

1 **Influence of Vitamin D Supplementation by Simulated Sunlight or Oral D<sub>3</sub> on**  
2 **Respiratory Infection during Military Training**

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12 **ABSTRACT**

13 **Purpose:** To determine the relationship between vitamin D status and upper respiratory tract  
14 infection (URTI) of physically active men and women across seasons (study 1). Then, to  
15 investigate the effects on URTI and mucosal immunity of achieving vitamin D sufficiency  
16 ( $25(\text{OH})\text{D} \geq 50 \text{ nmol} \cdot \text{L}^{-1}$ ) by a unique comparison of safe, simulated-sunlight or oral  $\text{D}_3$   
17 supplementation in winter (study 2). **Methods:** In study 1, 1,644 military recruits were  
18 observed across basic military training. In study 2, a randomized controlled trial, 250 men  
19 undertaking military training received either placebo, simulated-sunlight (1.3x standard  
20 erythemal dose, three-times-per-week for 4-weeks and then once-per-week for 8-weeks) or  
21 oral vitamin  $\text{D}_3$  ( $1,000 \text{ IU} \cdot \text{day}^{-1}$  for 4-weeks and then  $400 \text{ IU} \cdot \text{day}^{-1}$  for 8-weeks). URTI was  
22 diagnosed by physician (study 1) and Jackson common cold questionnaire (study 2). Serum  
23  $25(\text{OH})\text{D}$ , salivary secretory immunoglobulin A (SIgA) and cathelicidin were assessed by  
24 LC-MS/MS and ELISA. **Results:** In study 1, only 21% of recruits were vitamin D sufficient  
25 during winter. Vitamin D sufficient recruits were 40% less likely to suffer URTI than recruits  
26 with  $25(\text{OH})\text{D} < 50 \text{ nmol} \cdot \text{L}^{-1}$  (OR (95% CI) = 0.6 (0.4–0.9)); an association that remained  
27 after accounting for sex and smoking. Each URTI caused on average 3 missed training days.  
28 In study 2, vitamin D supplementation strategies were similarly effective to achieve vitamin  
29 D sufficiency in almost all ( $\geq 95\%$ ). Compared to placebo, vitamin D supplementation  
30 reduced the severity of peak URTI symptoms by 15% and days with URTI by 36% ( $P <$   
31  $0.05$ ). These reductions were similar with both vitamin D strategies ( $P > 0.05$ ).  
32 Supplementation did not affect salivary SIgA or cathelicidin. **Conclusion:** Vitamin D  
33 sufficiency reduced the URTI burden during military training.  
34 **Keywords:** cholecalciferol, 25-hydroxyvitamin D, exercise, UVB, immunity, virus.

## 35 **INTRODUCTION**

36 Athletes and military personnel experience arduous training and nutritional  
37 inadequacy that may compromise host defense and increase their susceptibility to respiratory  
38 illness such as the common cold, particularly during the autumn-winter (1, 2). The  
39 immunomodulatory effects of vitamin D are considered to play a role in the seasonal stimulus  
40 for upper respiratory tract infection (URTI) (3, 4). This has fuelled considerable interest in  
41 potential prophylactic benefits of vitamin D supplementation on URTI. Vitamin D can be  
42 obtained from diet but is primarily synthesized by skin exposure to sunlight ultraviolet B  
43 (UVB) radiation. As dietary vitamin D intakes in the US and Europe (112–330 IU·day<sup>-1</sup>, (5-  
44 7)) are typically less than recommended (600 IU·day<sup>-1</sup>, (7, 8)) people who live at latitudes  
45 >35° or live indoors for the majority of sunlight hours and cover-up from the sun are at  
46 higher risk of vitamin D insufficiency. Indeed, epidemiological studies report vitamin D  
47 sufficiency (serum 25-hydroxyvitamin D (25(OH)D) ≥50 nmol·L<sup>-1</sup>) in only 40–65% of  
48 athletes and military personnel during the winter, when skin exposure to UVB radiation is  
49 negligible (9-11).

50 Vitamin D is widely accepted to influence both innate and adaptive immunity with  
51 implications for host defense (12, 13). 25(OH)D is converted in the kidney to the biologically  
52 active form 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D), which enhances the innate immune  
53 response by the induction of antimicrobial proteins like cathelicidin (13). Antimicrobial  
54 proteins help to prevent URTI as part of the first line of defense. The actions of vitamin D on  
55 adaptive immunity may also be anti-inflammatory or ‘tolerogenic’ (3). Immune tolerance has  
56 been described as the ability to dampen defense yet control infection at a non-damaging level  
57 (14); prompting the search for tolerogenic nutritional supplements to reduce URTI burden  
58 (3). URTI burden can be assessed by URTI prevalence, or the duration or severity of URTI.  
59 As such, maintaining or achieving vitamin D sufficiency may reduce URTI burden by

60 preventing URTI symptoms but also by reducing the duration and/or severity of URTI (3, 9,  
61 11)).

62 Large cross-sectional and randomized, placebo-controlled supplementation studies in  
63 the general population highlight that vitamin D reduces the burden of URTI (4, 15, 16).

64 However, cross-sectional studies in young healthy and athletic populations present  
65 conflicting findings (17-19), which might be explained by small samples with few URTI, a  
66 limited range of vitamin D concentrations due to single-season data collections, and a lack of  
67 control for factors known to independently influence URTI (e.g. sex and smoking).

