectrum similar to sunlight (λ: 290–400 nm; 95% UVA: 320–400 nm, 5% UVB: 290–320 nm) that was characterized by a spectroradiometer (USB2000+, Ocean Optics BV, Duiven, The Netherlands) radiometrically calibrated with traceability to UK national standards.

During each exposure, participants received a 1.3x standard erythemal dose (SED) whilst wearing shorts and a T-shirt to expose ~40% skin surface area. This dose is equivalent

to ~15 minutes, midday summer sun exposure six-times-per-week for a casually dressed individual in northern England (latitude 53.5°N) (28). A constant SSR dose was maintained during the study by monitoring irradiance using a spectroradiometer (USB2000+, Ocean Optics BV) and adjusting for any decrease in measured irradiance emitted by increasing exposure time, as described (28) (mean duration of SSR exposures was 222 ± 23 s). The exposure time was controlled by using an electronic timer on the irradiation cabinet. For the SSR-P participants, the number and duration of intervention exposures were the same as SSR, except the irradiation cabinet fluorescent tubes were covered with transparent UVR blocking film (DermaGard UV film, SunGard, Woburn, Massachusetts, USA). A spectroradiometer confirmed the UVR blocking film was effective at preventing transmission of 99.9% of UVR.

Oral vitamin D_3. Participants receiving the ORAL intervention consumed a vitamin D_3 capsule daily, containing 1,000 IU and 400 IU during the restoration and maintenance phases, respectively (Pure Encapsulations, Sudbury, Massachusetts, USA). The restoration dose was based on previous predictive modeling to achieve serum 25(OH)D ≥50 nmol·L^{-1} (29), and pilot investigations that showed it achieved similar serum 25(OH)D concentrations to SSR; and was less than the tolerable upper intake recommended by the IOM and EFSA (7, 8). The ORAL maintenance dose was shown in a pilot investigation to maintain serum 25(OH)D ≥50 nmol·L^{-1} and when accounting for typical habitual dietary intake (5-7) was similar to IOM and EFSA recommended dietary allowances (7, 8). For 12 weeks, ORAL-P participants consumed an identical-looking cellulose placebo capsule daily (Almac Group, County Armagh, UK). Independent analysis found the vitamin D_3 content of the 1,000 and 400 IU capsules to be 1,090 and 460 IU, respectively, and confirmed the placebo did not contain vitamin D (NSF International Laboratories, Ann Arbor, Michigan, USA).

URTI diagnosis (study 2). As in study 1, medical records were accessed to obtain data on physician-diagnosed URTI and lost training days due to URTI. However, URTI was principally monitored by self-reported daily symptoms recorded using the Jackson common cold questionnaire (30). A strength of the Jackson common cold questionnaire compared to physician-diagnosed URTI is that URTI duration and severity, as well as prevalence, can be assessed. Participants were asked to rate eight symptoms (sneezing, headache, feeling generally unwell, runny nose, blocked nose, sore throat, cough, chilliness) on a 4-point Likert scale (not at all = 0, mild = 1, moderate = 2, severe = 3). Data were included when participants completed ≥80% of their daily Jackson questionnaires. A URTI was defined by a daily total symptom score of ≥6 for two or more consecutive days (31). Further, average URTI duration (average duration of all URTI episodes), the peak URTI symptom severity (maximum URTI severity score on a single day of any URTI episode; maximum possible peak severity is 24 arbitrary units (AU)), and the total number of days with a URTI during basic military training for each participant (total days with URTI; military training is 84 days in total) were also determined. Self-reported URTI data was not reported back to the military and therefore did not influence physician diagnosis of URTI or lost training days due to URTI.

Blood analysis (study 1 and 2). Whole blood samples were collected by venipuncture from an antecubital vein into plain vacutainer tubes (Becton Dickinson, Oxford, UK), and left to clot for 1 hour. Subsequently, samples were centrifuged at 1500 *g* for 10 minutes at 4°C and the serum was aliquoted into universal tubes before being immediately frozen at -80°C for later analysis. Total serum 25(OH)D was measured with high-pressure liquid chromatography-tandem mass spectrometry. Analyses were performed in a Vitamin D External Quality Assurance Scheme certified laboratory (Bioanalytical Facility, University of

East Anglia, Norwich, UK). The mean intra-assay coefficient of variation (CV) for 25(OH)D_3 and 25(OH)D_2 were <10% and the lower limit of quantification was 0.1 nmol·L^{-1} (32).

Saliva collection and analysis (study 2). Saliva was collected for 5 min in a pre-weighed 30 mL tube using the passive dribble method (33). Samples were weighed immediately after collection, centrifuged at 1500 *g* and 4°C for 10 minutes, aliquoted, and then stored at -80°C. Samples were analyzed in duplicate by enzyme-linked immunosorbent assay for secretory IgA (SIgA) and cathelicidin concentration (Salimetrics, Pennsylvania, USA, and Hycult Biotech, Pennsylvania, USA). The mean intra-assay CV was 2.3% for saliva SIgA concentrations ranging from 0.02 to 0.51 mg·mL^{-1} and 10.2% for saliva cathelicidin concentrations ranging from 0.30 to 65.90 µg·L^{-1}. Assuming the density to be 1.00 g·mL^{-1} for saliva, the secretion rate was calculated by multiplying the saliva flow rate by concentration (33).

