Effect of a test-and-treat approach to vitamin D supplementation on risk of all cause acute respiratory tract infection and covid-19: phase 3 randomised controlled trial (CORONAVIT)

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ABSTRACT

OBJECTIVE
To determine the effect of population level implementation of a test-and-treat approach to correction of suboptimal vitamin D status (25-hydroxyvitamin D (25(OH)D) ‹75 nmol/L) on risk of all cause acute respiratory tract infection and covid 19.

DESIGN
Phase 3 open label randomised controlled trial.

SETTING
United Kingdom.

PARTICIPANTS
6200 people aged ≥16 years who were not taking vitamin D supplements at baseline.

INTERVENTIONS
Offer of a postal finger prick test of blood 25(OH)D concentration with provision of a six month supply of lower dose vitamin D (800 IU/day, n=1550) or higher dose vitamin D (3200 IU/day, n=1550) to those with blood 25(OH)D concentration ‹75 nmol/L, compared with no offer of testing or supplementation (n=3100). Follow-up was for six months.

MAIN OUTCOME MEASURES
The primary outcome was the proportion of participants with at least one swab test or doctor confirmed acute respiratory tract infection of any cause. A secondary outcome was the proportion of participants with swab test confirmed covid-19. Logistic regression was used to calculate odds ratios and associated 95% confidence intervals. The primary analysis was conducted by intention to treat.

RESULTS
Of 3100 participants offered a vitamin D test, 2958 (95.4%) accepted and 2674 (86.3%) had 25(OH)D concentrations ‹75 nmol/L and received vitamin D supplements (n=1328 lower dose, n=1346 higher dose). Compared with 136/2949 (4.6%) participants in the no offer group, at least one acute respiratory tract infection of any cause occurred in 87/1515 (5.7%) in the lower dose group (odds ratio 1.26, 95% confidence interval 0.96 to 1.66) and 76/1515 (5.0%) in the higher dose group (1.09, 0.82 to 1.46). Compared with 78/2949 (2.6%) participants in the no offer group, 55/1515 (3.6%) developed covid-19 in the lower dose group (1.39, 0.98 to 1.97) and 45/1515 (3.0%) in the higher dose group (1.13, 0.78 to 1.63).

CONCLUSIONS
Among people aged 16 years and older with a high baseline prevalence of suboptimal vitamin D status, implementation of a population level test-and-treat approach to vitamin D supplementation was not associated with a reduction in risk of all cause acute respiratory tract infection or covid-19.

TRIAL REGISTRATION
ClinicalTrials.gov NCT04579640.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Vitamin D metabolites support innate immune responses to SARS-CoV-2 and other respiratory pathogens

Suboptimal vitamin D status (25-hydroxyvitamin D ‹75 nmol/L) is associated with increased susceptibility to all cause acute respiratory tract infections and covid-19

Phase 3 randomised controlled trials of vitamin D to prevent covid-19 have not yet reported

WHAT THIS STUDY ADDS

This phase 3 randomised controlled trial (6200 participants) shows that implementation of a population level test-and-treat approach to oral vitamin D replacement at daily doses of 800 IU or 3200 IU was not associated with a reduction in risk of all cause acute respiratory tract infection or covid-19 among people aged 16 years and older with a high baseline prevalence of suboptimal vitamin D status

Introduction

The covid-19 pandemic has refocused attention on strategies to prevent acute respiratory tract infection. Although vaccination against SARS-CoV-2 represents the mainstay for disease control, its effectiveness at a global level is compromised by factors such as cost, availability, vaccine hesitancy, vaccine failure, and vaccine escape.[1-3] Complementary, low cost approaches to enhance immunity to SARS-CoV-2 and other pathogens responsible for acute respiratory tract infections are needed.

Vitamin D metabolites have long been recognised to support innate immune responses to respiratory viruses and bacteria, and regulate immunopathological inflammation.[4-6] The vitamin D inducible antimicrobial peptides cathelicidin LL-37 and human β defensin 2 bind to the SARS-CoV-2 spike protein and inhibit viral binding to angiotensin converting enzyme 2, its

cellular receptor.[7] [8] Longitudinal studies investigating potential associations between higher vitamin D status or vitamin D supplement use and reduced risk of SARS-CoV-2 infection have shown mixed results, with some reporting protective associations and others reporting null or negative associations[9-14]; meta-analyses including these and other observational studies report protective associations overall.[15] [16] Findings of randomised controlled trials of vitamin D supplementation to prevent acute respiratory tract infections caused by pathogens other than SARS-CoV-2 have also been heterogeneous.[17-21] Meta-analysis of these and other randomised controlled trials shows a small but statistically significant protective effect that is strongest when modest daily doses of vitamin D (400-1000 IU) are given for periods of up to one year.[22] A phase 2 randomised controlled trial in 321 healthcare workers in Mexico reported a large protective effect of daily oral vitamin D supplementation against covid-19 (relative risk 0.23, 95% confidence interval 0.09 to 0.55).[23] However, phase 3 clinical trials of prophylactic vitamin D to reduce the incidence and severity of covid-19 are lacking, as are studies comparing the effectiveness of different doses of vitamin D supplements for the prevention of acute respiratory tract infections of any cause among adults. There is also a dearth of studies designed to evaluate the effectiveness of practical approaches to identification and treatment of vitamin D deficiency at scale in the general population to improve health outcomes. We therefore established a phase 3 pragmatic randomised controlled trial (CORONAVIT) to evaluate the effectiveness of a test-and-treat approach to identification and treatment of suboptimal vitamin D status to prevent acute respiratory tract infection and covid-19 in UK adults from December 2020 to June 2021—a period when the incidence of covid-19 was high and SARS-CoV-2 vaccine coverage was initially low.

Methods

Study design

We conducted a three arm, parallel, open label individually randomised controlled trial nested within the population based COVIDENCE UK cohort study (NCT04330599), using trial-within-cohort methodology.[24] COVIDENCE UK was established to determine risk factors for incident covid-19,[12] [13] characterise the clinical course of the disease, evaluate impacts of covid-19 on physical and mental health and economic vulnerability,[25] and provide a platform from which to conduct clinical trials of interventions to reduce the incidence and severity of acute respiratory tract infections. The study was launched on 1 May 2020 and closed to recruitment on 6 October 2021; a total of 19 981 participants enrolled.[26]

Participants

Trial participants were drawn from those taking part in COVIDENCE UK who provided information for the following variables using an online questionnaire completed at enrolment: age, sex, ethnic origin, postal address, highest educational level attained, occupational status, weight, height, history of SARS-CoV-2 infection, covid-19 vaccination status, chronic health conditions, tobacco smoking history, alcohol consumption, intake of micronutrient supplements, hours spent outdoors, and dietary consumption of oily fish and red meat. Inclusion criteria for the trial were current residence in the UK, age 16 years or older at screening, enrolment in COVIDENCE UK, and online provision of informed consent. Exclusion criteria were taking vitamin D supplements, digoxin, alfacalcidol, calcitriol, dihydrotachysterol or paricalcitol; diagnosis of sarcoidosis, primary hyperparathyroidism, nephrolithiasis, or renal failure requiring dialysis; allergy to any ingredient in the study capsules; and pregnancy.

Randomisation

A randomly selected subset of 6200 cohort participants were assessed as eligible on the basis of their enrolment questionnaire data and report of no supplemental vitamin D intake at baseline. Using a computer program (Stata v14.2), the trial statistician individually randomised participants to receive no offer of vitamin D testing or supplementation (no offer group) or an offer of a postal vitamin D test with supply of 800 IU/day vitamin D supplements if blood 25-hydroxyvitamin D (25(OH)D) concentrations were <75 nmol/L (lower dose group) or 3200 IU/day if blood concentrations were <75 nmol/L (higher dose group). To implement the randomisation, the study statistician downloaded unique identifiers of all participants in the COVIDENCE UK longitudinal study with questionnaire responses indicating eligibility to participate in the CORONAVIT trial. The trial statistician then used Stata software to randomly select 6200 of those reporting no supplemental vitamin D intake and to randomly assign them to no offer, lower dose offer, or higher dose offer groups in a 2:1:1 ratio in blocks of 10. Overall, 3100 participants were randomised to the no offer group and 1550 participants each were randomised to each of the lower or higher dose offer groups. Treatment allocation was not concealed, and randomisation was not stratified.

