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Influence of military preventive policy for recruit training on COVID-19 seroconversion: The IMPACT-COVID-19 study

Influence of Military Preventive Policy for ReCruit Training on COVID-19 seroconversion: the IMPACT-COVID-19 study

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Running Head: Vitamin D and COVID-19 in young adults

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Abstract

Introduction

Recruitment and training is vital to maintaining the size, deployability and effectiveness of armed forces, but was threatened early in the COVID-19 pandemic. Reports suggested asymptomatic seroconversion driving SARS-CoV-2 transmission in young adults. Potential association between lower vitamin D status and increased infection risk was also highlighted. We aimed to prospectively determine seroconversion and test the hypothesis that this would vary with vitamin D supplementation in representative populations.

Methods

Two cohorts were recruited from Yorkshire, Northern England. Infantry recruits received daily oral vitamin D (1000 IU for four weeks, followed by 400 IU for the remaining 22 weeks of training) in institutional countermeasures to facilitate ongoing training/co-habitation. Controls were recruited from an un-supplemented University population, subject to social distancing and household restrictions. Venous blood samples (baseline and Week 16) were assayed for vitamin D and anti-SARS-CoV-2 spike glycoprotein antibodies, with additional serology (weeks 4, 9, 12) by dried blood spot. Impact of supplementation was analysed on an intention-to-treat basis in volunteers completing testing at all timepoints and remaining unvaccinated against SARS-CoV-2. Variation in seroconversion with vitamin D change was explored across, and modelled within, each population.

Results

In the military (n=333) and University (n=222) cohorts, seroconversion rates were 44.4% vs 25.7% (P=0.003). At week 16, military recruits showed higher vitamin D (60.5 ± 19.5 mmol.L-1 vs. 53.5 ± 22.4 mmol.L-1, p < 0.001), despite <50% supplementation adherence. A statistically significant (p=0.005) effect of negative change in vitamin D (%) on seroconversion in recruits (OR of 0.991 and 95% CI of 0.984-0.997) was not evidenced in the University cohort.

Conclusion

Among unvaccinated populations, SARS-CoV-2 infection of infantry recruits was not reduced by institutional countermeasures, versus civilians subject to national restrictions. Vitamin D supplementation improved serum levels, but implementation did not have a clinically meaningful impact on seroconversion during military training.

What is already known on this topic

- Laboratory investigations and observational human studies have linked vitamin D deficiency with an increased risk of COVID-19 infection.
- By contrast, randomised controlled trials of vitamin D supplementation for COVID-19 prevention have not shown consistent evidence of benefit.
- Establishing the role of vitamin D deficiency and the impact of supplementation would be of potential value to maintaining military training pipelines in the event of future outbreaks of acute respiratory illnesses.

What this study adds

- Among military recruits unvaccinated against SARS-CoV-2, daily oral vitamin D supplementation maintained serum levels, relative to the observed seasonal decline in un-supplemented young adults
- Change in serum vitamin D showed no clinically meaningful impact on risk of seroconversion.

How this study might affect research, practice and policy.

- The continuation of recruit training during a period of high coronavirus infectivity in the general population is associated with high rates of seroconversion, likely due to the close working environment inherent with military activities.
- Routinely supplementing with vitamin D for prevention of infection by SARS-CoV-2 is not supported by these findings

Introduction

At the outbreak of the COVID 19 pandemic, there was widespread debate about whether vitamin D status influenced susceptibility to infection with SARS-CoV-19. In winter, 39% of the United Kingdom (UK) adult population are vitamin D deficient (VDD), with this being more prevalent at higher latitudes.[2] Viral outbreaks are known to occur preferentially in winter months. Vitamin D3 is primarily produced in the skin following sunlight exposure,

leading to speculation that susceptibility to COVID-19 infection may be related to seasonal variation in vitamin D status.[3] There also appeared to be some overlap between risk factors for being VDD and COVID-19 (obesity, age and ethnicity)[4] with black and ethnic minority groups at increased risk of developing infection and severe manifestations[5,6,7]