Statistical analysis. Statistical analyses were performed using SPSS Version 25 (IBM Corp, NY, US). Data points that were more than three times the interquartile range were deemed as outliers and removed. Where data were not normally distributed they were transformed using square-root calculation. Significance was set at $P < 0.05$. For study 1, an estimated minimum required sample size of 1,286 was calculated, using a type 1 error (one-tailed) of 5%, a power of 80%, and an anticipated odds ratio of 1.5 (equivalent to a small effect size), and including a binomial variable at 20%. This was based on previous literature describing the difference in URTI prevalence between individuals with low and high vitamin D status whereby, 20% of individuals with high vitamin D status reported a URTI (4), whilst also anticipating that 20% of individuals would have low vitamin D status across the whole year (34). Logistic regression were used to compare vitamin D status (25(OH)D ≥50 vs <50 nmol·L^{-1} and ≥75 vs <30, ≥50–<75 and <75 nmol·L^{-1}) with URTI prevalence during twelve-weeks military

training, and the first three weeks of military training; circulating 25(OH)D has an estimated three-week half-life (35, 36). Sex and smoking were included as covariates as they have previously been shown to influence URTI susceptibility (37, 38). Chi-square tests were used to compare URTI prevalence between vitamin D sufficient participants and those with serum 25(OH)D <50 nmol·L^{-1}, and the proportion of vitamin D sufficient participants between seasons. We used one-way ANOVA to compare 25(OH)D between seasons. For study 2, an estimated minimum required sample size of 74 (37 in each comparison group) was calculated, using the anticipated odds ratio of 0.3 for URTI prevalence between vitamin D and placebo supplemented individuals with low vitamin D status (15), and that 60% would self-report URTI during basic military training (18, 31, 39), with a type 1 error (one-tailed) of 5%, and a power of 80%. URTI prevalence between vitamin D (SSR and ORAL) and placebo (SSR-P and ORAL-P) supplementation groups was compared by logistic regression. Independent samples *t*-tests (2 groups (SSR and ORAL combined, SSR-P and ORAL-P combined)) were used to compare vitamin D and placebo supplementation effects on average URTI duration, total days with URTI, peak URTI severity, saliva flow rate, SIgA, and cathelicidin. Serum 25(OH)D, total days with URTI, URTI duration, URTI severity, saliva flow rate, SIgA, and cathelicidin, were compared between vitamin D strategies, and placebo groups, by mixed-model ANOVA ((4 groups (SSR, ORAL, SSR-P, and ORAL-P) × 3-time points (baseline, week 5 and 12)). Sunlight exposure and dietary vitamin D intake between SSR, ORAL, SSR-P, and ORAL-P groups were compared by one-way ANOVA. Cohen's *d* effect sizes (*d*) are presented to indicate the meaningfulness of group differences for total days with URTI, URTI duration, and URTI severity; whereby, values greater than 0.2, 0.5, and 0.8 represent small, medium and large effects, respectively (40).

RESULTS

Study one

Low proportion of wintertime vitamin D sufficiency in healthy young men and women

Baseline serum 25(OH)D concentration was lower in winter than all other seasons ($P < 0.01$, Figure 2A); when only 21% of participants were vitamin D sufficient (baseline serum 25(OH)D ≥50 nmol·L^{-1}; Figure 2B).

Vitamin D sufficiency associated with reduced URTI prevalence

A total of 110 URTI episodes were recorded with 7% of participants having at least one physician-diagnosed URTI. On average, each URTI resulted in 3.4 ± 3.3 lost training days (4% of total training days). Vitamin D sufficient participants at baseline were 40% less likely to have a physician-diagnosed URTI, during 12 weeks of training, than participants with baseline serum 25(OH)D <50 nmol·L^{-1} (6% vs 9%, respectively, OR (95% CI) = 0.6 (0.4–0.9), $P < 0.05$, Figure 2C). Vitamin D sufficient participants at baseline were half as likely to have a URTI within the first three weeks of training than participants with a baseline serum 25(OH)D <50 nmol·L^{-1} (2% vs 5%, OR (95% CI) = 0.5 (0.3–0.8), $P < 0.05$); approximately half of all URTI episodes occurred during this period of training (47%, 52 URTI episodes). The association between vitamin D status and URTI prevalence remained when controlling for sex and smoking ($P < 0.05$). URTI prevalence was not different between participants with a baseline serum 25(OH)D ≥75 nmol·L^{-1} and baseline serum 25(OH)D of <30, ≥50–<75, or <75 nmol·L^{-1} ($P > 0.05$).

Study two

A flow diagram detailing the number of participants assessed, recruited, and excluded from the analysis is provided in Figure 3. There were no differences between treatment or control groups in demographics, anthropometrics, or serum total 25(OH)D at baseline (Table 1 and Figure 4). During the 12-week intervention, daily sunlight exposure (0.35 ± 0.56 SED·d^{-1}) and dietary vitamin D were not different between groups (153 ± 136 IU·day^{-1}, $P > 0.05$). Participants were sufficiently blinded to the intervention since only 38.4% correctly guessed their allocated group, 27.3% were incorrect, and 34.3% said they did not know whether they had received an active or placebo intervention.

Winter simulated sunlight and oral vitamin D_3 increased vitamin D sufficiency

At baseline, before wintertime vitamin D supplementation began, only one-quarter (27%) of participants were vitamin D sufficient. Both SSR and ORAL supplementation strategies were successful in achieving vitamin D sufficiency in almost all by week 5 (≥95%). Week 5 and 12 serum 25(OH)D concentrations in the SSR and ORAL groups were higher than in the respective placebo groups ($P < 0.001$, Figure 4).