Intervention

Consenting participants randomised to either intervention arm of the trial were sent a blood spot testing kit through the post for determination of 25(OH)D concentrations in capillary blood, as described previously.[27] Those found to have concentrations <75 nmol/L received a six month supply of capsules containing either 800 IU/day or 3200 IU/day vitamin D_3 according to their treatment allocation. The 75 nmol/L threshold for 25(OH)D is widely considered to discriminate between those with optimal vitamin D status and those with suboptimal status.[28-30] We adopted a test-and-treat approach to avoid the potential risk of inducing hypervitaminosis D by providing higher dose supplementation to individuals with baseline

25(OH)D concentrations >75 nmol/L, and in response to feedback from our patient and public involvement group who advised that participants might be better motivated to adhere to supplementation if they knew that their baseline vitamin D status was suboptimal.

Participants in both intervention groups were supplied with D-Pearls capsules (Pharma Nord, Vejle, Denmark), unless they expressed a preference for a vegetarian or vegan supplement, in which case they received Pro D_3 vegan capsules (Synergy Biologics, Walsall, UK). Participants with 25(OH)D concentrations ≥75 nmol/L at initial testing were offered a second postal vitamin D test two months after the first test: those whose second 25(OH)D result was <75 nmol/L were offered a postal supply of vitamin D supplements. Participants receiving study supplements were instructed to take one capsule daily until the capsules were finished. Administration of supplements was not supervised.

Follow-up assessments

Follow-up was for six months, from 17 December 2020 to 16 June 2021. At monthly intervals we emailed the participants links to follow-up online questionnaires, which captured information on: incident swab test positive or doctor diagnosed acute respiratory tract infections, including covid-19; hospital admission for treatment of acute respiratory tract infections, including covid-19; requirement for ventilatory support; prescription of antibiotics for acute respiratory tract infection; incidence of acute exacerbations of asthma or chronic obstructive pulmonary disease, and other adverse events; uptake of covid-19 vaccination; presence or absence of ongoing covid-19 symptoms at four weeks post-diagnosis and at six month follow-up (participants with swab test positive covid-19); and MRC (Medical Research Council) dyspnoea score,[31] FACIT (Functional Assessment of Chronic Illness Therapy) Fatigue Score,[32] and Post-Covid Physical Health Symptom Score[33] at six month follow-up (participants with swab test positive covid-19). The Post-Covid Physical Health Symptom Score was based on the participants' responses to questions on 16 symptoms commonly experienced after covid-19: excessive shortness of breath, cough, unusual tiredness or fatigue, problems with sleeping, memory problems, difficulty concentrating, joint or muscle pain, problems with taste or smell, diarrhoea, stomach (abdominal) pains, changes to voice, hair loss, unusual racing of the heart, light-headedness or dizziness, unusual sweating, and episodes of ringing or buzzing in the head or in one or both ears for more than five minutes. Participants were given four response options to each question: "No" (1 point), "Yes, but improving" (2 points), "Yes, but not improving or worsening" (3 points), and "Yes, and worsening" (4 points). We summed the responses to provide the symptom score (lower scores signifying fewer post-covid-19 symptoms).

Details of incident swab test confirmed or doctor confirmed acute respiratory tract infections, admissions to hospital, and deaths were also captured through electronic linkage to routinely collected medical record data. End trial postal vitamin D testing was offered to a randomly selected subset of 1600 participants who received study supplements (800 each in the lower and higher dose arms) and 400 participants who were randomised to no offer. Participants randomised to no offer who were found to have 25(OH)D concentrations <50 nmol/L at the end of the trial received a 60 day supply of capsules, each containing 2500 IU vitamin D_3 (Cytoplan, Hanley Swan, UK).

Each monthly questionnaire contained the following advice encouraging participants with symptoms of covid-19 to be tested for SARS-CoV-2 infection: "If you currently have symptoms of coronavirus (a high temperature, a new, continuous cough or loss of or altered sense of smell or taste), call NHS111 or visit https://www.nhs.uk/conditions/coronavirus-covid-19/ for more information." This wording was identical for questionnaires sent to all participants. In addition to the monthly questionnaires, we sent participants in the intervention arms a link to an online adherence questionnaire on 31 March 2021. This captured information on frequency of study supplement use.

Laboratory testing

Black Country Pathology Services (Sandwell General Hospital, West Bromwich, UK) performed the 25(OH)D assays; this laboratory participates in the UK National External Quality Assessment Service (NEQAS) for Vitamin D and the Vitamin D External Quality Assessment Scheme (DEQAS) for serum 25(OH)D. Concentrations of $25(OH)D_3$ and $25(OH)D_2$ were determined in dried blood spot eluates using liquid chromatography tandem mass spectrometry (Acquity UPLC-TQS or TQS-Micro Mass Spectrometers; Waters, Milford, MA) after derivatisation and liquid-liquid extraction as described elsewhere.[27] The results were summed to give total 25(OH)D concentrations. Good overall agreement has been observed between using the blood spot method and plasma 25(OH)D concentrations in paired capillary and venous samples,[27] showing a minimal overall bias of −0.2% (bias range −16.9-26.7%). For the current study, we used the following reference ranges for total 25(OH)D concentrations: deficient or suboptimal <75.0 nmol/L, adequate 75.0-220.0 nmol/L, and high >220.0 nmol/L. The between day coefficients of variation for $25(OH)D_3$ were 11.1% at 16.9 nmol/L, 8.2% at 45.5 nmol/L, 6.9% at 131.7 nmol/L, and for $25(OH)D_2$ were 7.0% at 222.2 nmol/L, 13.7% at 18.1 nmol/L, 7.5% at 42.7 nmol/L, and 6.4% at 127.3 nmol/L. The mean bias of dried blood spot compared with serum $25(OH)D_3$ concentrations during 2018-21 was 4.0%. Limits of quantitation were 7.5 nmol/L for $25(OH)D_3$ and 2.8 nmol/L for $25(OH)D_2$.

Main outcome measures

The primary outcome was the proportion of participants who had at least one swab test confirmed

or doctor confirmed acute respiratory tract infection of any cause. These events were captured either through self-report using structured monthly online questionnaires and/or through linkage to one or more of the following databases containing routinely collected virology test results and medical record data from primary, secondary, and tertiary healthcare facilities: the UK Office for National Statistics mortality database, the UK National Health Service Hospital Episode Statistics, the COVID-19 Hospitalisation in England Surveillance System, the General Practice Extraction Service, and the Second Generation Surveillance Service. The last of these is a national laboratory reporting system used to capture routine laboratory data on infectious diseases. Supplementary table S1 shows the wording of questions in monthly follow-up questionnaires that was used to capture self-reported swab test confirmed or doctor confirmed acute respiratory tract infections, along with the algorithms used to define these events. Supplementary table S2 lists ICD-10 (international classification of diseases, 10th revision) codes for acute respiratory tract infections that were used in computerised searches to capture events documented in medical records.

Secondary efficacy outcomes were the proportion of all participants developing covid-19 confirmed by reverse transcription polymerase chain reaction or antigen testing; the proportion of all participants admitted to hospital for treatment of covid-19; the proportion of participants admitted to hospital for treatment of covid-19 who required ventilatory support; the proportion of all participants dying with covid-19; the proportion of participants developing test confirmed covid-19 who reported symptoms lasting more than four weeks; the proportion of participants developing test confirmed covid-19 who reported ongoing symptoms at the end of the study; the proportion of all participants prescribed one or more courses of antibiotics for treatment of an acute respiratory tract infection of any cause; the proportion of all participants admitted to hospital for treatment of an acute respiratory tract infection of any cause; the proportion of all participants dying of an acute respiratory tract infection of any cause; the proportion of participants with asthma or chronic obstructive pulmonary disease developing at least one severe acute exacerbation; mean values for the MRC dyspnoea score,[31] the FACIT Fatigue Scale score,[32] and the Post-Covid Physical Health Symptom Score[33] among participants developing test confirmed covid-19 who reported ongoing symptoms at the end of the study; and mean end study 25(OH)D concentrations in the subset of participants for whom this was measured. Safety outcomes were incidence of death, serious adverse events, adverse events leading to discontinuation of study drugs, and other monitored safety conditions: hypercalcaemia (serum corrected calcium concentration >2.65 mmol/L), hypervitaminosis D (25(OH)D concentration >220 nmol/L), and nephrolithiasis.