Despite the global pandemic, there was an imperative requirement to maintain UK military recruit training. Consequently, a "Military Judgement Panel" (MJP) sat to debate several mitigating measures, including the relative merits of supplementing Service Personnel (SP) with vitamin D given the paucity of data. UK-specific data show that 24% of Royal Marine recruits have levels <25 nmol/L at the start of training[8] and only 21% of infantry recruits are vitamin D sufficient (≥50 nmol/l) during the winter.[9] Balancing the evidence,[10] and in the context of some plausible mechanistic data, the MJP decided in favour of vitamin D supplementation alongside complementary countermeasures aimed at limiting SARS-CoV-2 transmission and severe COVID-19 illness in military training establishments. Data from the Office for National Statistics (ONS) at around the same time (May 28th 2020) showed that only 30% of those testing positive reported symptoms. Asymptomatic infection, especially common in the young, was acknowledged as a major driver of the pandemic and indeed was described as the "Achilles' heel" in control strategies.[11]

The primary aim of this study therefore was to investigate whether seroconversion to SARS-CoV-2 would vary with vitamin D supplementation in young adult. Secondary aims were to assess the background 'point' prevalence and subsequent rate of increase in seropositivity in healthy young adults; and concurrent trends in vitamin D levels over 4 months, including the effect of oral supplementation. These goals were to be realised at a time predating vaccination of the general population against SARS-CoV-2, during periods in which widespread social distancing and graded restrictions on household mixing were practised.

Methods

Ethical approval and clinical trial registration

This study received ethics approval from the [redacted for peer review] and [redacted for peer review]. Clinical Trial Registration number was [redacted for peer review].

Participants

Two cohorts of adults aged 16 to 30 years old were recruited from the Yorkshire region of northern England and studied over 16 weeks. Military recruits, receiving daily oral vitamin D supplementation, were enrolled at the Infantry Training Centre (ITC) Catterick (latitude 54.36669° N from the 18th October 2020 and had final week 16 measures in the week beginning 14th June 2021. A second comparator cohort not taking vitamin D were recruited from a geographically similar latitude in the vicinity of Leeds Beckett University (LBU) (53.8008° N) and consisted primarily of students. Recruitment commenced at LBU on the 7th October 2020 and final week 16 data were collected on the 20th May.

Usual infantry military training continued at ITC with minor modifications, where possible, to promote social distancing throughout the 26 week course. Additional countermeasures applied concurrent with the study including environmental decontamination with povidone-iodine application and disinfectant thermal fogging. The University was open to students from the 1st September 2020 and although the majority of teaching was online some teaching did take place in small groups. Changes in UK Government restrictions over time are displayed in supplementary Box 1 online.

Inclusion criteria included being 16-30 years of age and either enrolled on Phase 1 Army Training at ITC or able to access either of the Leeds Beckett University sites in Leeds. No participant was acutely unwell at the time of sample collection and all self-declared that they had not previously tested positive for SARS-CoV-2 or had knowingly experienced symptoms consistent with COVID-19. Exclusion criteria included being shown to have already seroconverted for SARS-CoV-2 on initial screening; use of over-the-counter (OTC) or prescribed vitamin D supplements, pregnancy, hypercalcaemia at baseline in the ITC cohort and having a condition conferring 'very high risk' or 'high risk' of severe COVID-19 according to UK government guidance at the time.

Vitamin D administration

The initial MJP decision had been made to supplement with vitamin D3 1000 IU per day, this was subsequently revised to 1000 IU per day for four weeks followed by 400 IU for the remaining 12 weeks of the study, as this was both in line with previous data showing an effect on Acute Respiratory Tract Infection (ARI) at these doses in UK SP,[9] consistent with benefit on ARI reported in a previous meta-analysis[12] and a regimen that had previously been shown to achieve vitamin D sufficiency (>50 nmol/L) in >95% SP over 12 weeks.[9,13,14] Supplies of vitamin D at ITC were issued by the camp Quartermaster to the Section Commanders who were then responsible for distribution to individual recruits. Adherence to the regimen was assessed by online-survey responses collected at weeks 4, 9, 12 and 16.

Participant measures

Baseline physical and demographic data included age, gender, ethnicity, body mass and body mass index (BMI), smoking and alcohol history and home post code. Contemporaneous UK

Government restrictions, over the course of the study period, were collected for context alongside an overview of any additional control measures in each cohort location.