Winter vitamin D supplementation reduced URTI burden

A total of 93 Jackson-defined URTI episodes were recorded with 69% of participants having at least one self-reported URTI. The URTI prevalence was similar in vitamin D and placebo supplementation groups for the restoration (weeks 1–4), maintenance (weeks 5–12), and entire 12 week period of training (ORAL and SSR vs ORAL-P and SSR-P 57% vs 63%, 29% vs 32%, and 71% vs 68%, respectively, $P > 0.05$). The URTI average duration were also similar in vitamin D and placebo supplementation groups (Figure 5A, $P > 0.05$). Winter vitamin D supplementation reduced URTI burden compared to placebo; whereby, participants had 15% lower peak URTI severity ($P < 0.05$; Figure 5B), and 36% fewer total days with a URTI ($P < 0.05$; Figure 5C). Participants beginning vitamin D supplementation

with serum 25(OH)D <50 nmol·L^{-1} had 33% shorter average URTI duration (P = 0.05; Figure 5D), 21% lower peak URTI severity (P < 0.05; Figure 5E) and 43% fewer total days with URTI (P < 0.05; Figure 5F), when receiving vitamin D rather than placebo supplementation. There was no difference in URTI prevalence, duration, severity or total days with URTI between vitamin D supplementation strategies, or between the different placebo groups (P > 0.05). Specifically, the ORAL and SSR vitamin D supplementation strategies effect on URTI burden was similar (ORAL vs SSR, URTI prevalence 70% vs 72%, total days with URTI 9.2 ± 8.4 vs 8.4 ± 6.7 days, URTI average duration 6.9 ± 5.0 vs 6.5 ± 5.7 days, peak URTI severity 10.8 ± 3.0 vs 12.3 ± 3.8 AU, all P > 0.05). A physician-diagnosed URTI was recorded for 8% of recruits, which was comparable to 8% prevalence in the same seasonal period in study 1, and resulted in 3.3 ± 1.3 training days lost.

Vitamin D supplementation and mucosal immunity

Vitamin D supplementation and placebo groups did not differ at baseline, and weeks 5 and 12, for saliva flow rate, SIgA concentration, SIgA secretion rate, cathelicidin concentration, and cathelicidin secretion rate (P > 0.05; Table 2).

DISCUSSION

The primary finding of these two studies was that vitamin D sufficiency reduced the burden of URTI in healthy young adults completing arduous military training. In study 1, vitamin D sufficient men and women were 40% less likely to suffer a physician-diagnosed URTI during training than those with serum 25(OH)D <50 nmol·L^{-1} (Figure 2). Given this finding, and that only 21% of participants were vitamin D sufficient during winter, study 2 examined the effect of winter vitamin D supplementation on URTI. Compared to placebo,

345 vitamin D supplementation reduced the severity of peak URTI symptoms by 15% and days
346 with URTI by 36% (Figure 5). Study 2 is the first to demonstrate the benefits of vitamin D
347 supplementation, in line with IOM and EFSA guidelines, on URTI in an active population.
348 These findings are timely as the nutrition and athletic performance position stands from the
349 International Olympic Committee and American College of Sports Medicine highlight that
350 vitamin D insufficiency is widespread in athletes (9, 41).

351 In study 1, vitamin D sufficient men and women were less likely to suffer a physician-
352 diagnosed URTI during training than those with serum 25(OH)D of <50 nmol·L^{-1} (Figure 2).
353 This finding can be considered robust as it was observed after accounting for sex and
354 smoking, which is a strength of this study when compared to previous research that has not
355 controlled for factors known to independently influence URTI (17-19). In study 1, the
356 association between baseline vitamin D status and URTI was stronger during the first three
357 weeks of the twelve-week training program, which might be expected given the high
358 incidence of URTI at this time and that 25(OH)D has approximately a three-week half-life
359 (35, 36). Study 1 extends our understanding of the relationship between vitamin D and URTI
360 in active populations as data was collected in a large sample, across all seasons, and with a
361 large range of serum 25(OH)D concentrations. The burden of URTI was evident as each
362 URTI resulted in an average of 3 days missed training.

363 In study 2 vitamin D supplementation by simulated-sunlight and oral vitamin D_3 was
364 similarly effective to achieve IOM and EFSA recommended vitamin D sufficiency in the
365 majority of individuals (≥95%, Figure 4). Vitamin D supplementation did not reduce self-
366 reported URTI prevalence or benefit mucosal immunity compared to placebo (Table 2).
367 However, vitamin D supplementation reduced URTI burden compared to placebo:
368 participants receiving vitamin D reported 15% lower peak URTI severity and 36% fewer

days with URTI compared to placebo (Figure 5). The magnitude of the reduction in URTI burden in study 2 can be considered meaningful as effect sizes were medium to large. These findings also broadly agree with the previous research in this area (20, 22), i.e., vitamin D supplementation reduced URTI symptoms (22) and absence from duty due to respiratory infection (20).

The different methods used to assess URTI in the studies may explain the difference between study 1 and 2 prevalence findings. The lower URTI prevalence in study 1 than study 2 (7% vs 69%) indicates that physician diagnosis of URTI compared to daily self-report likely missed more minor illnesses that did not warrant a medical visit. Further, study 2 physician-diagnosed URTI prevalence was 8%, which was the same as study 1, when controlling for season. Self-reported URTI data was not reported back to the military and therefore did not influence physician diagnosis of URTI or lost training days due to URTI. When considered carefully in the context of these different methods, the findings of studies 1 and 2 are complementary. In study 2, lower peak URTI severity and fewer days with URTI with vitamin D supplementation, compared to placebo, would be expected to translate to vitamin D sufficient individuals reporting less to medical services, and consequently having fewer physician-diagnosed URTI than those individuals with 25(OH)D <50 $nmol \cdot L^{-1}$. This is entirely consistent with the main finding of study 1: URTI prevalence was lower in vitamin D sufficient individuals than those with 25(OH)D <50 $nmol \cdot L^{-1}$ (Figure 2).