Sample size calculation

Using a graphical user interphase,[34] we determined that a total of 6200 participants would need to be randomised to detect a 20% reduction in the proportion of participants meeting the primary outcome with 84% marginal power[35] and 5% type 1 error rate. The calculation was based on the following assumptions: 20% risk of participants in the no offer group experiencing at least one swab test or doctor confirmed acute respiratory tract infection at six months, 25% loss to follow-up, and a 2:1:1 ratio of participants randomised to no offer, lower dose offer, or higher dose offer, respectively.

Statistical analysis

Statistical analyses were performed using Stata 14.2 (College Station, TX). Primary analyses were done according to intention to treat, with pairwise comparisons between each intervention arm and the no offer arm. The intention-to-treat analysis included participants with data for at least one follow-up time point. We used logistic regression to compare the proportions of participants experiencing dichotomous study endpoints between intervention and no offer arms, with treatment effects presented as odds ratios and corresponding 95% confidence intervals. Dunnett's test was used to adjust for multiple testing for the primary outcome (ie, owing to two comparisons),[36] with a critical P value threshold of 0.027. We compared continuous outcomes between the intervention and no offer arms using unpaired Student's t tests, with treatment effects presented as mean differences with corresponding 95% confidence intervals.

We prespecified subgroup analyses comparing the effect of the intervention on major outcomes in participants who received at least one dose of a covid-19 vaccine during follow-up with those who did not, and in those with lower versus higher actual or predicted baseline 25(OH)D concentrations. For each of these subgroup analyses, we prespecified testing for interactions between allocation and the potential effect modifier of interest. Additionally, we conducted a post hoc exploratory Cox regression analysis to determine effects of allocation before and after a first dose of covid-19 vaccine. Predicted baseline 25(OH)D concentrations for participants in the no offer arm were calculated according to published methodology[37] using baseline 25(OH)D values of participants randomised to either intervention arm and employing a random forest regression model[38] with 11 features (age, sex, body mass index, ethnicity, portions of red meat intake per week, portions of oily fish intake per week, use of multivitamin supplements (yes or no), use of vitamin D supplements (yes or no), use of cod liver oil supplements (yes or no), sunshine hours per week, and latitude). We obtained sunshine hours per week from HadUK's 2019 5 km gridded climate measurements using the mean value of sunshine hours per week from October to December 2019 in the postcode grid square for each participant. Participants with missing data on age, body mass index, sunshine hours per week, and

latitude were imputed with the median value of that covariate among participants with non-missing data. For all other covariates, participants with missing data were imputed with the mode of that covariate among participants with non-missing data. Using a training set comprising 75% of all participants with measured 25(OH)D values, we conducted a grid search over hyperparameter values using a cross validation procedure with five folds. We searched several hyperparameter values: split criterion (mean squared error and mean absolute error), maximum tree depth (3, 4, 5, or 6 layers), bootstrap sample size (50%, 65%, or 80% of participants in the training set), and number of trees (250, 500, 1000). The mean gamma deviance was used to evaluate hyperparameter combinations over all five folds of the cross validation split. The optimal hyperparameter combination produced a mean gamma deviance of 0.151 across all validation folds. To verify the generalisation accuracy of the model trained with these hyperparameters, we used a test set comprising 25% of all participants with measured 25(OH)D values, which yielded a gamma deviance of 0.141, showing that the out-of-sample performance observed in the cross validation was indicative of test set performance. The final model was trained on all instances with available data and used to impute baseline 25(OH)D concentrations for participants randomised to the no offer arm. These imputed baseline values were then compared with measured baseline 25(OH)D values for participants randomised to the supplement arms (see supplementary figure S1): the distribution of imputed 25(OH)D concentrations in the no offer arm at baseline (range 25.0-86.2 nmol/L, SD 8.2 nmol/L) was constrained compared with that of measured 25(OH)D concentrations in the lower dose arm (range 10.3-179.6 nmol/L, SD 17.8 nmol/L) and higher dose arm (range 10.3-122.0 nmol/L, SD 16.2 nmol/L). This finding questioned the validity of the imputation, which precluded conduct of subgroup analyses by baseline 25(OH)D concentration.

A sensitivity analysis was also prespecified, which excluded data from participants in either intervention arm who reported taking vitamin D capsules for less than half the time, as well as those in the no offer arm who reported any intake of supplemental vitamin D during follow-up. Two post hoc exploratory analyses were also performed in response to reviewer comments. The first compared mean end study 25(OH)D concentrations of participants in the no offer arm who contributed data to the intention-to-treat analysis but not to the sensitivity analysis (ie, participants randomised to no offer who reported use of off-trial vitamin D supplements) with those who contributed data to both intention-to-treat and sensitivity analyses (ie, participants randomised to no offer who reported no use of off-trial vitamin D supplements). The second evaluated the effect of allocation in the subset of participants who completed all six follow-up questionnaires.

No interim analysis was planned or performed owing to the short duration of the trial and because linkage to routinely collected health data was not done until the end of the trial. The independent data monitoring committee did, however, review accumulating data on serious adverse events by study arm on one occasion (three months into study follow-up), at which point it recommended continuation of the trial.

Patient and public involvement

Three patient and public involvement representatives were active in development of the research questions and the choice of outcome measures specified in the study protocol. One of them also led on development and implementation of strategies to maximise participant recruitment.

Results

Participants

A total of 17 700 participants in the COVIDENCE UK cohort study were assessed for eligibility to take part in the CORONAVIT trial in October 2020: 6200 of 6470 participants classified as eligible based on their responses to study questionnaires were randomly selected for invitation to the trial, and then randomly assigned to no offer versus lower dose offer versus higher dose offer groups (fig 1). Table 1 shows the baseline characteristics of participants by study arm. Median age was 60.2 years, 4156/6200 (67.0%) participants were female, and 77/6200 (1.2%) had received one dose or more of a covid-19 vaccine. Characteristics were balanced between the three groups. Of 3100 participants randomised to either lower dose or higher dose vitamin D, 2958 (95.4%) consented to receive a postal 25(OH)D test, and 2674 (86.3%) had blood 25(OH)D concentrations <75 nmol/L at baseline or two months and received vitamin D supplements (800 IU capsules to 1328 participants and 3200 IU capsules to 1346 participants). Follow-up was for six months, from December 2020 to June 2021; by the end of this period, 5523/6200 (89.1%) participants had received one dose or more of a covid-19 vaccine (see supplementary table S3). Self-reported adherence to study supplements among participants randomised to either intervention arm was good, with 90.9% of participants reporting that they took study supplements at least six times per week (see supplementary table S4). Availability of questionnaire and linkage data was also good, with 5979/6200 (96.4%) randomised participants contributing data to the intention-to-treat analysis. Compared with the no offer group, in the subset of participants included in the intention-to-treat analysis for whom measures of end study vitamin D status were available, mean 25(OH)D concentrations were significantly increased in the lower dose (79.4 v 66.6 nmol/L; mean difference 12.7 nmol/L, 9.8 to 15.6 nmol/L) and higher dose arms (102.9 v 66.6 nmol/L; mean difference 36.3 nmol/L, 95% confidence interval 32.9 to 39.6 nmol/L); table 2 and fig 2.