68 Randomized, controlled trials investigating the effect of vitamin D supplementation on URTI  
69 and immunity in military recruits and athletes are extremely limited and present a mixed  
70 picture (20-23). These studies show reduced URTI symptoms (22), improved mucosal  
71 immunity (i.e. salivary cathelicidin and immunoglobulin A (IgA)) (21, 23) and fewer missed  
72 training days due to URTI (20), as well as, no effect on URTI symptoms (20) or mucosal  
73 immunity (22, 23). The significant heterogeneity reported in these trials may stem from  
74 variations in participant baseline vitamin D status and dosing regimens; these factors are  
75 considered to modify the effect of vitamin D on immunity to respiratory pathogens (15). The  
76 participants in these studies were vitamin D sufficient at baseline (20, 21), which likely  
77 limited the need and potential benefit of vitamin D supplementation (11). Also participants  
78 were administered higher oral vitamin D doses than recommended by the Institute of  
79 Medicine (IOM) and European Food Safety Authority (EFSA) (21, 22) increasing the risk of  
80 adverse outcomes (tolerable upper intake  $4000 \text{ IU} \cdot \text{day}^{-1}$ ) (7, 8). Although vitamin D is  
81 derived from skin exposure to sunlight the effect of safe skin sunlight exposure on URTI  
82 burden and mucosal immunity has yet to be studied. Ultraviolet (UV) radiation has a range of  
83 vitamin D-dependent and -independent effects on immunity (24); however, whether there are  
84 additional benefits of safe sunlight exposure, compared to oral vitamin D supplementation, is

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

88 First the relationship between vitamin D status and URTI prevalence was determined  
89 in a large, prospective cohort study of young men and women commencing military training  
90 across all seasons (study 1). It was hypothesized that vitamin D sufficient recruits would be  
91 less likely to suffer URTI, compared to those who had serum 25(OH)D <50 nmol·L<sup>-1</sup>. Then,  
92 in a randomized, placebo-controlled trial (study 2), the effects on overall URTI burden  
93 (prevalence, duration and severity) and mucosal immunity of achieving vitamin D sufficiency  
94 by either simulated sunlight, following recommendations on safe, low-level sunlight exposure  
95 (25), or oral D<sub>3</sub> supplementation, in wintertime was investigated. Vitamin D sufficiency was  
96 targeted because maintaining serum 25(OH)D concentration ≥50 nmol·L<sup>-1</sup> has been  
97 recommended for health by the IOM and EFSA and is achievable using safe doses of oral  
98 vitamin D<sub>3</sub> and simulated sunlight (7, 8). It was hypothesized that achieving vitamin D  
99 sufficiency during winter by vitamin D supplementation would reduce URTI burden, and  
100 improve mucosal immunity, compared to placebo supplementation.

101

## 102 **METHODS**

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

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

## 116 **Study one**

117 **Participants and study design.** 1,644 men and women (n = 1,220 men: 95% white ethnicity,  
118 age  $21 \pm 3$  years; body mass  $75.3 \pm 9.9$  kg, height  $1.77 \pm 0.06$  m, body mass index (BMI)  
119  $24.0 \pm 2.7$  kg·m<sup>-2</sup>, 38% smokers; n = 424 women: 95% white ethnicity, age  $22 \pm 3$  years,  
120 body mass  $64.8 \pm 8.2$  kg, height  $1.65 \pm 0.06$  m, BMI  $23.7 \pm 2.4$  kg·m<sup>-2</sup>, 24% smokers)  
121 participated in this prospective cohort study between January 2014 and September 2015.  
122 Participants were included if they gave baseline blood samples and URTI data was available  
123 during the entire 12 weeks of military training.

124 **Experimental procedures.** Baseline measures were collected from each participant during  
125 the initial medical assessment; including a venous blood sample for determination of serum  
126 25(OH)D; height and body mass; ethnicity and smoking history by self-reported  
127 questionnaire (Figure 1). Medical records were accessed to obtain physician-diagnosed URTI  
128 and lost training days due to URTI. The URTI were diagnosed by a single general practice-  
129 trained physician. A lost training day was recorded when a recruit was unavailable for normal  
130 military training.

## 131 **Study two**

132 **Participants and study design.** 250 men (age  $22 \pm 7$  years, body mass  $76.3 \pm 10.8$  kg, height  
133  $1.77 \pm 0.06$  m, BMI  $24.2 \pm 3.0$  kg·m<sup>-2</sup>) participated in this double-blind, randomized,  
134 placebo-controlled trial (Figure 1). Participants were recruited at the start of 12 weeks of  
135 Basic Military Training during January and February of 2016 and 2017; when ambient UVB  
136 is negligible at UK latitudes (50–60°N), and serum 25(OH)D is at its annual nadir.  
137 Participants were eligible to participate if they had sun-reactive skin type of I to IV on the  
138 Fitzpatrick Skin Type Scale (26), were not consuming supplements containing vitamin D, and  
139 had not used a sunbed or traveled to a sunny climate in the 3 months before the study.

140 **Experimental procedures.** Participants were randomized within their platoons to one of four  
141 intervention groups: 1) oral vitamin D<sub>3</sub> supplementation (ORAL); 2) oral placebo  
142 supplementation (ORAL-P); 3) solar simulated radiation (SSR); or, 4) solar simulated  
143 radiation placebo (SSR-P). Block randomization was used ([www.randomiser.org](http://www.randomiser.org)) to achieve  
144 an equal distribution of intervention groups within each platoon so any differences in training  
145 conditions between platoons did not influence the outcomes of the study. The intervention  
146 strategy for the SSR and ORAL groups was to restore and then maintain IOM and EFSA  
147 recommended vitamin D sufficiency (serum 25(OH)D  $\geq 50$  nmol·L<sup>-1</sup>). Participants completed  
148 a 4-week restoration phase, necessary because serum 25(OH)D was at its annual wintertime  
149 nadir, followed by an 8-week maintenance phase.

150 At baseline, during the routine initial medical assessment, height and body mass were  
151 measured, a venous blood sample was collected for the determination of serum 25(OH)D,  
152 and a lifestyle questionnaire was completed to determine smoking and alcohol use.