Study 2 findings are notable as they highlight that vitamin D supplementation may reduce URTI burden, rather than prevent URTI. Vitamin D supplementation did not influence the innate mucosal antimicrobial proteins SIgA and cathelicidin that form an important part of the first line of defense against URTI. Based on these findings it is speculated that the tolerogenic effects of vitamin D may reduce URTI burden by limiting inflammation in

response to an infection (i.e., controlling infection at a non-damaging level) (3, 14, 42), which subsequently leads to a reduction in self-reported URTI severity and duration (14). Future research is warranted to investigate the effect of vitamin D supplementation on URTI and circulating anti-inflammatory cytokines (3). To better understand the influence of vitamin D supplementation on the immune pathway these studies should examine serum $1,25(OH)_2D$, the biologically active form, as well as 25(OH)D. It is also worth noting that women were not included in study 2, and therefore future work should determine the influence of vitamin D supplementation on URTI burden in women.

The pathological determination of URTI using nasopharyngeal throat swabs would have provided assurance that URTIs reported in study 1 and 2 were infection by origin, rather than due to some other cause e.g., allergy. Nonetheless, previous research has shown that infectious pathogens of URTI identified by self-reported questionnaire methods were confirmed in 82% of recreationally active men and women (31), and in 75% of Winter Olympic Games athletes (43). Furthermore, study 2 was completed during winter when common cold and flu are prevalent, and symptoms caused by summer allergies are rare. Rejecting self-reported URTI for pathogen recognition is not advocated, rather future research is advised to use a blended approach incorporating the infectious etiology with real-world URTI symptomology. Study 2 findings highlight the importance of the daily assessment of URTI symptoms to monitor URTI duration and severity as well as prevalence, regardless of whether pathogen recognition is available. The assessment of URTI duration and severity will be important in future studies wishing to further examine potential tolerogenic effects of vitamin D on immune health. Future research should also adopt the blended approach to more fully understand the effectiveness of other potential treatments for URTI.

Currently, there is no consensus for the optimal vitamin D threshold or dose for immune health (13). Participants beginning supplementation with serum 25(OH)D <50 nmol·L^{-1} reported shorter URTI duration when receiving vitamin D compared to placebo supplementation. Further evidence that participants with serum 25(OH)D <50 nmol·L^{-1} benefitted more from vitamin D supplementation than the entire sample is clear when examining the effect sizes between vitamin D and placebo for URTI outcomes; small-medium effect sizes for the entire sample, compared to medium and large effect sizes for participants with serum 25(OH)D <50 nmol·L^{-1} (Figure 5). Compared to IOM and EFSA recommended vitamin D sufficiency, no additional protection from URTI of higher vitamin D status, including a previously proposed optimal threshold (serum 25(OH)D >75 nmol·L^{-1}) (44) was revealed. These findings alongside, other findings from this research program that show benefits of vitamin D sufficiency on *in vivo* immunity (45), support 25(OH)D ≥50 nmol·L^{-1} for immune health. Further, the current studies highlight that exercise performance may indirectly benefit from maintaining vitamin D sufficiency by reducing lost training days to URTI.

No additional benefit of SSR compared to oral vitamin D_3 supplementation was shown on URTI, immune function (this study and (45)), or exercise performance (10). Consequently, active people are advised to take the 400 IU·day^{-1} oral vitamin D_3 dose, from the maintenance phase of study 2, to maintain vitamin D sufficiency when exposure to ambient UVB is inadequate: between early autumn and late winter, and for those that live and/or exercise indoors for the majority of sunlight hours or cover-up from the sun. When accounting for typical dietary vitamin D intake, this oral vitamin D_3 supplementation approach corresponds with current IOM and EFSA recommendations (600 IU·day^{-1}) for bone and general health and, unlike simulated sunlight, there is no time burden for an individual; no requirement for bulky irradiation cabinets; and oral vitamin D_3 supplementation is

effective regardless of sun-reactive skin type. Nevertheless, low-level sunlight may provide benefits to human health, additional to vitamin D synthesis, and this remains an area of active research (24).

CONCLUSIONS

Vitamin D sufficiency reduced URTI burden in military recruits during arduous training. In study 1, vitamin D sufficient recruits were less likely to have a URTI compared to those with serum 25(OH)D <50 nmol·L^{-1}. In study 2, winter vitamin D supplementation, which achieved vitamin D sufficiency in almost all (≥95%), reduced peak URTI severity, and total days with URTI compared to placebo. To reduce the burden of URTI, maintaining vitamin D sufficiency is recommended for military personnel and other active populations, such as athletes who participate in arduous training.

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FIGURE 1. A schematic of the prospective cohort study (study 1) that investigated the association between vitamin D status (serum 25(OH)D), upper respiratory tract infection (URTI) and days lost from training, and the randomized controlled trial (study 2) that investigated the effects of vitamin D supplementation by solar simulated radiation (SSR), oral vitamin D_3 (ORAL), or placebo (SSR-P or ORAL-P) on URTI and mucosal immunity. Blood samples were collected at baseline (study 1 and 2), week 5, and the end of week 12 (study 2). Saliva samples were collected at baseline, week 5 and the end of week 12 (study 2). The syringe icon represents the blood sample; the head and tube icon represent the saliva sample.