Blood sampling and serological testing

To reduce face-to-face contact during the study a validated dried blood spot (DBS) method was employed for serology. DBS sampling and subsequent assay were performed by the Clinical Immunology Service at the University of Birmingham as previously described.[15] Briefly, capillary blood samples were obtained using finger-prick lancets and collected onto forensic-grade 226 DBS cards (Ahlstrom-Munksjo, https://www.ahlstrom-munksjo.com). DBS cards were stored at room temperature in individual sample bags with desiccant. Eluate was collected and subsequently stored at 4°C until use.

DBS sampling was utilised for serological testing at baseline, weeks 4, 9, 12 and 16. DBS technique was taught at baseline. In the LBU cohort subsequent DBS samples were completed by the participant and either posted or delivered to the study site. DBS samples at ITC were done under the supervision of study investigators.

Venous blood samples were taken at baseline and week 16. Serum was separated by centrifugation at 1,600×g for 10 minutes at room temperature and aliquots were stored at -20 ^{o}C until use. These venous samples were used for supplemental venous blood serology and vitamin D assay in both cohorts. In addition, calcium was assayed in the ITC cohort, as a condition of safely observing the effects of vitamin D supplementation without prior knowledge of individual baseline levels.

Serum samples and DBS eluates were tested for anti-SARS-CoV-2 spike glycoprotein antibodies as previously described[13] using a commercially available IgGAM ELISA that measures total antibody responses (MK654, The Binding Site (TBS), Birmingham, UK).

Commercial kits for albumin and calcium were adapted for use on a Cobas Fara centrifugal analyser (Roche Diagnostics Ltd, Welwyn Garden City, UK). Serum total vitamin D3 was measured on the Roche Cobas automated immunoanalyser, using a proprietary serum assay kit (Elecsys® Vitamin D total II, Roche Diagnostics, Indianapolis, Indiana, USA) according to the manufacturer's instructions. The analytical sensitivity was 12.5nmol/L with CV ≤7.0%.

Data interpretation and statistical analysis

Serum vitamin D <25 nmol/l was taken to indicate VDD and, for safety purposes, an upper threshold of 185 nmol/l was considered high. Having a SARS-CoV-2 IgGAM ratio of ≥1.0 indicated seroconversion. Based broadly on previous investigations into ARI, seroconversion of 20% was assumed over the study duration and a reduction to 15% with vitamin D supplementation was targeted. This required a total sample size of 470 to give 80% power with an alpha of 0.05.

All statistical analyses were conducted using a commercial statistical software (SPSS version 27, IBM® SPSS, IBM Corp., New York, USA). The normality distribution was assessed using the Kolmogorov-Smirnov test and through visual inspection of normality plots. Baseline demographic characteristics for the ITC and LBU cohorts were summarised and compared chi-square tests for gender, ethnicity and lifestyle behaviors and Mann-Whitney U test for age, height, weight and body mass index (BMI) (Table 1). Between cohorts comparisons performed with independent-samples t-test or Mann-Whitney U test as appropriate. Within group comparisons were performed using paired samples T-test. The percentage changes in vitamin D levels pre-intervention to post-intervention, were calculated using the following formula:

(post-intervention value – baseline value)/baseline value × 100

Binomial regression models were constructed separately for the ITC and LBU cohorts with factors thought likely to affect seroconversion, based upon those being reported as potentially relevant in the literature at the time, as well as that might unavoidably differ between the groups e.g. alcohol consumption, ethnicity. These included age, anthropometric variables, ethnicity, BMI, smoking status, alcohol consumption and percentage change in vitamin D. Data expressed as mean ± SD or otherwise stated. The level of significance was set at $P < 0.05$.

Results

1176 potential participants (726 at ITC, Catterick, 450 at LBU) were recruited and screened at baseline. At the end of the study 333 of the ITC cohort and 222 of the LBU cohort had completed serological testing at all time points and remained unvaccinated against SARS-CoV-2. These were included in the final analysis. A greater proportion of recruits were lost to follow up primarily due to them exercising the option at week 4 of their military training to DAOR ("Discharge as of Right"). Eighty-seven of the ITC cohort and 94 of the LBU cohort were seropositive at initial screening and excluded. Eighty-four of the ITC cohort and 29 of the LBU cohort were excluded due to being vaccinated before the end of the study.

Baseline demographics of those completing the study are shown in Table 1. The ITC cohort were predominantly male versus a more balanced University group (98.2% vs. 41.4%, $P<0.001$), and slightly younger, with a greater rate of smoking, but lower rate of alcohol consumption. They were also slightly heavier with no difference in BMI, due to them being slightly taller.