Among those included in the sensitivity analysis (ie, excluding non-adherent participants randomised to either intervention, and participants in the no offer arm who took vitamin D supplements), mean
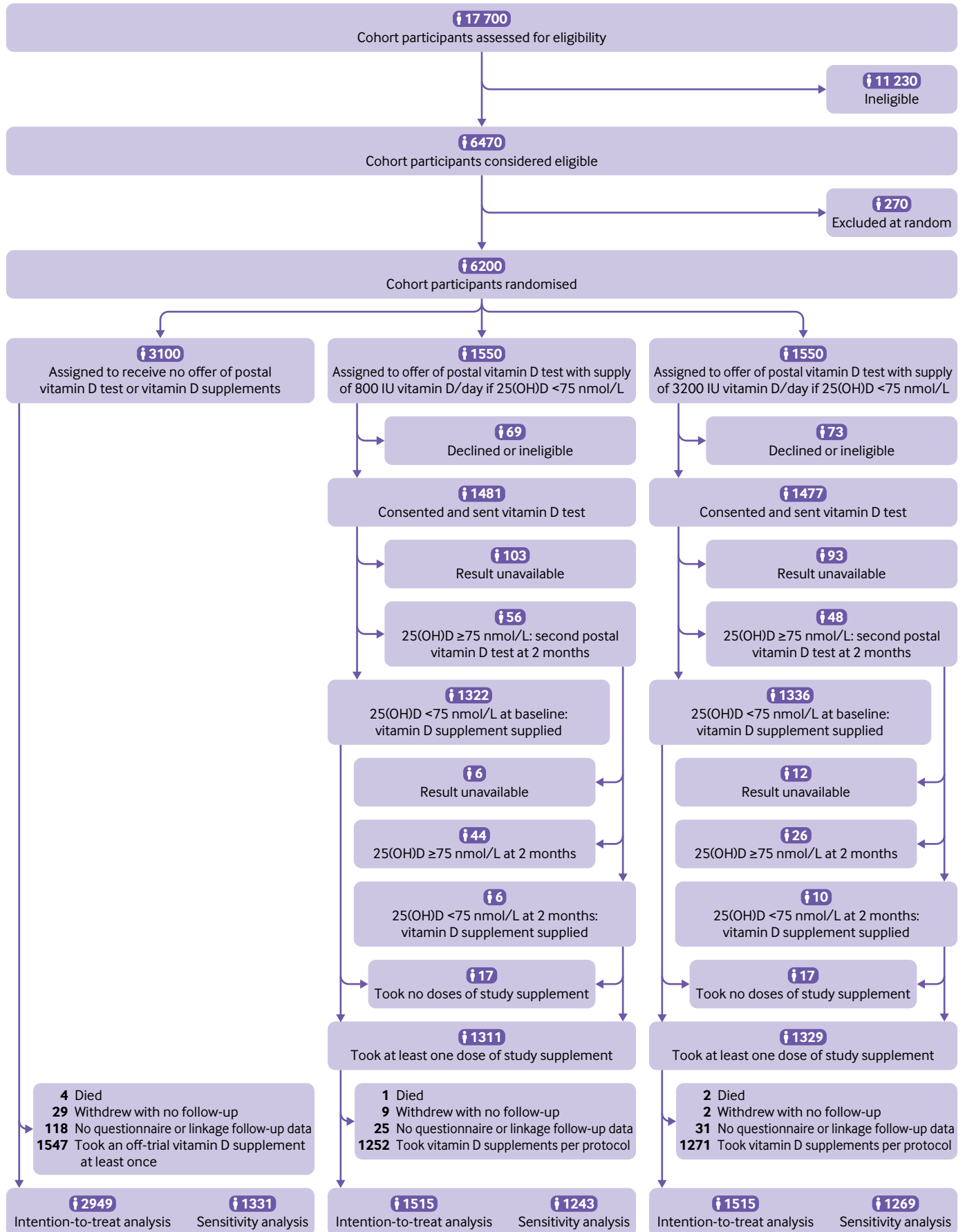
17 700
Cohort participants assessed for eligibility

11 230
Ineligible

6470
Cohort participants considered eligible

270
Excluded at random

6200
Cohort participants randomised

3100
Assigned to receive no offer of postal vitamin D test or vitamin D supplements

1550
Assigned to offer of postal vitamin D test with supply of 800 IU vitamin D/day if 25(OH)D <75 nmol/L

1550
Assigned to offer of postal vitamin D test with supply of 3200 IU vitamin D/day if 25(OH)D <75 nmol/L

69
Declined or ineligible

73
Declined or ineligible

1481
Consented and sent vitamin D test

1477
Consented and sent vitamin D test

103
Result unavailable

93
Result unavailable

56
25(OH)D ≥75 nmol/L: second postal vitamin D test at 2 months

48
25(OH)D ≥75 nmol/L: second postal vitamin D test at 2 months

1322
25(OH)D <75 nmol/L at baseline: vitamin D supplement supplied

1336
25(OH)D <75 nmol/L at baseline: vitamin D supplement supplied

6
Result unavailable

12
Result unavailable

44
25(OH)D ≥75 nmol/L at 2 months

26
25(OH)D ≥75 nmol/L at 2 months

6
25(OH)D <75 nmol/L at 2 months: vitamin D supplement supplied

10
25(OH)D <75 nmol/L at 2 months: vitamin D supplement supplied

17
Took no doses of study supplement

17
Took no doses of study supplement

1311
Took at least one dose of study supplement

1329
Took at least one dose of study supplement

4 Died
29 Withdrew with no follow-up
118 No questionnaire or linkage follow-up data
1547 Took an off-trial vitamin D supplement at least once

1 Died
9 Withdrew with no follow-up
25 No questionnaire or linkage follow-up data
1252 Took vitamin D supplements per protocol

2 Died
2 Withdrew with no follow-up
31 No questionnaire or linkage follow-up data
1271 Took vitamin D supplements per protocol

2949
Intention-to-treat analysis

1331
Sensitivity analysis

1515
Intention-to-treat analysis

1243
Sensitivity analysis

1515
Intention-to-treat analysis

1269
Sensitivity analysis

Fig 1 | Flow of participants through study

Table 1 | Baseline characteristics of participants by allocation. Values are numbers (percentages) unless stated otherwise