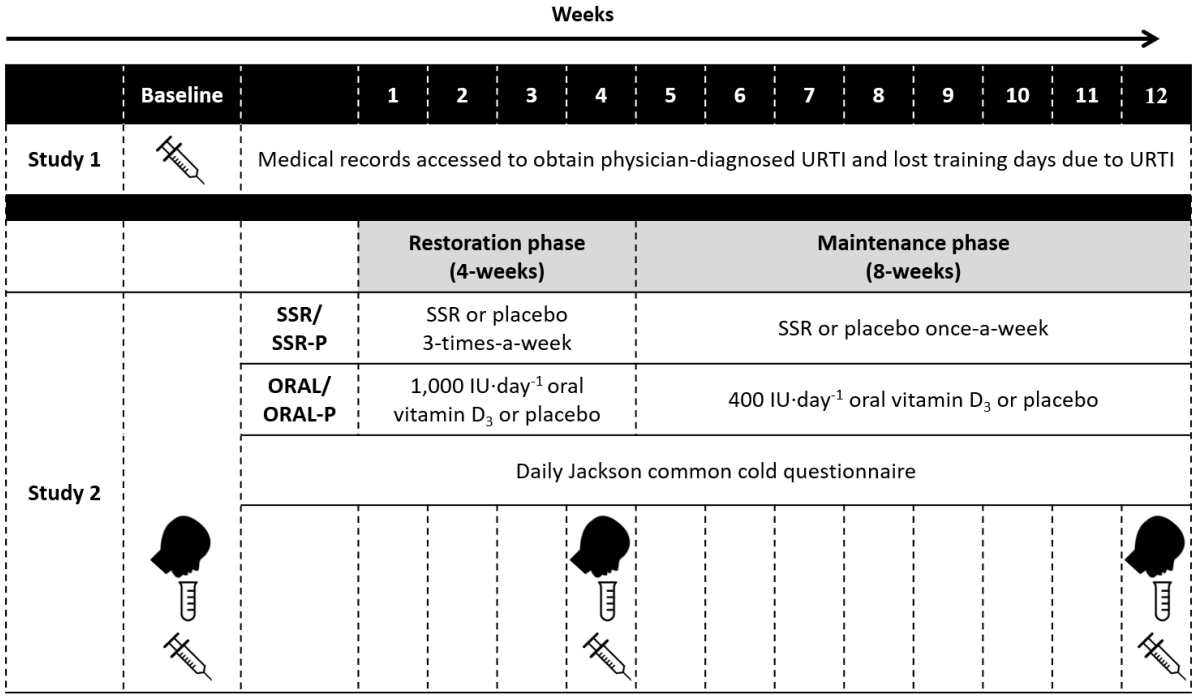

FIGURE 2. Seasonal variation in serum 25(OH)D (panel A), vitamin D sufficiency prevalence (serum 25(OH)D ≥50 nmol·L^{-1}; panel B), and the URTI prevalence when serum 25(OH)D ≥50 nmol·L^{-1} or <50 nmol·L^{-1} (panel C) in 1,644 men and women during 12-weeks of military training. a, lower than summer, $P < 0.05$. b, lower than autumn, $P < 0.05$. c, lower than spring, $P < 0.05$. *, lower than participants with serum 25(OH)D <50 nmol·L^{-1}, $P < 0.05$. Panel A data are mean ± SD. Panels B and C are percentages represented by vertical bars.