153 Additional blood samples were obtained at week 5, and week 12. At baseline, week 5, and  
154 week 12 saliva samples were collected in the evening, between 18:00 and 21:30 h, at least 15  
155 minutes postprandial. Participants were excluded from analysis if they did not achieve  $\geq 80\%$

156 compliance with the intervention. Compliance with the interventions was calculated from  
157 researcher weekly counts of oral capsules remaining in recruit pill boxes and SSR cabinet  
158 visit records. Vitamin D from the diet was estimated in week 12 using a food frequency  
159 questionnaire, and solar UVR exposure was measured in weeks 4 and 11 using polysulphone  
160 badges, worn on the upper chest/anterior shoulder region on the outer clothes, as described  
161 (10, 27). The change in absorbance of the badges due to exposure was measured using a  
162 spectrophotometer and related to the erythema effective UVR (sunburning) through a  
163 standard polynomial relationship; data are expressed as standard erythema dose per day (27).  
164 Participant dietary vitamin D intake was calculated excluding the oral D<sub>3</sub> supplement  
165 participants received in the ORAL group. On completion of the study, to confirm participant  
166 blinding, participants were asked to guess the intervention they had received.

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

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



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

191 **Oral vitamin D<sub>3</sub>.** Participants receiving the ORAL intervention consumed a vitamin D<sub>3</sub>  
192 capsule daily, containing 1,000 IU and 400 IU during the restoration and maintenance phases,  
193 respectively (Pure Encapsulations, Sudbury, Massachusetts, USA). The restoration dose was  
194 based on previous predictive modeling to achieve serum 25(OH)D  $\geq 50$  nmol·L<sup>-1</sup> (29), and  
195 pilot investigations that showed it achieved similar serum 25(OH)D concentrations to SSR;  
196 and was less than the tolerable upper intake recommended by the IOM and EFSA (7, 8). The  
197 ORAL maintenance dose was shown in a pilot investigation to maintain serum 25(OH)D  $\geq 50$   
198 nmol·L<sup>-1</sup> and when accounting for typical habitual dietary intake (5-7) was similar to IOM  
199 and EFSA recommended dietary allowances (7, 8). For 12 weeks, ORAL-P participants  
200 consumed an identical-looking cellulose placebo capsule daily (Almac Group, County  
201 Armagh, UK). Independent analysis found the vitamin D<sub>3</sub> content of the 1,000 and 400 IU  
202 capsules to be 1,090 and 460 IU, respectively, and confirmed the placebo did not contain  
203 vitamin D (NSF International Laboratories, Ann Arbor, Michigan, USA).

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

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

228 East Anglia, Norwich, UK). The mean intra-assay coefficient of variation (CV) for 25(OH)D<sub>3</sub>  
229 and 25(OH)D<sub>2</sub> were <10% and the lower limit of quantification was 0.1 nmol·L<sup>-1</sup> (32).

230 **Saliva collection and analysis (study 2).** Saliva was collected for 5 min in a pre-weighed 30  
231 mL tube using the passive dribble method (33). Samples were weighed immediately after  
232 collection, centrifuged at 1500 g and 4°C for 10 minutes, aliquoted, and then stored at -80°C.  
233 Samples were analyzed in duplicate by enzyme-linked immunosorbent assay for secretory  
234 IgA (SIgA) and cathelicidin concentration (Salimetrics, Pennsylvania, USA, and Hycult  
235 Biotech, Pennsylvania, USA). The mean intra-assay CV was 2.3% for saliva SIgA  
236 concentrations ranging from 0.02 to 0.51 mg·mL<sup>-1</sup> and 10.2% for saliva cathelicidin  
237 concentrations ranging from 0.30 to 65.90 µg·L<sup>-1</sup>. Assuming the density to be 1.00 g·mL<sup>-1</sup> for  
238 saliva, the secretion rate was calculated by multiplying the saliva flow rate by concentration  
239 (33).

240 **Statistical analysis.** Statistical analyses were performed using SPSS Version 25 (IBM Corp,  
241 NY, US). Data points that were more than three times the interquartile range were deemed as  
242 outliers and removed. Where data were not normally distributed they were transformed using  
243 square-root calculation. Significance was set at  $P < 0.05$ . For study 1, an estimated minimum  
244 required sample size of 1,286 was calculated, using a type 1 error (one-tailed) of 5%, a power  
245 of 80%, and an anticipated odds ratio of 1.5 (equivalent to a small effect size), and including  
246 a binomial variable at 20%. This was based on previous literature describing the difference in  
247 URTI prevalence between individuals with low and high vitamin D status whereby, 20% of  
248 individuals with high vitamin D status reported a URTI (4), whilst also anticipating that 20%  
249 of individuals would have low vitamin D status across the whole year (34). Logistic  
250 regression were used to compare vitamin D status (25(OH)D  $\geq 50$  vs  $< 50$  nmol·L<sup>-1</sup> and  $\geq 75$  vs  
251  $< 30$ ,  $\geq 50$ — $< 75$  and  $< 75$  nmol·L<sup>-1</sup>) with URTI prevalence during twelve-weeks military