Characteristics	Overall (n=6200)	No offer (n=3100)	800 IU/day offer (n=1550)	3200 IU/day offer (n=1550)
Age				
Median (IQR) age (years)	60.2 (49.8-67.8)	60.8 (49.9-68.2)	59.8 (50.3-67.4)	60.7 (50.2-68.5)
Age range (years)	16.1-89.8	16.1-89.8	16.5-88.2	16.4-88.6
Sex				
Male	2044 (33.0)	1040 (32.5)	498 (32.1)	506 (32.6)
Female	4156 (67.0)	2060 (64.4)	1052 (67.9)	1044 (67.4)
Ethnicity				
White	5867 (94.6)	2925 (94.4)	1473 (95.0)	1469 (94.8)
Asian, Asian British	142 (2.3)	79 (2.5)	32 (2.1)	31 (2.0)
Black, African, Caribbean, black British	33 (0.5)	12 (0.4)	10 (0.6)	11 (0.7)
Mixed, multiple, other	154 (2.5)	83 (2.6)	34 (2.2)	37 (2.4)
Country of residence				
England	5515 (89.0)	2757 (86.2)	1384 (89.3)	1374 (88.6)
Northern Ireland	123 (2.0)	61 (1.9)	33 (2.1)	29 (1.9)
Scotland	340 (5.5)	169 (5.3)	74 (4.8)	97 (6.3)
Wales	222 (3.6)	113 (3.5)	59 (3.8)	50 (3.2)
Highest educational attainment				
Primary or secondary school	52 (0.8)	23 (0.7)	12 (0.8)	17 (1.1)
Higher or further education (A levels)	924 (14.9)	476 (14.9)	233 (15.0)	215 (13.9)
College	2740 (44.2)	1366 (42.7)	700 (45.2)	674 (43.5)
Postgraduate	1817 (29.3)	885 (27.7)	459 (29.6)	473 (30.5)
Occupational status				
Employed, health or social care worker	566 (9.1)	270 (8.7)	147 (9.5)	149 (9.6)
Employed, other frontline worker	755 (12.2)	381 (12.3)	192 (12.4)	182 (11.8)
Employed, non-frontline worker	1406 (22.7)	722 (23.3)	348 (22.5)	336 (21.7)
Self-employed	564 (9.1)	266 (8.3)	144 (9.3)	154 (9.9)
Retired	2504 (40.4)	1263 (39.5)	606 (39.1)	635 (41.0)
Furloughed	141 (2.3)	66 (2.1)	43 (2.8)	32 (2.1)
Unemployed	126 (2.0)	50 (1.6)	42 (2.7)	34 (2.2)
Student	150 (2.4)	83 (2.6)	34 (2.2)	33 (2.1)
Other	147 (2.4)	76 (2.4)	36 (2.3)	35 (2.3)
Body mass index				
‹25	2903 (46.8)	1440 (45.0)	724 (46.7)	739 (47.7)
25-30	2036 (32.8)	1026 (32.1)	496 (32.0)	514 (33.2)
›30	1249 (20.1)	630 (19.7)	322 (20.8)	297 (19.2)
Medical conditions				
Hypertension	227 (3.7)	120 (3.8)	54 (3.5)	53 (3.4)
Diabetes mellitus	259 (4.2)	122 (3.8)	56 (3.6)	81 (5.2)
Heart disease	1207 (19.5)	590 (18.4)	298 (19.2)	319 (20.6)
Asthma	946 (15.3)	466 (14.6)	265 (17.1)	215 (13.9)
COPD	114 (1.8)	61 (1.9)	27 (1.7)	26 (1.7)
Smoking status				
Never smoker	3460 (55.8)	1709 (53.4)	887 (57.2)	864 (55.7)
Former smoker	2346 (37.8)	1210 (37.8)	553 (35.7)	583 (37.6)
Current smoker	393 (6.3)	181 (5.7)	109 (7.0)	103 (6.6)
Alcohol intake (units/wk)				
None	1651 (26.6)	857 (26.8)	411 (26.5)	383 (24.7)
1-14	3403 (54.9)	1665 (52.0)	865 (55.8)	873 (56.3)
≥15	1145 (18.5)	577 (18.0)	274 (17.7)	294 (19.0)
Covid-19 vaccine status				
Unvaccinated	5774 (93.1)	2826 (91.2)	1465 (94.5)	1483 (95.7)
Partially vaccinated	55 (0.9)	27 (0.9)	19 (1.2)	9 (0.6)
Fully vaccinated	22 (0.4)	11 (0.4)	5 (0.3)	6 (0.4)
Not known or missing data	349 (5.6)	236 (7.6)	61 (3.9)	52 (3.4)
25(OH)D				
Mean (SD) concentration (nmol/L); range*	−†	−†	41.5 (18.0); 10.3-179.6	40.9 (16.4); 10.3-122.0
25(OH)D category (nmol/L)				
‹25.0	−†	−†	232 (15.0)	216 (13.9)
25.0-49.9	−†	−†	759 (49.0)	797 (51.4)
50-74.9	−†	−†	337 (21.7)	333 (21.5)
≥75.0	−†	−†	43 (2.7)	28 (1.8)
Not determined	−†	3100 (100.0)	179 (11.6)	176 (11.4)

COPD=chronic obstructive pulmonary disease; IQR=interquartile range; SD=standard deviation; 25(OH)D=25-hydroxyvitamin D.
*Data missing for 189 participants in 3200 IU/day arm and 198 participants in 800 IU/day arm.
†Not determined for participants randomised to no offer arm.

differences in end study 25(OH)D concentrations between intervention versus no offer arms were greater (for lower dose versus no offer group, 25.8 nmol/L 22.0 to 29.5 nmol/L; for higher dose versus no offer group, 49.7 nmol/L, 95% confidence interval 45.1 to 54.2 nmol/L; table 3 and fig 2).

Table 2 | Primary and secondary outcomes, by allocation: intention-to-treat analysis. Values are percentages (number with event/number in group) unless specified otherwise

	No offer	800 IU/day offer	800 IU/day v no offer				3200 IU/day offer	3200 IU/day v no offer			
			Odds ratio (95% CI)	P value	Mean difference (95% CI)	P value		Odds ratio (95% CI)	P value	Mean difference (95% CI)	P value
Primary outcome											
≥1 swab test or doctor confirmed ARI of any cause*	4.6 (136/2949)	5.7 (87/1515)	1.26 (0.96 to 1.66)	0.10	–	–	5.0 (76/1515)	1.09 (0.82 to 1.46)	0.55	–	–
Secondary outcomes											
Acute covid-19 outcomes:											
Swab test confirmed covid-19†	2.6 (78/2949)	3.6 (55/1515)	1.39 (0.98 to 1.97)	0.07	–	–	3.0 (45/1515)	1.13 (0.78 to 1.63)	0.53	–	–
Admitted to hospital for covid-19	1.4 (40/2949)	1.6 (24/1515)	1.17 (0.70 to 1.95)	0.55	–	–	1.9 (29/1515)	1.42 (0.88 to 2.30)	0.16	–	–
In-hospital ventilatory support for covid-19‡	2.5 (1/40)	4.2 (1/24)	1.70 (0.10 to 28.43)	0.71	–	–	3.4 (1/29)	1.39 (0.08 to 23.23)	0.82	–	–
Deaths with covid-19	0.0 (0/2949)	0.0 (0/1515)	–§	–	–§	–	0.0 (0/1515)	–§	–	–	–
Long covid outcomes:											
Swab test confirmed covid-19: symptoms for >4 weeks†	24.4 (19/78)	38.2 (21/55)	1.92 (0.91 to 4.06)	0.09	–	–	24.4 (11/45)	1.00 (0.43 to 2.36)	0.99	–	–
Swab test confirmed covid-19: symptoms at study end	9.0 (7/78)	20.0 (11/55)	2.54 (0.91 to 7.03)	0.07	–	–	17.8 (8/45)	2.19 (0.74 to 6.52)	0.16	–	–
Mean (SD) MRC dyspnoea score (No)	2.14 (1.46) (7)	1.55 (0.93) (11)	–	–	–0.60 (–2.00 to 0.90)	0.36	2.13 (0.64) (8)	–	–	–0.02 (–1.40 to 1.36)	0.98
Mean (SD) FACIT Fatigue Scale score (No)	28.43 (7.25) (7)	25.22 (5.74) (9)	–	–	–3.21 (–10.54 to 4.13)	0.36	28.00 (8.75) (8)	–	–	–0.43 (–9.36 to 8.50)	0.92
Mean Post-COVID Physical Health Symptom Score (No)	30.71 (10.16) (7)	31.90 (10.70) (10)	–	–	1.19 (–9.83 to 12.20)	0.82	33.25 (12.33) (8)	–	–	2.54 (–10.02 to 15.09)	0.67
All cause ARI outcomes:*											
≥1 courses of antibiotics for ARI of any cause*¶	0.8 (22/2864)	0.3 (5/1489)	0.44 (0.16 to 1.15)	0.09	–	–	0.9 (14/1498)	1.22 (0.62 to 2.39)	0.57	–	–
Admitted to hospital for ARI of any cause*	0.4 (12/2949)	0.5 (7/1515)	1.14 (0.45 to 2.89)	0.79	–	–	0.7 (11/1515)	1.79 (0.79 to 4.07)	0.16	–	–
Death due to ARTI of any cause*	0.0 (0/2949)	0.0 (0/1515)	–§	–	–§	–	0.0 (0/1515)	–§	–	–	–
Airways disease outcomes:											
≥1 severe acute asthma exacerbation in patients with asthma**	4.9 (21/431)	3.1 (8/255)	0.63 (0.28 to 1.45)	0.28	–	–	6.7 (14/209)	1.40 (0.70 to 2.82)	0.34	–	–
≥1 severe acute COPD exacerbation in patients with COPD††	15.9 (10/63)	21.4 (6/28)	1.45 (0.47 to 4.46)	0.52	–	–	7.4 (2/27)	0.42 (0.09 to 2.08)	0.29	–	–
Biochemical outcome:											
Mean (SD) end study 25(OH)D concentration (nmol/L)‡‡ (No)	66.6 (28.6) (306)	79.4 (18.3) (742)	–	–	12.7 (9.8 to 15.6)	<0.001	102.9 (23.6) (741)	–	–	36.3 (32.9 to 39.6)	<0.001