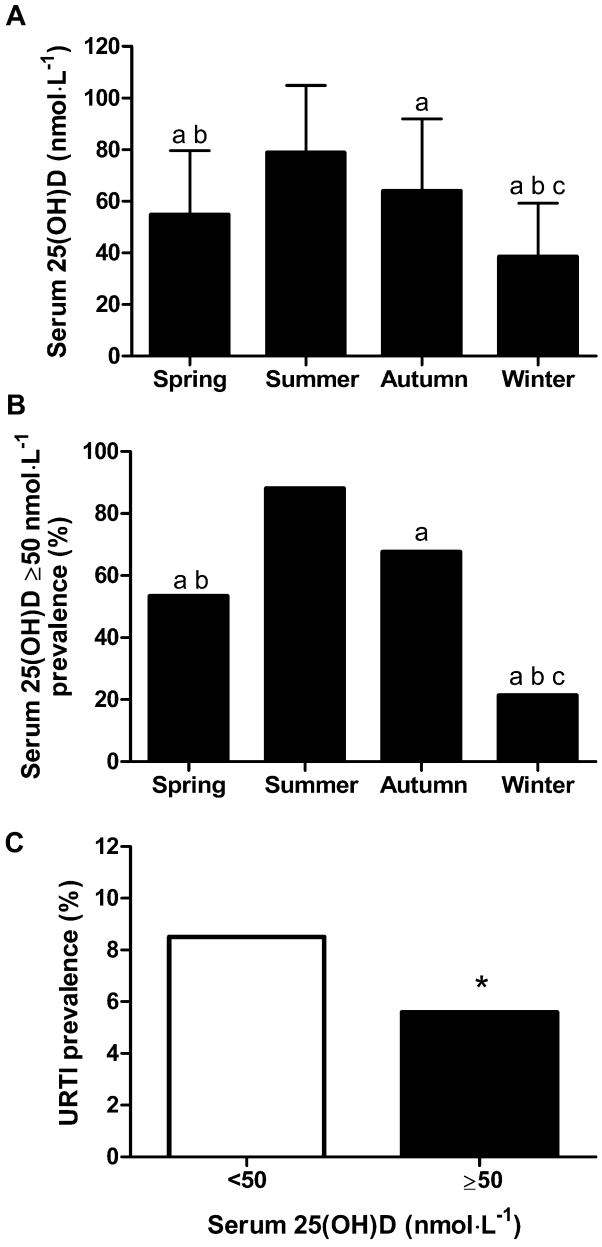

FIGURE 3. Flow diagram of the randomized controlled trial (study 2) investigating the effects of vitamin D supplementation on upper respiratory tract infection (URTI) and mucosal immunity. Flow diagram indicates the number of participants assessed, randomized to solar simulated radiation (SSR) or oral vitamin D_3 (ORAL), or a placebo (solar simulated radiation placebo (SSR-P) or oral placebo (ORAL-P)), and statistically analyzed for URTI, salivary secretory immunoglobulin A (SIgA), and cathelicidin.

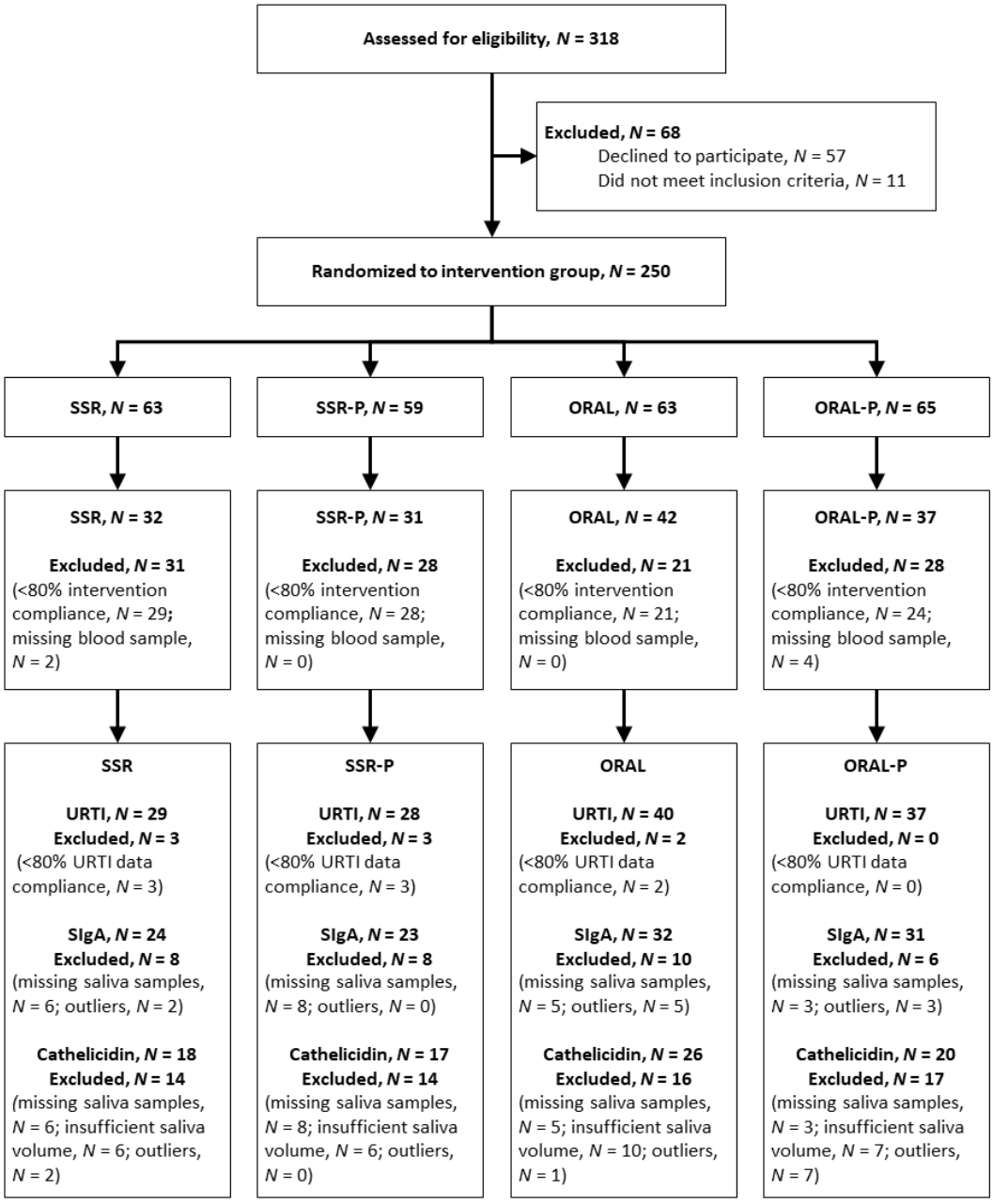

FIGURE 4. Serum 25(OH)D in men completing military training whilst receiving 12-weeks of vitamin D supplementation (solar simulated radiation (SSR) or oral vitamin D_3 (ORAL)) or a placebo (solar simulated radiation placebo (SSR-P) or oral placebo (ORAL-P)). Combined vitamin D interventions (SSR and ORAL) vs combined placebo (SSR-P and ORAL-P; panel A), ORAL vs ORAL-P (panel B), and SSR vs SSR-P (panel C). *, greater than placebo, $P < 0.05$. †, greater than baseline, $P < 0.05$. ‡, greater than week 5, $P < 0.05$. Data are mean ± SD.