The proportion of the total cohort at ITC fully adherent to vitamin D supplementation by the end of the study was 46.4%. The proportion not taking any vitamin D at week 4, 9, 12 and

16 was 22.9%, 22.1%, 24.6%, and 25.7%. Those with reasonable compliance (4-14 tablets missed) at the same time points were 29.1%, 26.2%, 17.8%, and 19.2%.

At baseline there was a significant lower Vitamin D in the ITC vs LBU cohort (54.4 ± 23.9 vs 62.4 ± 27.0 $nmol.L^{-1}$, $p< 0.001$). At week 16 this trend was reversed, with vitamin D higher in the ITC cohort (60.5 ± 19.5 vs 53.5 ± 22.4 $nmol.L^{-1}$). 6.3% of the ITC cohort were vitamin D deficient at baseline but only 0.6% (two participants) by week 16, whereas in the LBU cohort the percentage deficient rose from 5.9% to 7.2%. No individual exceeded the upper vitamin D safety threshold and there was no significant rise in calcium over the 16 weeks in the vitamin D treated cohort (baseline calcium 2.46 ± 0.12, week 16 2.36 ± 0.18).

Rates of seroconversion by interval are shown in Table 2. At the end of the study 44.4% of the ITC Cohort and 25.7% of the LBU cohort had seroconverted (P=0.003). In the ITC cohort there was a significant difference in percentage change in vitamin D over the course of the study and risk of seroconversion. An increase of 13.0 ± 3.4% in vitamin D over the course of the study was associated with seroconversion, whereas the relative change was 28.5 ± 3.5% in those who remained uninfected according to antibody status ($p = 0.003$) (Figure 1). In the LBU cohort there was no difference between percentage change in vitamin D (-11.5 ± 1.9% vs -9.2 ± 4.3%, $p > 0.05$) and seroconversion (Figure 2).

The binomial regression model did not show any effect of vitamin D change in the LBU cohort or indeed if the cohorts were combined. In the ITC cohort while the overall model was not significant, after controlling for the other variables it did show a statistically significant ($p=0.005$) but very minor effect of % reduction in vitamin D on seroconversion (OR of 0.991 and 95% CI of 0.984-0.997), (Table 3).

Discussion

This study, with high seroconversion rates, demonstrated that while vitamin D increased in the ITC recruits, their overall infection rate was higher than that of the University cohort, in which vitamin D levels fell over the course of the study. While we hoped that two similar aged cohorts, living at a similar latitude, studied over a similar period, may allow for a relatively balanced comparison, we were unable to predict how UK Government restrictions would evolve once enrolment and sampling had commenced. Throughout the period of changes in policy, legally-enforced, to which the University cohort were subject, recruits at ITC continued to cohabitate and undergo near-normal training, whereas the LBU cohort, primarily composed of students, largely remained at home with the majority of learning conducted online. As such, it is unsurprising that a greater overall rate of seroconversion occurred among recruits at ITC. In view of this key difference it seems most appropriate to consider the cohorts to be effectively two different populations.

In the ITC cohort, the supplementation regimen was sufficient to produce a mean vitamin D level above the conventional 50 nmol/L considered sufficient. In keeping with previous work in British Army recruits,[14] serum concentrations increased significantly, with levels greater in the ITC than un-supplemented LBU cohort by the study end. Furthermore, despite suboptimal compliance across the study, only two participants at ITC were found to be vitamin D deficient (<25 nmol/l) by its completion and no participant suffered significant hypervitaminosis D or hypercalcaemia. Under supplementation at ITC, those that seroconverted showed a rise in vitamin D that was significantly lower than those who did not seroconvert. Nevertheless, when this is examined in the context of other factors in the

regression model the effect, while statistically significant, is of a size that cannot be considered clinically meaningful.

Subsequent to the start of this study an updated meta-analysis and systematic review[15] of vitamin D supplementation and ARI again showed an overall modest protective effect compared with placebo. Greater benefit was seen in those using a daily dosing regimen, between 400–1000 IU, and in those with the lowest vitamin D status at baseline. While we used a daily dosing regimen in line with this, the fact that both of our cohorts entered the study with vitamin D levels considered sufficient (>50 mmol/L) may have impeded our chances of observing any effect from supplementation.