ARI=acute respiratory tract infection; CI=confidence interval; COPD=chronic obstructive pulmonary disease; FACIT=Functional Assessment of Chronic Illness Therapy; MRC=Medical Research Council; 25(OH)D=25-hydroxyvitamin D.
†Confirmed using reverse transcriptase polymerase chain reaction and/or antigen testing for SARS-CoV-2.
‡Invasive and non-invasive respiratory support.
§Not calculable because of zero events.
¶Data on antibiotics from self-report only.
**Acute worsening of asthma symptoms requiring treatment with oral corticosteroids and/or requiring hospital treatment.
††Acute worsening of COPD symptoms requiring treatment with oral corticosteroids and/or antibiotics and/or requiring hospital treatment.
‡‡End study 25(OH)D concentrations available for 1789 participants (741 randomised to 3200 IU/day, 742 randomised to 800 IU/day, 306 randomised to no offer).
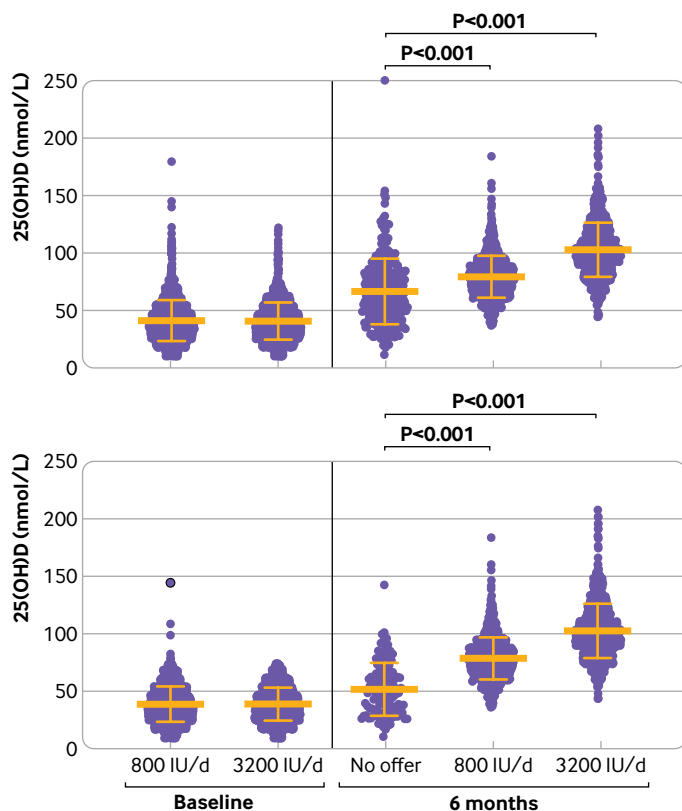
Fig 2 | 25-hydroxyvitamin D (25(OH)D) concentrations by time point and allocation for participants included in the intention-to-treat analysis (top panel) and for participants included in the sensitivity analysis (bottom panel), which excludes data from those randomised to either intervention arm who reported taking vitamin D capsules less than half the time, and those randomised to the no offer arm who reported any intake of supplemental vitamin D during follow-up. Bars show mean and standard deviation for each group. P values are from unpaired Student's *t* tests

Primary and secondary outcomes

Overall, 299 participants experienced the primary end point of at least one episode of swab test confirmed or doctor confirmed acute respiratory tract infection. Compared with the no offer group, no statistically significant difference was found in proportions of participants with these events in the lower dose offer arm (87/1515 (5.7%) *v* 136/2949 (4.6%); 1.26, 0.96 to 1.66, P=0.10) or higher dose offer arm (76/1515 (5.0%) *v* 136/2949 (4.6%); odds ratio 1.09, 95% confidence interval 0.82 to 1.46, P=0.55; table 2).

No statistically significant differences in outcomes relating to incidence or severity of acute covid-19 or prolonged symptoms of covid-19 were seen between those randomised to either the lower or the higher dose offer group compared with the no offer group (table 2). We also found no evidence to suggest that allocation to either offer compared with no offer influenced prescription of antibiotics for acute respiratory tract infection, admission to hospital, or death from all cause acute respiratory tract infection, or incidence of acute exacerbations of asthma or chronic obstructive pulmonary disease (table 2).

Subgroup analyses

In subgroup analysis we found no evidence to suggest that covid-19 vaccination modified the effect of allocation on incidence of covid-19 or prolonged covid-19 symptoms (see supplementary table S5). Planned subgroup analysis by baseline vitamin D status was not conducted, as the range and distribution of imputed 25(OH)D concentrations in the no offer arm at baseline did not match those of measured concentrations in participants randomised to the lower dose or higher dose arm (see supplementary figure S1), questioning the validity of the imputation. Exploratory survival analysis to determine effects of allocation before versus after administration of a first dose of covid-19 vaccine did not show protective effects of the intervention during either phase of follow-up (for lower dose versus no offer, pre-vaccination phase, hazard ratio 1.37, 95% confidence interval 0.90 to 2.07, P=0.14; post-vaccination phase, 1.68, 0.87 to 3.22, P=0.12; for higher dose versus no offer, pre-vaccination phase, 0.93, 0.58 to 1.49, P=0.73; post-vaccination phase, 1.57, 0.81 to 3.05, P=0.18; see supplementary figure S2).

Sensitivity analyses

Of 3100 people randomised to the no offer group, 1547 (49.9%) reported taking supplemental vitamin D on at least one occasion during the study, whereas 2523/2674 (94.4%) participants supplied with study supplements reported taking the supplements more than half the time. Results of sensitivity analyses excluding the former group and including the latter (table 3) were not materially different to those yielded by intention-to-treat analyses (table 2). A post hoc exploratory analysis comparing mean end study 25(OH)D concentrations in participants in the no offer arm who contributed data to the intention-to-treat analysis but not to the sensitivity analysis (ie, participants randomised to no offer who reported use of off-trial vitamin D supplements) compared with those who contributed data to both the intention-to-treat and the sensitivity analyses (ie, participants randomised to no offer who reported no use of off-trial vitamin D supplements) showed mean end trial 25(OH)D concentrations to be higher in the former group compared with the latter group (74.5 *v* 53.7 nmol/L, 95% confidence interval for difference, 14.6 to 27.0 nmol/L, P<0.001). A post hoc exploratory sensitivity analysis evaluating the effect of allocation among participants who completed all six follow-up questionnaires showed null results (higher dose *v* no offer, odds ratio 1.01, 95% confidence interval 0.73 to 1.39, P=0.96; lower dose *v* no offer 1.16, 0.85 to 1.56, P=0.35).

Adverse events

Seven participants (four in the no offer group, one in the lower dose group, and two in the higher dose group) died during the study, and 313 (143 in the no offer group and 85 in each of the intervention groups) experienced one or more serious adverse events (see supplementary

Table 3 | Primary and secondary outcomes, by allocation: sensitivity analyses.* Values are percentages (number with event/number in group) unless specified otherwise