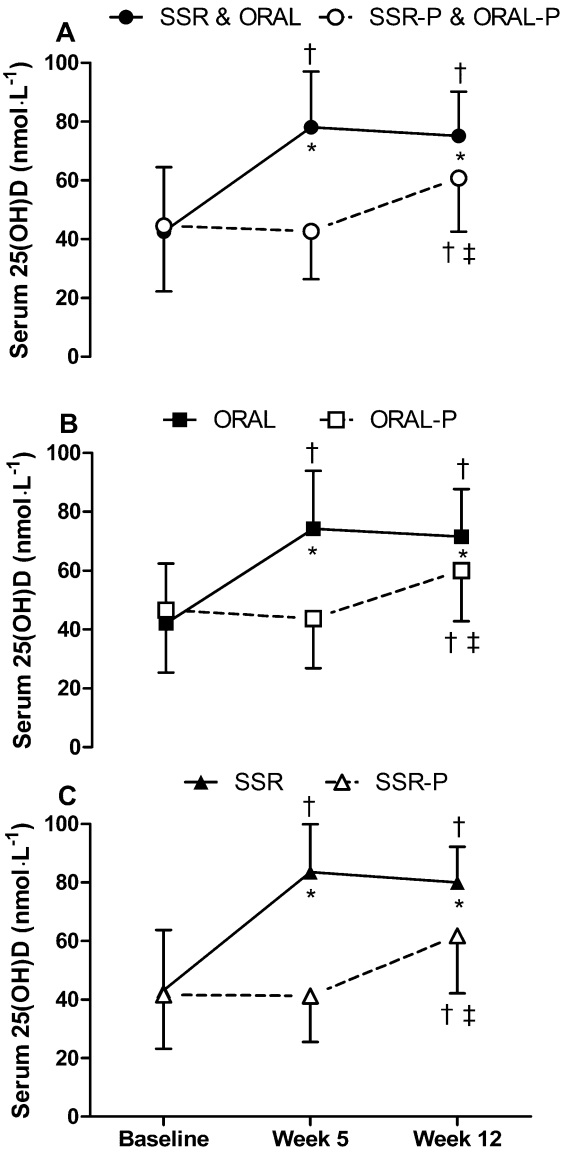

FIGURE 5. Upper respiratory tract infection (URTI) average duration (panel A & D), peak URTI severity (panel B & E), and total days with URTI during military training (panel C & F), in the vitamin D supplementation (SSR and ORAL) vs placebo supplementation groups (SSR-P and ORAL-P) in all participants (left-hand column) and participants with a baseline 25(OH)D <50 nmol·L^{-1} (N = 62; right-hand column). * and #, lower than placebo, $P < 0.05$ and $P = 0.05$, respectively. Data are mean ± SD. d = Cohen's d effect size. [a] maximum possible peak severity (24 arbitrary units (AU)), [b] total number of days for military training (84 days).