Compared with systematic reviews and meta-analyses specific to COVID-19 and vitamin D in the context of critical illness, fewer meta-analyses have been done focussing risk of infection without hospitalisation, but one included just over 91,000 participants, and found that vitamin D deficiency was associated with an 80% greater risk of developing COVID-19 than those who were vitamin D sufficient.[16] There remain few prospective studies. Since we commenced our study COVIDENCE UK has reported on over 15,000 participants with baseline information on potential risk factors for COVID-19. These were collected by online questionnaire monthly between May 2020 and Feb 2021. Although infection rate was relatively low (446 cases, 2.9%) consumption of a vitamin D supplement gave a crude OR of 0.8 but a fully adjusted OR did not show any significant benefit.[7]

There have been few published randomised-controlled trials (RCT) looking specifically at vitamin D and COVID-19. A small RCT (n=40) of high dose vitamin D (60,000 IU per day for 7 days) versus placebo in mildly symptomatic/asymptomatic patients showed a greater rate of SRAS-CoV-2 RNA negativity by day 21.[17] The largest RCT (CORONAVIT) regarding vitamin D and COVID-19/ARI was published in 2022[18] and examined the

implementation of an open-label “test-and-treat” approach to correct suboptimal vitamin D status in a UK population (mean vitamin D in those tested 39.7 nmol/L). Across the range of vitamin D (up to 3200 IU per day) supplemented formally, there were no difference in OR between groups for ARI or COVID-19 (2.6 - 3.0 for developing COVID-19, a finding perhaps limited by much lower levels of COVID-19 infection (<3% participants) than the study was powered for (20% ARI rate anticipated at six months).

We acknowledge that while our study was clearly not a blinded RCT it has both its strengths and limitations. In terms of strengths, we uniquely focused on a younger population which were free from comorbidities (that might otherwise influence vitamin D levels and act as confounders). An additional advantage of a younger population was that relatively few had received any vaccine by the end of the study, as opposed to studies in older populations where up to 89% had received at least one vaccination.[18] We were also able to include participants with a wide range of vitamin D at baseline and week 16. Compared to other studies with infection rates of <4%[7,18] we had high infection rates as evidenced by seroconversion in the order of 44% in the ITC cohort and 26% in the LBU cohort.

In terms of limitations, our study, as with most studies in relation to COVID-19, the influence of varying degrees of Government restrictions act as a significant confounder. This meant the two recruited cohorts had to be analysed as individual groups and meant that no comparative analysis of the impact of vitamin D supplementation could be undertaken. While participants with medical conditions judged to confer ‘high’ or ‘very high’ risk of COVID-19 were excluded from recruitment, we could not exclude an impact from lower risk health conditions - which may have been present to a lesser extent in the ITC cohort screened and selected for military service - upon our analyses. Despite these potential confounders, the high seroconversion rate allowed for exploration of vitamin D supplementation and COVID-19 seroconversion in both cohorts separately. While compliance within the ITC cohort issued

vitamin D was not optimal it was in line with medication compliance generally.[19] While a higher vitamin D dose may have been more efficacious, the dose used was in line with previous data in a military population and resulted in sufficiency which can reasonably expected to have been reached prior to the infection surge noted by tracking websites from December 2020.

Conclusions

This prospective cohort study in two young adult populations showed no clinically meaningful effect of vitamin D sufficiency or supplementation on COVID-19 seroconversion. This finding is in line with current evidence that vitamin D supplementation has insufficient evidence to support use in the prevention of COVID-19. Armed forces should not be falsely reassured that the ability to demonstrate continuity of effective training, as with the countermeasures described, will at the same time associate with insignificant risks of infection and onwards transmission of SARS-CoV-2, or similar infectious threats. Future work should target a population with known vitamin D deficiency, using active case-finding with regular serological testing in an RCT setting, as the strategy most likely to be fruitful during a period of high infection rates.