252 training, and the first three weeks of military training; circulating 25(OH)D has an estimated  
253 three-week half-life (35, 36). Sex and smoking were included as covariates as they have  
254 previously been shown to influence URTI susceptibility (37, 38). Chi-square tests were used  
255 to compare URTI prevalence between vitamin D sufficient participants and those with serum  
256 25(OH)D <50 nmol·L<sup>-1</sup>, and the proportion of vitamin D sufficient participants between  
257 seasons. We used one-way ANOVA to compare 25(OH)D between seasons. For study 2, an  
258 estimated minimum required sample size of 74 (37 in each comparison group) was  
259 calculated, using the anticipated odds ratio of 0.3 for URTI prevalence between vitamin D  
260 and placebo supplemented individuals with low vitamin D status (15), and that 60% would  
261 self-report URTI during basic military training (18, 31, 39), with a type 1 error (one-tailed) of  
262 5%, and a power of 80%. URTI prevalence between vitamin D (SSR and ORAL) and placebo  
263 (SSR-P and ORAL-P) supplementation groups was compared by logistic regression.  
264 Independent samples *t*-tests (2 groups (SSR and ORAL combined, SSR-P and ORAL-P  
265 combined)) were used to compare vitamin D and placebo supplementation effects on average  
266 URTI duration, total days with URTI, peak URTI severity, saliva flow rate, SIgA, and  
267 cathelicidin. Serum 25(OH)D, total days with URTI, URTI duration, URTI severity, saliva  
268 flow rate, SIgA, and cathelicidin, were compared between vitamin D strategies, and placebo  
269 groups, by mixed-model ANOVA ((4 groups (SSR, ORAL, SSR-P, and ORAL-P) × 3-time  
270 points (baseline, week 5 and 12)). Sunlight exposure and dietary vitamin D intake between  
271 SSR, ORAL, SSR-P, and ORAL-P groups were compared by one-way ANOVA. Cohen's *d*  
272 effect sizes (*d*) are presented to indicate the meaningfulness of group differences for total  
273 days with URTI, URTI duration, and URTI severity; whereby, values greater than 0.2, 0.5,  
274 and 0.8 represent small, medium and large effects, respectively (40).

275

276 **RESULTS**

277 **Study one**

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

279 Baseline serum 25(OH)D concentration was lower in winter than all other seasons ( $P < 0.01$ ,  
280 Figure 2A); when only 21% of participants were vitamin D sufficient (baseline serum  
281 25(OH)D  $\geq 50$  nmol·L<sup>-1</sup>; Figure 2B).

282 **Vitamin D sufficiency associated with reduced URTI prevalence**

283 A total of 110 URTI episodes were recorded with 7% of participants having at least one  
284 physician-diagnosed URTI. On average, each URTI resulted in  $3.4 \pm 3.3$  lost training days  
285 (4% of total training days). Vitamin D sufficient participants at baseline were 40% less likely  
286 to have a physician-diagnosed URTI, during 12 weeks of training, than participants with  
287 baseline serum 25(OH)D  $< 50$  nmol·L<sup>-1</sup> (6% vs 9%, respectively, OR (95% CI) = 0.6 (0.4–  
288 0.9),  $P < 0.05$ , Figure 2C). Vitamin D sufficient participants at baseline were half as likely to  
289 have a URTI within the first three weeks of training than participants with a baseline serum  
290 25(OH)D  $< 50$  nmol·L<sup>-1</sup> (2% vs 5%, OR (95% CI) = 0.5 (0.3–0.8),  $P < 0.05$ ); approximately  
291 half of all URTI episodes occurred during this period of training (47%, 52 URTI episodes).  
292 The association between vitamin D status and URTI prevalence remained when controlling  
293 for sex and smoking ( $P < 0.05$ ). URTI prevalence was not different between participants with  
294 a baseline serum 25(OH)D  $\geq 75$  nmol·L<sup>-1</sup> and baseline serum 25(OH)D of  $< 30$ ,  $\geq 50$ – $< 75$ , or  
295  $< 75$  nmol·L<sup>-1</sup> ( $P > 0.05$ ).

296

297 **Study two**

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

### 306 **Winter simulated sunlight and oral vitamin D<sub>3</sub> increased vitamin D sufficiency**

307 At baseline, before wintertime vitamin D supplementation began, only one-quarter (27%) of  
308 participants were vitamin D sufficient. Both SSR and ORAL supplementation strategies were  
309 successful in achieving vitamin D sufficiency in almost all by week 5 ( $\geq 95\%$ ). Week 5 and  
310 12 serum 25(OH)D concentrations in the SSR and ORAL groups were higher than in the  
311 respective placebo groups ( $P < 0.001$ , Figure 4).

### 312 **Winter vitamin D supplementation reduced URTI burden**

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

322 with serum 25(OH)D  $<50 \text{ nmol}\cdot\text{L}^{-1}$  had 33% shorter average URTI duration ( $P = 0.05$ ; Figure  
323 5D), 21% lower peak URTI severity ( $P < 0.05$ ; Figure 5E) and 43% fewer total days with  
324 URTI ( $P < 0.05$ ; Figure 5F), when receiving vitamin D rather than placebo supplementation.  
325 There was no difference in URTI prevalence, duration, severity or total days with URTI  
326 between vitamin D supplementation strategies, or between the different placebo groups ( $P >$   
327  $0.05$ ). Specifically, the ORAL and SSR vitamin D supplementation strategies effect on URTI  
328 burden was similar (ORAL vs SSR, URTI prevalence 70% vs 72%, total days with URTI  $9.2$   
329  $\pm 8.4$  vs  $8.4 \pm 6.7$  days, URTI average duration  $6.9 \pm 5.0$  vs  $6.5 \pm 5.7$  days, peak URTI  
330 severity  $10.8 \pm 3.0$  vs  $12.3 \pm 3.8$  AU, all  $P > 0.05$ ). A physician-diagnosed URTI was  
331 recorded for 8% of recruits, which was comparable to 8% prevalence in the same seasonal  
332 period in study 1, and resulted in  $3.3 \pm 1.3$  training days lost.

### 333 **Vitamin D supplementation and mucosal immunity**

334 Vitamin D supplementation and placebo groups did not differ at baseline, and weeks 5 and  
335 12, for saliva flow rate, SIgA concentration, SIgA secretion rate, cathelicidin concentration,  
336 and cathelicidin secretion rate ( $P > 0.05$ ; Table 2).