	No offer	800 IU/day v no offer					3200 IU/day v no offer				
		800 IU/day offer	Odds ratio (95% CI)	P value	Mean difference (95% CI)	P value	3200 IU/day offer	Odds ratio (95% CI)	P value	Mean difference (95% CI)	P value
Primary outcome											
≥1 swab test or doctor confirmed ARI of any cause†	4.4 (59/1331)	5.3 (66/1243)	1.21 (0.84 to 1.73)	0.30	–	–	4.3 (55/1269)	0.98 (0.67 to 1.42)	0.90	–	–
Secondary outcomes											
Acute covid-19 outcomes:											
Swab test confirmed covid-19‡	2.5 (34/1331)	3.1 (39/1243)	1.24 (0.77 to 1.97)	0.37	–	–	2.5 (32/1269)	0.99 (0.61 to 1.61)	0.96	–	–
Admitted to hospital for covid-19	1.5 (20/1331)	1.4 (17/1243)	0.91 (0.47 to 1.74)	0.77	–	–	1.6 (21/1269)	1.10 (0.59 to 2.04)	0.31	–	–
In-hospital ventilatory support for covid-19§	5.0 (1/20)	0.0 (0/17)	–¶	–	–	–	4.8 (1/21)	0.95 (0.55 to 16.29)	0.97	–	–
Deaths with covid-19	0.0 (0/1331)	0.0 (0/1243)	–¶	–	–	–	0.0 (0/1269)	–¶	–	–	–
Long covid outcomes:											
Swab test confirmed covid-19: symptoms for >4 weeks‡	26.5 (9/34)	35.9 (14/39)	1.56 (0.57 to 4.25)	0.39	–	–	31.2 (10/32)	1.26 (0.43 to 3.67)	0.67	–	–
Swab test confirmed covid-19: symptoms at study end	5.9 (2/34)	20.5 (8/39)	4.13 (0.81 to 21.00)	0.09	–	–	18.7 (6/32)	3.69 (0.69 to 19.85)	0.13	–	–
Mean (SD) MRC dyspnoea score (No)	1.00** (1)	1.29 (0.76) (7)	–	–	0.29††	–	2.29 (0.49) (7)	–	–	1.29††	–
Mean (SD) FACIT Fatigue Scale score (No)	34.00** (1)	23.00 (4.69) (6)	–	–	–11.00††	–	25.29 (4.53) (7)	–	–	–8.71††	–
Mean (SD) Post-COVID Physical Health Symptom Score (No)	29.00** (1)	30.50 (11.33) (6)	–	–	1.50††	–	30.57 (10.50) (7)	–	–	1.57††	–
All cause ARI outcomes*:											
≥1 courses of antibiotics for ARI of any cause†§§	1.0 (13/1331)	0.3 (4/1243)	0.33 (0.11 to 1.00)	0.05	–	–	0.6 (7/1269)	0.56 (0.22 to 1.41)	0.22	–	–
Admitted to hospital for ARI of any cause*	0.4 (6/1331)	0.2 (3/1243)	0.53 (0.13 to 2.14)	0.38	–	–	0.8 (10/1269)	1.75 (0.64 to 4.84)	0.28	–	–
Death due to ARTI of any cause†	0.0 (0/1331)	0.0 (0/1243)	–¶	–	–	–	0.0 (0/1269)	–¶	–	–	–
Airways disease outcomes:											
≥1 severe acute asthma exacerbation in patients with asthma ‡‡	6.1 (11/180)	3.3 (7/210)	0.53 (0.20 to 1.40)	0.20	–	–	4.7 (8/169)	0.76 (0.30 to 1.95)	0.57	–	–
≥1 severe acute COPD exacerbation in patients with COPD¶¶	17.9 (5/28)	25.0 (6/24)	1.53 (0.40 to 5.84)	0.53	–	–	4.5 (1/22)	0.22 (0.02 to 2.03)	0.18	–	–
Biochemical outcome:											
Mean (SD) end study 25(OH)D concentration (nmol/L)***(No)	53.7 (23.1) (116)	79.5 (18.3) (736)	–	–	25.8 (22.0 to 29.5)	<0.001	103.4 (23.3) (729)	–	–	49.7 (45.1 to 54.2)	<0.001

ARI=acute respiratory tract infection; CI=confidence interval; COPD=chronic obstructive pulmonary disease; FACIT=Functional Assessment of Chronic Illness Therapy; MRC=Medical Research Council; 25(OH)D=25-hydroxyvitamin D.

*Excludes data from participants randomised to either supplement arm who reported taking vitamin D capsules less than half the time as well as those randomised to no offer arm who reported any intake of supplemental vitamin D during follow-up.

†Includes covid-19 and other ARIs.

‡Confirmed using reverse transcriptase polymerase chain reaction and/or antigen testing for SARS-CoV-2.

§Invasive and non-invasive respiratory support.

¶Not calculable because of zero events.

**Standard deviation not calculable as only one participant had the outcome in the no offer arm.

††95% confidence interval not calculable as the no offer arm had one participant with outcome.

‡‡Acute worsening of asthma symptoms requiring treatment with oral corticosteroids and/or requiring hospital treatment.

§§Data on antibiotics for acute respiratory tract infection from self-report only.

¶¶Acute worsening of COPD symptoms requiring treatment with oral corticosteroids and/or antibiotics and/or requiring hospital treatment.

***End study 25(OH)D concentrations available for 1789 participants (741 randomised to 3200 IU/day, 742 randomised to 800 IU/day, 306 randomised to no offer).

table S6). Supplementary table S7 lists these events: none were considered to be related to vitamin D supplementation. Four participants in the higher dose group developed hypercalcaemia (serum corrected calcium >2.65 mmol/L): the study supplements were discontinued, and the hypercalcaemia and symptoms resolved. One participant in the no offer group had asymptomatic hypervitaminosis D (25(OH)D concentration 250 nmol/L) at six month follow-up, after taking a non-study vitamin D supplement at a dose of 4000 IU/day. One participant in the higher dose group was twice admitted to hospital with renal colic due to nephrolithiasis. A total of 47 non-severe adverse events led to discontinuation of study supplements (23 v 24 in lower and higher dose offer groups, respectively; see supplementary table S8).

Discussion

We present results of the first phase 3 randomised controlled trial to evaluate the effectiveness of a test-and-treat approach for correction of suboptimal vitamin D status to prevent acute respiratory tract infections. The trial also investigated whether vitamin D supplementation is associated with a reduction in risk of covid-19. Among participants randomised to receive an offer of postal vitamin D testing, uptake of testing and supplementation was good, prevalence of 25(OH)D concentrations <75 nmol/L was high, and end study 25(OH)D concentrations were increased compared with the group not offered testing or supplementation, providing objective evidence of a high level of adherence. However, no statistically significant effect of either dose was seen on the primary outcome of swab test or doctor confirmed acute respiratory tract infection, or on the major secondary outcome of swab test confirmed covid-19. Oral vitamin D supplementation was safe and well tolerated at both doses investigated: incidence of adverse events was balanced between arms, and no serious adverse event was attributed to study supplements.

Comparison with other studies

The design of our study was informed by findings from a recent meta-analysis, suggesting that protective effects of vitamin D against acute respiratory tract infection might be strongest when daily doses of 400-1000 IU were given for up to one year.[22] The results from the current study do not support the hypothesis that such regimens offer protection against acute respiratory tract infection, and our null findings are consistent with those of several other recent phase 3 trials of vitamin D supplementation that have reported no effect of vitamin D supplementation on risk of acute respiratory tract infections.[20 21 39] The null result for the major secondary outcome of incident covid-19 in this trial is consistent with our finding of no independent association between intake of supplementary vitamin D and risk of covid-19 in a prospective observational study undertaken in this cohort before initiation of this trial,[12] as well as null results from a mendelian

randomisation study that tested for associations between genetically predicted 25-hydroxyvitamin D concentrations and susceptibility to covid-19.[40] However, the result contrasts with recent findings from a phase 2 randomised controlled trial in healthcare workers in Mexico, which reported a strong protective effect of a daily dose of 4000 IU vitamin D.[23] The difference in findings might be because participants in the Mexican trial had not been vaccinated against SARS-CoV-2, or it may reflect the relatively short duration of follow-up (one month). The former hypothesis is not supported by results from subgroup analysis in the current study, showing no effect of vitamin D on risk of covid-19 either before or after covid-19 vaccination.