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Table 1. Baseline characteristics of participants who completed the study

	Whole cohort (*n* = 555)	**Recruits (*n* = 333)**	**Civilians (*n* = 222)**	***P value***
Demographics				
Age (years)	21.4 ± 3.5	20.9 ± 3.3	22.2 ± 3.6	< 0.001
Gender, male [n (%)]	419 (75.5%)	327 (98.2%)	92 (41.4%)	<0.001
Gender, female [n (%)]	136 (24.5%)	6 (1.8%)	130 (58.6%)	<0.001
Ethnicity, Caucasian [n (%)]	501 (90.1%)	302 (90.4%)	199 (89.6%)	0.0053
Ethnicity, Mixed/Multiple ethnic groups [n (%)]	24 (4.3%)	15 (4.5%)	9 (4.1%)	
Ethnicity, Asian/Asian British [n (%)]	16 (2.9%)	4 (1.2%)	12 (5.4%)	
Ethnicity, Black/African/Caribbean/Black British [n (%)]	15 (2.7%)	13 (3.9%)	2 (0.9%)	
Anthropometrics				
Weight (Kg)	74.4 ± 12.8	76.4 ± 11.4	71.4 ± 14.2	< 0.001

Height (m)	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	< 0.001
BMI ($kg.m^2$)	24.3 ± 3.4	24.4 ± 3.0	24.2 ± 3.9	> 0.05
Lifestyle behaviours				
Smoker [n (%)]	118 (21.3)	104 (31.2%)	14 (6.3%)	<0.0001
Non-smoker [n (%)]	369 (66.5%)	175 (52.6%)	194 (87.4%)	
Ex-smoker [n (%)]	68 (12.3%)	54 (16.2%)	14 (6.3%)	
Alcohol user [n (%)]	433 (78%)	245 (73.6%)	188 (84.7%)	<0.001
Non-alcohol user [n (%)]	122 (22%)	88 (26.4%)	34 (15.3%)	< 0.001

Data expressed as mean ± SD (%).

Table 2. New seroconversion cases (%) for participants who completed the trial

	Whole cohort	**Recruits**	**Civilians**
Week 5	14.2	18.3	8.1
Week 9	9.5	10.5	8.1
Week 12	6.7	7.5	5.4
Week 16	6.5	8.1	4.1
No seroconversion	63.1	55.6	74.3

Table 3. Binomial logistic regression predicting the likelihood of seroconversion in recruits.

Variable	*B*	SE	Wald	*df*	*P*	Odds Ratio	95% CI for Odds Ratio	
							Lower	Upper
Age	-0.005	0.039	0.018	1	0.892	0.995	0.922	1.073
BMI	0.029	0.042	0.462	1	0.497	1.029	0.947	1.119
Race/Ethnicity	0.112	0.208	0.292	1	0.589	1.119	0.744	1.683
Smoking status	0.044	0.193	0.053	1	0.819	1.045	0.717	1.524
Alcohol status	-0.026	0.284	0.008	1	0.927	0.974	0.558	1.700
Vit-D %change	-0.009	0.003	7.800	1	**0.005**	0.991	0.984	0.997
Constant	-1.071	1.228	0.760	1	0.383	0.343		

The overall model was not significant (x^2 (6) = 10.272, p = 0.114) and the model explained only 4.8% of the variance (Nagelkerke R^2) in the likelihood of seroconversion while correctly classifying 61.1% of the cases.

Figure 1 In the ITC cohort vitamin D increased over the 16 weeks and there was a significant difference in percentage change in vitamin D over the course of the study and the risk of seroconversion. Seroconversion was associated with only a 13.0 % increase in vitamin D over the course of the study whereas no seroconversion was associated with a 28.5 % increase in vitamin D over the course of the study ($p = 0.003$)

Figure 2 In the LBU cohort vitamin D reduced over the course of the 16 weeks and there was no difference between percentage change in vitamin D (-11.5 % vs -9.2 %, $p > 0.05$) and seroconversion.