337

## 338 **DISCUSSION**

339 The primary finding of these two studies was that vitamin D sufficiency reduced the  
340 burden of URTI in healthy young adults completing arduous military training. In study 1,  
341 vitamin D sufficient men and women were 40% less likely to suffer a physician-diagnosed  
342 URTI during training than those with serum 25(OH)D  $<50 \text{ nmol}\cdot\text{L}^{-1}$  (Figure 2). Given this  
343 finding, and that only 21% of participants were vitamin D sufficient during winter, study 2  
344 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  $<50 \text{ nmol}\cdot\text{L}^{-1}$  (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<sub>3</sub> was  
364 similarly effective to achieve IOM and EFSA recommended vitamin D sufficiency in the  
365 majority of individuals ( $\geq 95\%$ , 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



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

374         The different methods used to assess URTI in the studies may explain the difference  
375 between study 1 and 2 prevalence findings. The lower URTI prevalence in study 1 than study  
376 2 (7% vs 69%) indicates that physician diagnosis of URTI compared to daily self-report  
377 likely missed more minor illnesses that did not warrant a medical visit. Further, study 2  
378 physician-diagnosed URTI prevalence was 8%, which was the same as study 1, when  
379 controlling for season. Self-reported URTI data was not reported back to the military and  
380 therefore did not influence physician diagnosis of URTI or lost training days due to URTI.  
381 When considered carefully in the context of these different methods, the findings of studies 1  
382 and 2 are complementary. In study 2, lower peak URTI severity and fewer days with URTI  
383 with vitamin D supplementation, compared to placebo, would be expected to translate to  
384 vitamin D sufficient individuals reporting less to medical services, and consequently having  
385 fewer physician-diagnosed URTI than those individuals with 25(OH)D <50 nmol·L<sup>-1</sup>. This is  
386 entirely consistent with the main finding of study 1: URTI prevalence was lower in vitamin D  
387 sufficient individuals than those with 25(OH)D <50 nmol·L<sup>-1</sup> (Figure 2).

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

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

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

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

432           No additional benefit of SSR compared to oral vitamin D<sub>3</sub> supplementation was  
433 shown on URTI, immune function (this study and (45)), or exercise performance (10).  
434 Consequently, active people are advised to take the 400 IU·day<sup>-1</sup> oral vitamin D<sub>3</sub> dose, from  
435 the maintenance phase of study 2, to maintain vitamin D sufficiency when exposure to  
436 ambient UVB is inadequate: between early autumn and late winter, and for those that live  
437 and/or exercise indoors for the majority of sunlight hours or cover-up from the sun. When  
438 accounting for typical dietary vitamin D intake, this oral vitamin D<sub>3</sub> supplementation  
439 approach corresponds with current IOM and EFSA recommendations (600 IU·day<sup>-1</sup>) for bone  
440 and general health and, unlike simulated sunlight, there is no time burden for an individual;  
441 no requirement for bulky irradiation cabinets; and oral vitamin D<sub>3</sub> supplementation is