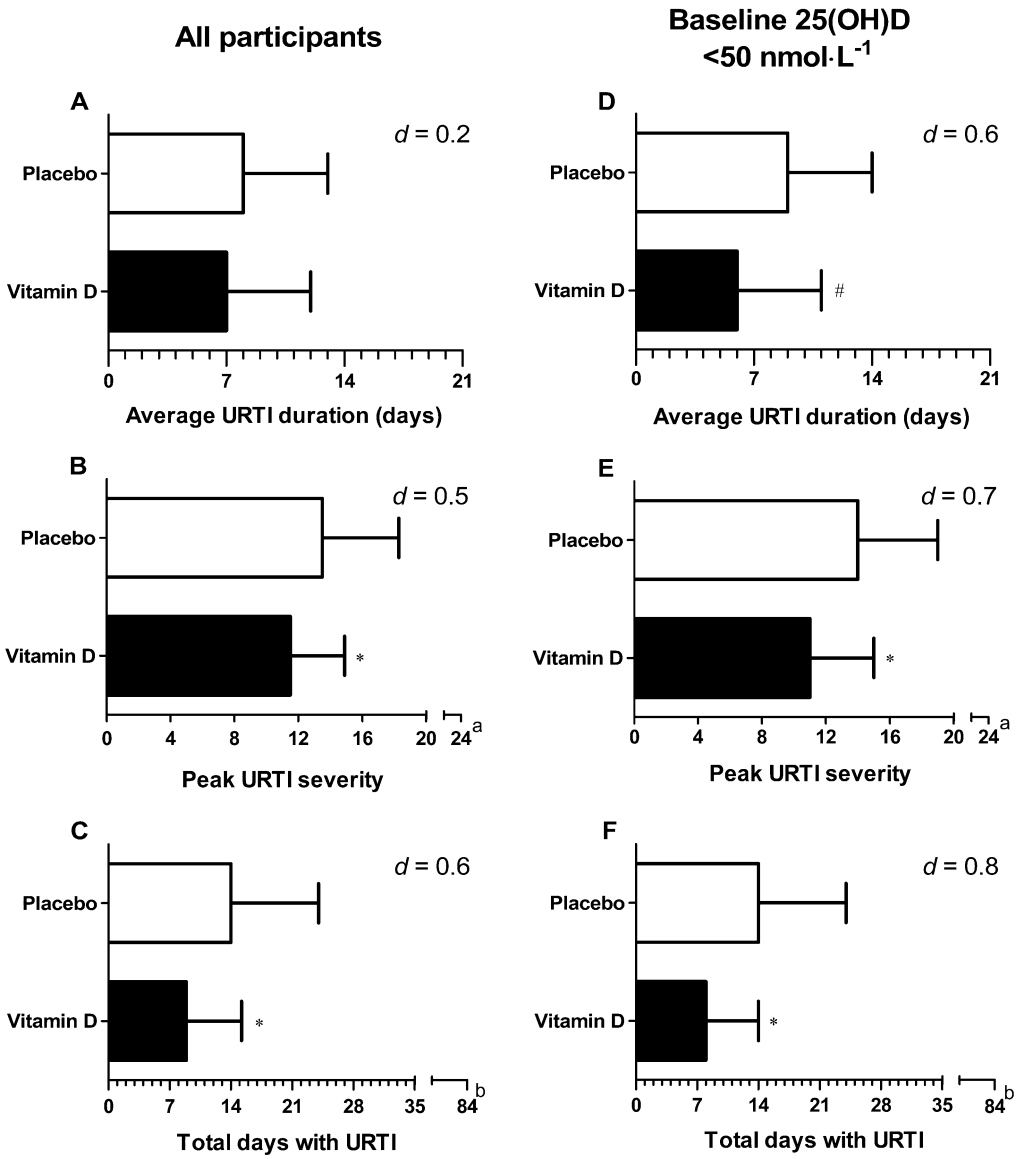

TABLE 1. Study 2 baseline participant demographics, anthropometrics, and lifestyle behaviors in solar simulated radiation (SSR), SSR placebo (SSR-P), oral vitamin D_3 (ORAL), and oral placebo (ORAL-P) supplemented groups.

626 Data are presented as mean ± SD unless otherwise stated. There were no differences in demographics,
627 anthropometrics, or lifestyle behaviors between groups ($P > 0.05$).

628

	SSR ($N = 63$)	SSR-P ($N = 59$)	ORAL ($N = 63$)	ORAL-P ($N = 65$)
Demographics				
Age (years)	21 ± 3	22 ± 3	21 ± 3	23 ± 12
Ethnicity (White Caucasian) [*n* (%)]	61 (98)	57 (97)	63 (100)	65 (100)
Skin type (I, II, III, IV) [*n* (%)]	4 (7), 16 (26), 33 (53), 9 (15)	4 (7), 16 (27), 28 (48), 11 (19)	5 (8), 18 (29), 33 (52), 7 (11)	3 (5), 19 (29), 29 (45), 14 (22)
Anthropometrics				
Height (m)	1.78 ± 0.06	1.78 ± 0.06	1.77 ± 0.07	1.78 ± 0.06
Body mass (kg)	76 ± 11	77 ± 11	75 ± 11	77 ± 10
BMI ($kg \cdot m^{-2}$)	24 ± 3	24 ± 3	24 ± 3	24 ± 3
Lifestyle behaviors				
Alcohol user [*n* (%)]	51 (82)	47 (80)	55 (87)	51 (78)
Smoker [*n* (%)]	23 (37)	25 (42)	26 (41)	21 (32)

TABLE 2. Influence of 12-weeks solar simulated radiation (SSR), placebo solar simulated radiation (SSR-P), oral vitamin D_3 (ORAL), and oral placebo (ORAL-P) on saliva flow rate (FR), SIgA concentration, SIgA secretion rate (SR), cathelicidin concentration and cathelicidin SR.

		SSR	SSR-P	ORAL	ORAL-P
FR ($\mu L \cdot min^{-1}$)	Baseline	205 ± 128	184 ± 181	260 ± 214	241 ± 173
	ΔBaseline to week 5	+5 ± 124	+26 ± 160	-36 ± 159	-5 ± 208
	ΔBaseline to week 12 † ‡	+69 ± 125	+124 ± 207	+24 ± 243	+64 ± 201
SIgA concentration ($mg \cdot mL^{-1}$)	Baseline	0.14 ± 0.08	0.12 ± 0.06	0.13 ± 0.06	0.12 ± 0.05
	ΔBaseline to week 5 †	+0.01 ± 0.08	+0.04 ± 0.09	+0.02 ± 0.09	+0.02 ± 0.07
	ΔBaseline to week 12 †	+0.00 ± 0.05	+0.03 ± 0.06	+0.03 ± 0.1	+0.03 ± 0.09
SIgA SR ($\mu g \cdot min^{-1}$)	Baseline	27 ± 17	18 ± 11	26 ± 19	25 ± 17
	ΔBaseline to week 5	-2 ± 22	+12 ± 16	+1 ± 18	+1 ± 20
	ΔBaseline to week 12 † ‡	+9 ± 16	+25 ± 31	+10 ± 22	+14 ± 24
Cathelicidin concentration ($\mu g \cdot L^{-1}$)	Baseline	14 ± 11	14 ± 14	13 ± 13	12 ± 11
	ΔBaseline to week 5	-8 ± 16	+6 ± 18	-2 ± 10	-1 ± 15
	ΔBaseline to week 12	-5 ± 14	+1 ± 19	-4 ± 16	-1 ± 17
Cathelicidin SR ($ng \cdot min^{-1}$)	Baseline	3.25 ± 3.04	1.69 ± 1.91	2.42 ± 2.28	3.13 ± 4.79
	ΔBaseline to week 5	-0.82 ± 3.82	+0.96 ± 1.81	-0.54 ± 1.78	-1.35 ± 4.25
	ΔBaseline to week 12	-0.70 ± 4.10	+2.15 ± 3.61	+0.14 ± 2.45	-0.64 ± 5.60

Main effect of time vs baseline, † $P < 0.05$. Main effect of time vs week 5, ‡ $P < 0.05$. Data are mean ± SD.