Figure 1

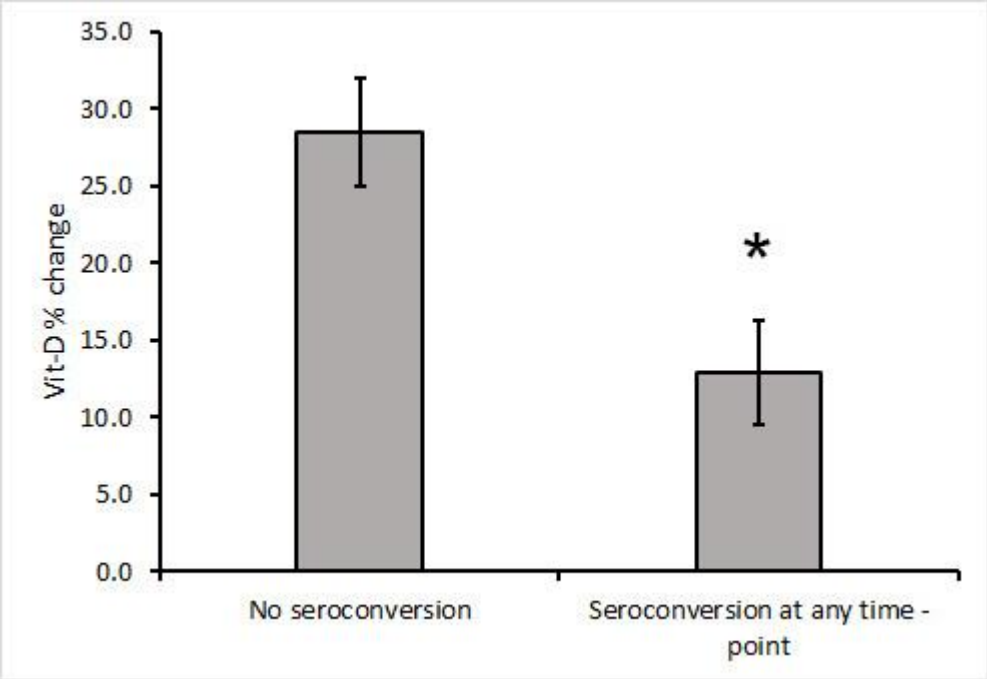
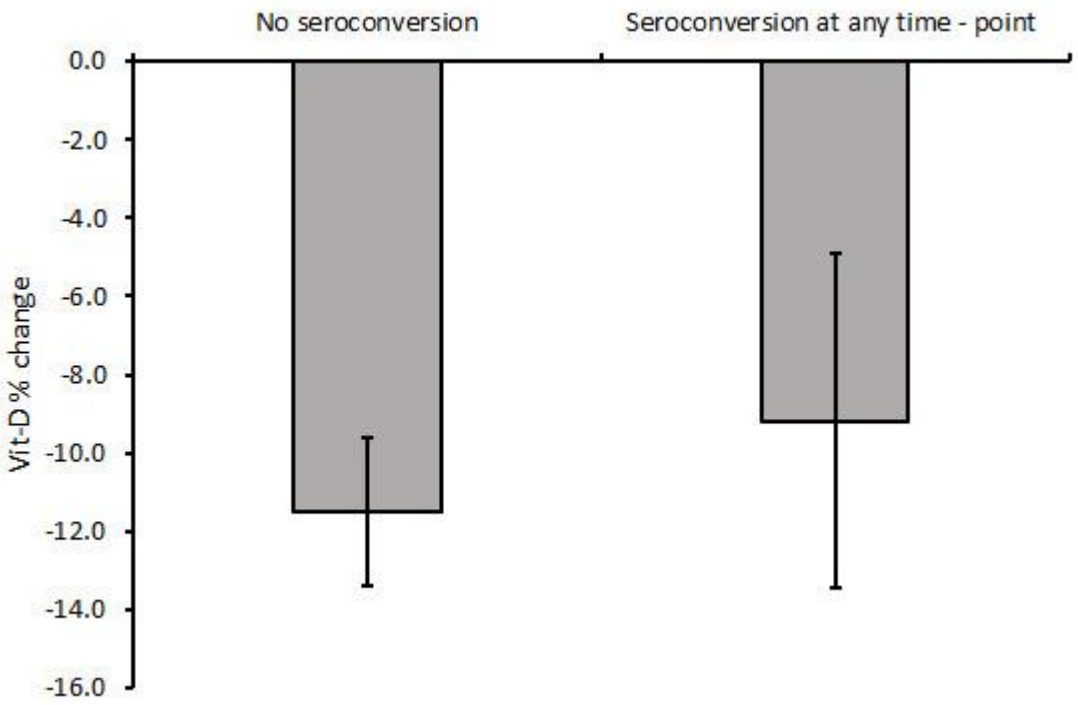

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Vit-D % change
*
No seroconversion
Seroconversion at any time - point

Figure 2


No seroconversion
Seroconversion at any time - point
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
Vit-D % change