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

## 445 **CONCLUSIONS**

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

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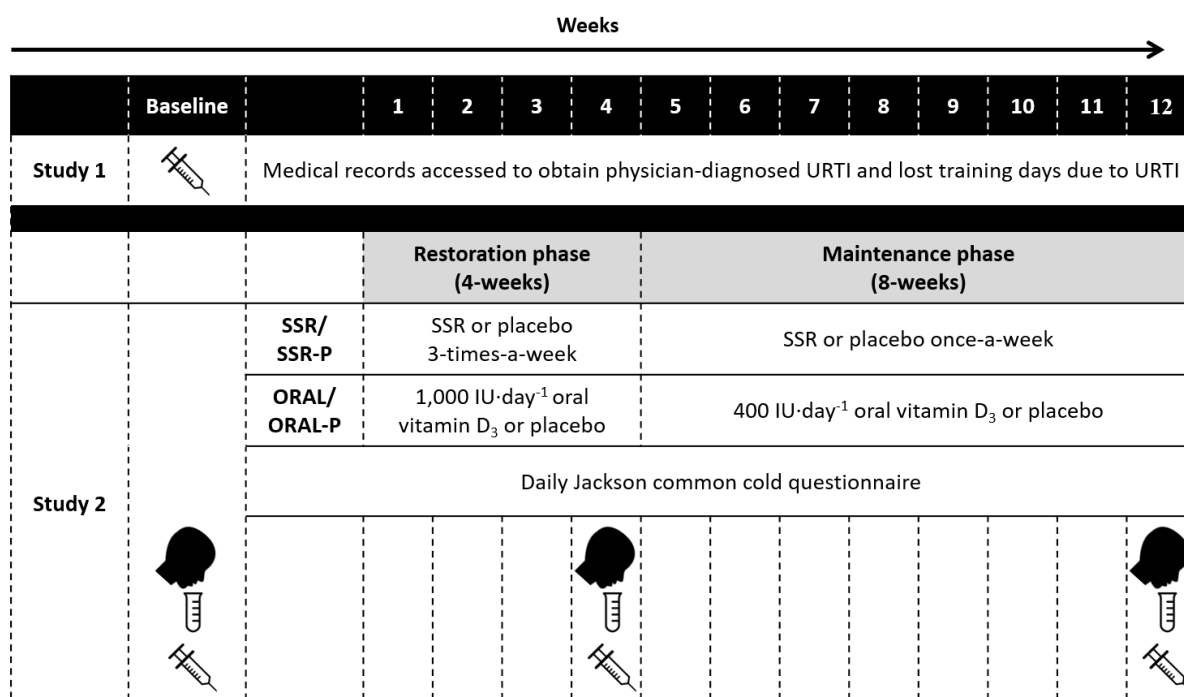
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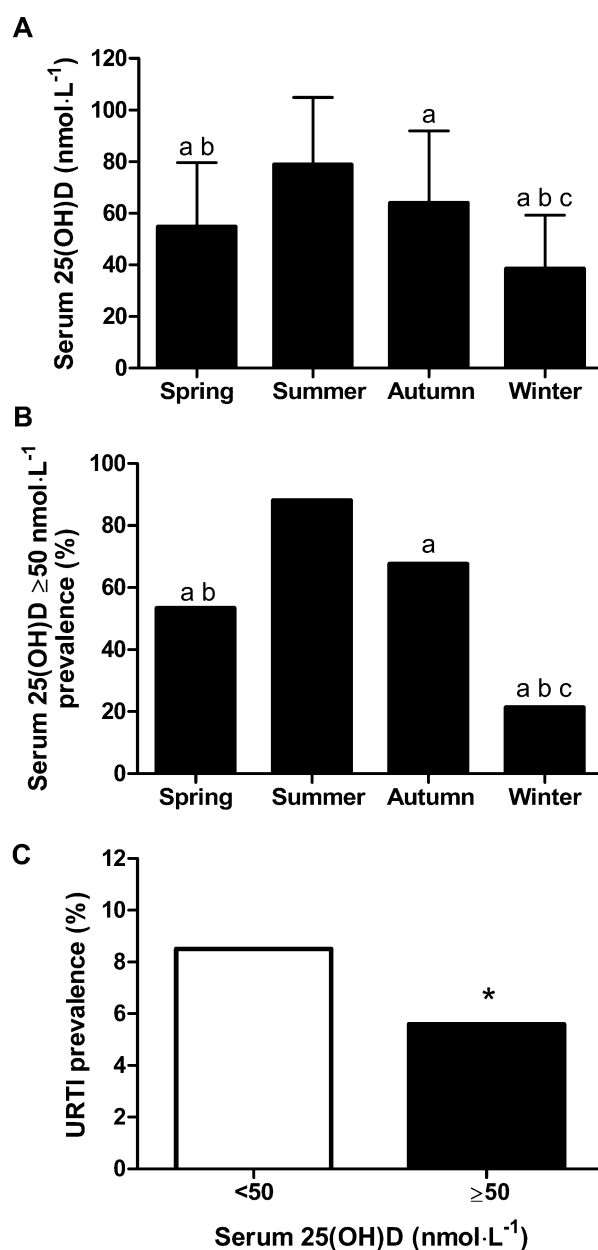
581 **FIGURE 1.** A schematic of the prospective cohort study (study 1) that investigated the  
 582 association between vitamin D status (serum 25(OH)D), upper respiratory tract infection  
 583 (URTI) and days lost from training, and the randomized controlled trial (study 2) that  
 584 investigated the effects of vitamin D supplementation by solar simulated radiation (SSR), oral  
 585 vitamin D<sub>3</sub> (ORAL), or placebo (SSR-P or ORAL-P) on URTI and mucosal immunity. Blood  
 586 samples were collected at baseline (study 1 and 2), week 5, and the end of week 12 (study 2).  
 587 Saliva samples were collected at baseline, week 5 and the end of week 12 (study 2). The  
 588 syringe icon represents the blood sample; the head and tube icon represent the saliva sample.



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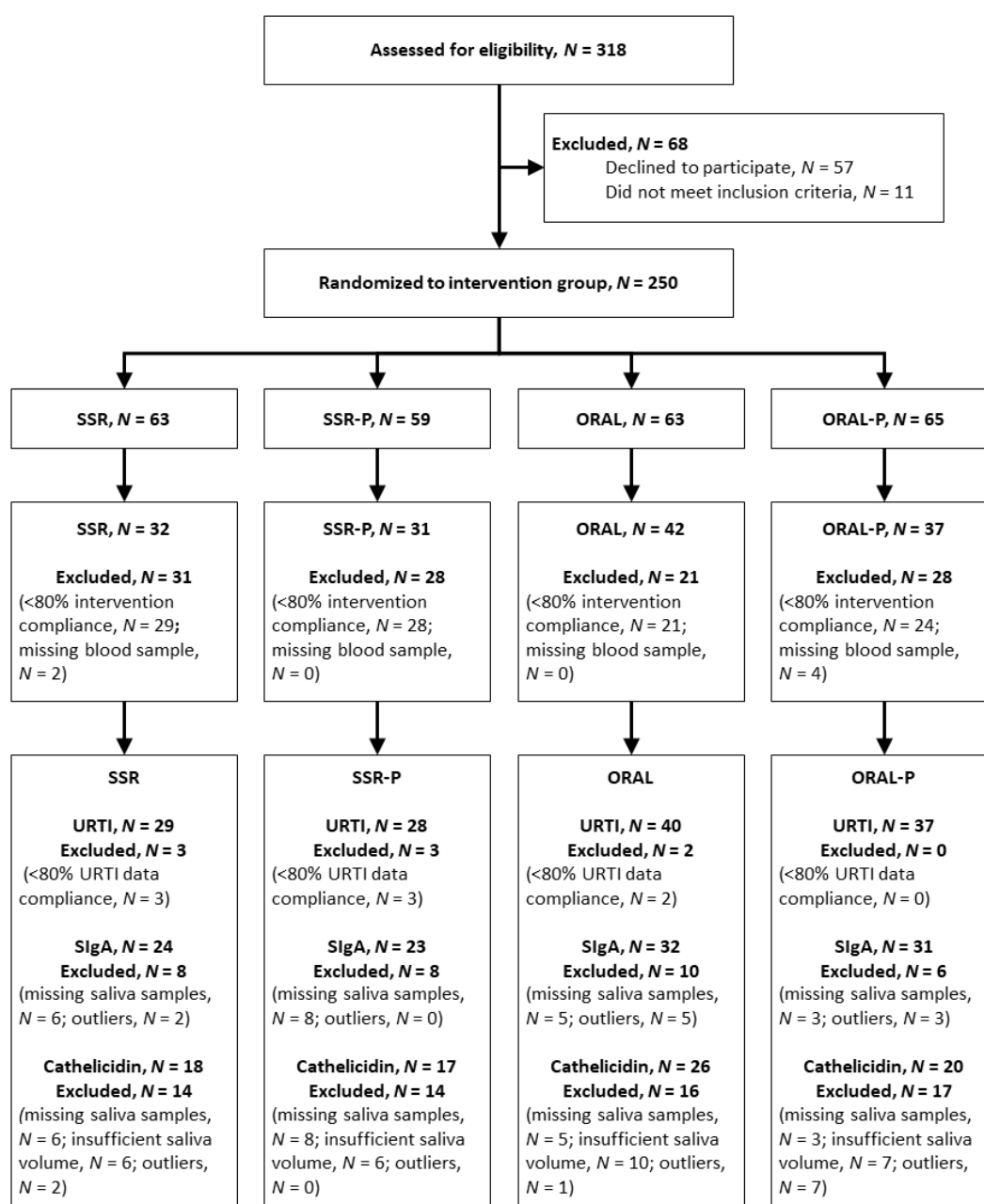
590

591 **FIGURE 2.** Seasonal variation in serum 25(OH)D (panel A), vitamin D sufficiency  
592 prevalence (serum 25(OH)D  $\geq 50$  nmol·L<sup>-1</sup>; panel B), and the URTI prevalence when serum  
593 25(OH)D  $\geq 50$  nmol·L<sup>-1</sup> or  $< 50$  nmol·L<sup>-1</sup> (panel C) in 1,644 men and women during 12-weeks  
594 of military training. a, lower than summer,  $P < 0.05$ . b, lower than autumn,  $P < 0.05$ . c, lower  
595 than spring,  $P < 0.05$ . \*, lower than participants with serum 25(OH)D  $< 50$  nmol·L<sup>-1</sup>,  $P <$   
596 0.05. Panel A data are mean  $\pm$  SD. Panels B and C are percentages represented by vertical  
597 bars.

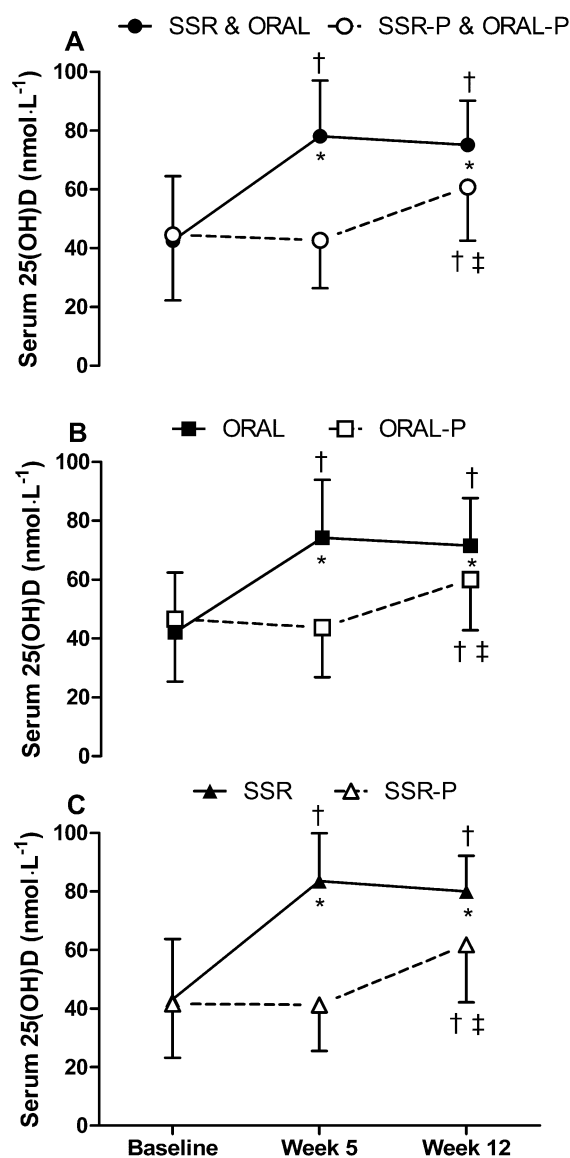


598

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

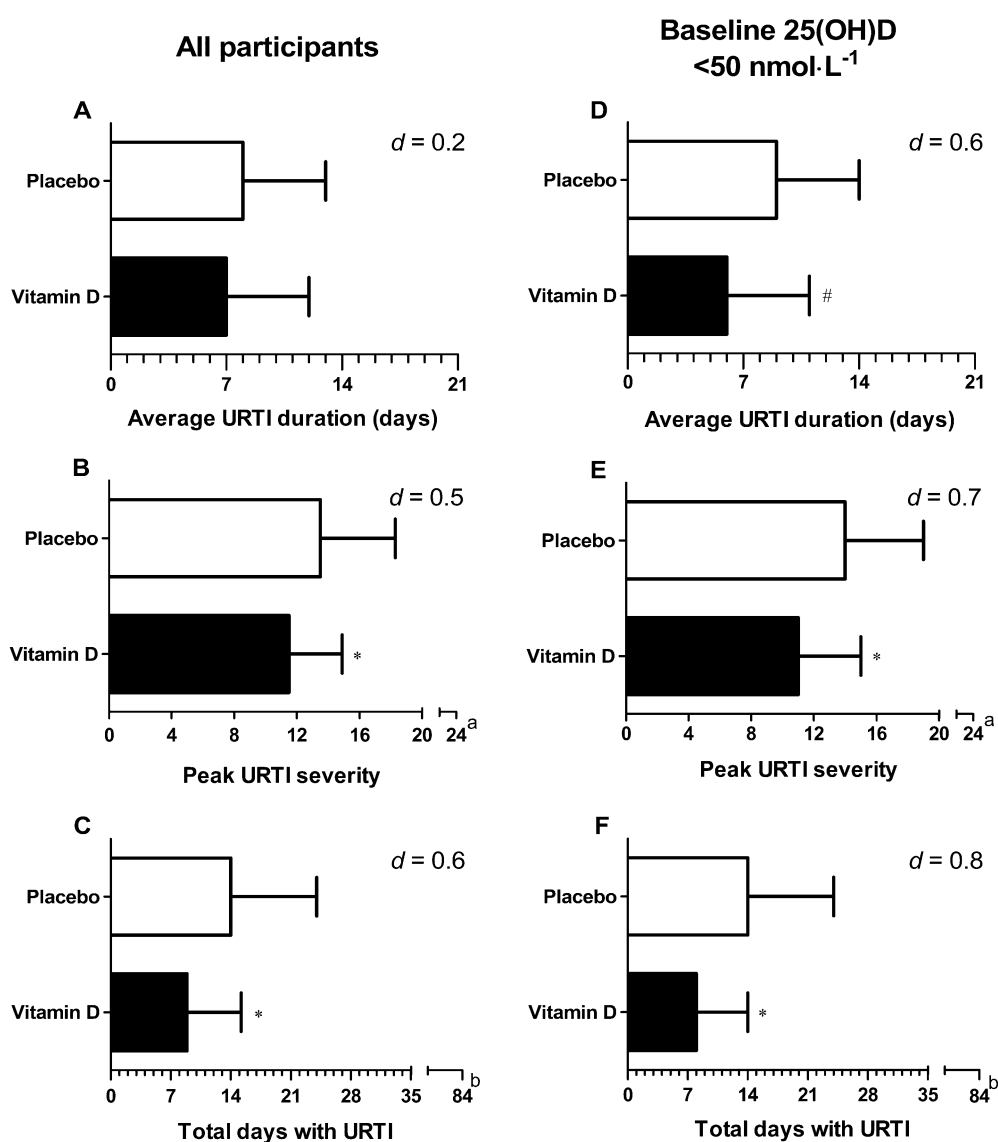


606 **FIGURE 4.** Serum 25(OH)D in men completing military training whilst receiving 12-weeks  
607 of vitamin D supplementation (solar simulated radiation (SSR) or oral vitamin D<sub>3</sub> (ORAL))  
608 or a placebo (solar simulated radiation placebo (SSR-P) or oral placebo (ORAL-P)).  
609 Combined vitamin D interventions (SSR and ORAL) vs combined placebo (SSR-P and  
610 ORAL-P; panel A), ORAL vs ORAL-P (panel B), and SSR vs SSR-P (panel C). \*, greater  
611 than placebo,  $P < 0.05$ . †, greater than baseline,  $P < 0.05$ . ‡, greater than week 5,  $P < 0.05$ .  
612 Data are mean  $\pm$  SD.



613

614 **FIGURE 5.** Upper respiratory tract infection (URTI) average duration (panel A & D), peak  
615 URTI severity (panel B & E), and total days with URTI during military training (panel C &  
616 F), in the vitamin D supplementation (SSR and ORAL) vs placebo supplementation groups  
617 (SSR-P and ORAL-P) in all participants (left-hand column) and participants with a baseline  
618 25(OH)D <50 nmol·L<sup>-1</sup> (N = 62; right-hand column). \* and #, lower than placebo, P < 0.05  
619 and P = 0.05, respectively. Data are mean ± SD. d = Cohen's d effect size. <sup>a</sup> maximum  
620 possible peak severity (24 arbitrary units (AU)), <sup>b</sup> total number of days for military training  
621 (84 days).



622

623

624 **TABLE 1.** Study 2 baseline participant demographics, anthropometrics, and lifestyle  
 625 behaviors in solar simulated radiation (SSR), SSR placebo (SSR-P), oral vitamin D<sub>3</sub> (ORAL),  
 626 and oral placebo (ORAL-P) supplemented groups.

627 Data are presented as mean  $\pm$  SD unless otherwise stated. There were no differences in demographics,

	SSR (N = 63)	SSR-P (N = 59)	ORAL (N = 63)	ORAL-P (N = 65)
<i>Demographics</i>				
Age (years)	21 $\pm$ 3	22 $\pm$ 3	21 $\pm$ 3	23 $\pm$ 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)
<i>Anthropometrics</i>				
Height (m)	1.78 $\pm$ 0.06	1.78 $\pm$ 0.06	1.77 $\pm$ 0.07	1.78 $\pm$ 0.06
Body mass (kg)	76 $\pm$ 11	77 $\pm$ 11	75 $\pm$ 11	77 $\pm$ 10
BMI (kg·m <sup>-2</sup> )	24 $\pm$ 3	24 $\pm$ 3	24 $\pm$ 3	24 $\pm$ 3
<i>Lifestyle behaviors</i>				
Alcohol user [n (%)]	51 (82)	47 (80)	55 (87)	51 (78)
Smoker [n (%)]	23 (37)	25 (42)	26 (41)	21 (32)

628 anthropometrics, or lifestyle behaviors between groups ( $P > 0.05$ ).

629

630 **TABLE 2.** Influence of 12-weeks solar simulated radiation (SSR), placebo solar simulated  
 631 radiation (SSR-P), oral vitamin D<sub>3</sub> (ORAL), and oral placebo (ORAL-P) on saliva flow rate  
 632 (FR), SIgA concentration, SIgA secretion rate (SR), cathelicidin concentration and  
 633 cathelicidin SR.

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

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

635  $\pm$  SD.