Strengths and limitations of this study

Our study has several strengths. In contrast with some recent large clinical trials of vitamin D supplementation for the prevention of acute respiratory tract infections,[21 39] the prevalence of suboptimal vitamin D status at baseline in our study population was high, with 25(OH)D concentrations <75 nmol/L in 97.4% of those tested. We investigated daily dosing regimens (thereby avoiding large and non-physiological fluctuations in 25(OH)D that are seen with intermittent bolus dosing),[41] and adherence was good (evidenced by self-report and by statistically significant differences in end study 25(OH)D concentrations between arms). The trial-within-cohort design allowed a rapid and efficient evaluation of a pragmatic approach to boosting vitamin D status in the general population to provide a timely answer to the pressing question of whether or not vitamin D supplements reduce risk of covid-19. Linkage with routinely collected data from medical records allowed comprehensive capture of outcomes in those who did not complete study questionnaires, allowing us to minimise loss to follow-up and to capture important events that precluded completion of questionnaires, such as severe illness and death. The trial was initiated before the widespread roll-out of covid-19 vaccination, and follow-up coincided with the second wave of covid-19 in the UK: both factors contributed to the appreciable number of participants who experienced covid-19 during follow-up, which allowed for potential effects of vitamin D on prevention of this specific cause of acute respiratory tract infection to be investigated. Other strengths include a rigorous case definition for the primary outcome that required objective confirmation of acute respiratory tract infection (as opposed to self-report of symptoms) and use of an externally accredited laboratory to measure 25(OH)D concentrations using liquid chromatography-tandem mass spectrometry, which is the gold standard assay for this determination.

Our study also has limitations. Provision of supplements to participants randomised to intervention was contingent on their having inadequate vitamin D status: thus, a subset (13.7%) of participants randomised to intervention did not receive study supplements. Conversely, another subset (49.9%) of participants randomised to the no offer

of supplementation group reported taking a vitamin D supplement on one or more occasions during follow-up. This could have led to increases in 25(OH)D concentrations in the no offer arm over the course of the study, although seasonal effects (sampling in June versus in December) will also have contributed. Together, these factors could have diluted any effect of vitamin D supplementation in the primary intention-to-treat analysis. We sought to overcome this by conducting a sensitivity analysis, which included only those randomised to offer versus no offer who did versus did not take supplemental vitamin D, respectively. That this analysis showed no effect of vitamin D supplementation on all outcomes investigated provides some reassurance that the null result yielded by the intention-to-treat analysis is valid. Ultimately, however, this trial was designed to investigate the effectiveness of a pragmatic test-and-treat approach to boosting population vitamin D status, rather than biological efficacy of vitamin D to prevent acute respiratory tract infections, and our findings should be interpreted accordingly: specifically, we highlight that our results are not inconsistent with findings from meta-analyses of placebo controlled trials of vitamin D to prevent acute respiratory tract infection,[22 42] which better address questions of efficacy. The open label design might have introduced ascertainment bias by influencing the likelihood of participants completing follow-up questionnaires. This potential problem was offset by the use of medical record linkage, which allowed us to capture outcomes in participants who did not complete all follow-up questionnaires. Moreover, a post hoc sensitivity analysis restricted to participants who completed all follow-up questionnaires yielded null results, consistent with those of the primary analysis. The proportion of those randomised to the no offer arm who experienced the primary outcome (4.6%) was lower than the 20% anticipated in the sample size calculation, possibly reflecting the impact of public health measures to control transmission of SARS-CoV-2 (such as lockdowns, social distancing, and mask wearing) on incidence of other acute respiratory tract infections.[43] Alternatively, it might reflect a reduction in consultations for acute respiratory tract infection because of participants' reluctance to attend a doctor's surgery with respiratory symptoms, as face-to-face consultations were actively discouraged during the pandemic, and the general public being reluctant to over-burden an already over-stretched health service.[44 45] This could have compromised power; however, the lower bounds for the 95% confidence intervals of odds ratios relating to the effect of lower and higher dose vitamin D supplements on our primary outcome (0.96 and 0.82, respectively) effectively rule out relative reductions in odds of acute respiratory tract infection of more than 4% and 18%, respectively. Some may not consider effects of this size or less to be of sufficient magnitude to implement the study intervention for the purpose of preventing acute respiratory tract infections. Incidence of some secondary outcomes, including admission to hospital for acute respiratory tract infection, was low: our study therefore lacked power to detect an effect of the intervention on severity of covid-19 and other acute respiratory tract infections. Prevalence of profound vitamin D deficiency (25(OH)D <25 nmol/L) at baseline was also low, and therefore our study lacked power to detect an effect of the intervention in participants in this group, who may be more likely to derive clinical benefit from vitamin D supplementation than those with higher baseline 25(OH)D concentrations.[46] Men, people from ethnic minorities, and those with lower educational attainment were relatively under-represented among study participants compared with the general population, which may have compromised the generalisability of our findings. We also highlight that other groups at increased risk of severe covid-19 were over-represented among trial participants, of whom 35.6% were aged ≥65 years (compared with 18.3% of the UK population)[47] and 19.5% had heart disease (compared with 3-4% of the UK population).[48] This over-representation, along with the presence of many participants who were unvaccinated or partially vaccinated during follow-up, might explain why a relatively high proportion of participants with covid-19 required admission to hospital.

Conclusions

This study found that implementation of a test-and-treat approach to correct suboptimal vitamin D status in the UK population was safe and effective in increasing 25(OH)D concentrations in people aged 16 years and older with baseline concentrations <75 nmol/L. This was not, however, associated with protection against all cause acute respiratory tract infection or covid-19.

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1 Wouters OJ, Shadlen KC, Salcher-Konrad M, et al. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. *Lancet* 2021;397:1023-34. doi:10.1016/S0140-6736(21)00306-8

2 Dror AA, Eisenbach N, Taiber S, et al. Vaccine hesitancy: the next challenge in the fight against COVID-19. *Eur J Epidemiol* 2020;35:775-9. doi:10.1007/s10654-020-00671-y

3 Williams TC, Burgers WA. SARS-CoV-2 evolution and vaccines: cause for concern? *Lancet Respir Med* 2021;9:333-5. doi:10.1016/S2213-2600(21)00075-8

4 Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015;7:4240-70. doi:10.3390/nu7064240

5 L Bishop E, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. *JBMR Plus* 2020;5:e10405. doi:10.1002/jbm4.10405

6 Chauss D, Freiwald T, McGregor R, et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of T_H1 cells. *Nat Immunol* 2022;23:62-74. doi:10.1038/s41590-021-01080-3

7 Roth A, Lütke S, Meinberger D, et al. LL-37 fights SARS-CoV-2: The Vitamin D-Inducible Peptide LL-37 Inhibits Binding of SARS-CoV-2 Spike Protein to its Cellular Receptor Angiotensin Converting Enzyme 2 In Vitro. *BioRxiv* 2020. doi:10.1101/2020.12.02.408153

8 Zhang L, Ghosh SK, Basavarajappa SC, et al. Molecular dynamics simulations and functional studies reveal that hBD-2 binds SARS-CoV-2 spike RBD and blocks viral entry into ACE2 expressing cells. *bioRxiv* 2021. doi:10.1101/2021.01.07.425621

9 Merzon E, Tworowski D, Gorohovski A, et al. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *FEBS J* 2020;287:3693-702. doi:10.1111/febs.15495

10 Raisi-Estabragh Z, McCracken C, Bethell MS, et al. Greater risk of severe COVID-19 in Black, Asian and Minority Ethnic populations is not explained by cardiometabolic, socioeconomic or behavioural factors, or by 25(OH)-vitamin D status: study of 1326 cases from the UK Biobank. *J Public Health (Oxf)* 2020;42:451-60. doi:10.1093/pubmed/fdaa095

11 Ma H, Zhou T, Heianza Y, Qi L. Habitual use of vitamin D supplements and risk of coronavirus disease 2019 (COVID-19) infection: a prospective study in UK Biobank. *Am J Clin Nutr* 2021;113:1275-81. doi:10.1093/ajcn/nqaa381

12 Holt H, Talaei M, Greenig M, et al. Risk factors for developing COVID-19: a population-based longitudinal study (COVIDENCE UK). *Thorax* 2021; published online 30 Nov. thoraxjnl-2021-217487. doi:10.1136/thoraxjnl-2021-217487

13 Talaei M, Faustini S, Holt H, et al. Determinants of pre-vaccination antibody responses to SARS-CoV-2: a population-based longitudinal study (COVIDENCE UK). *BMC Med* 2022;20:87. doi:10.1186/s12916-022-02286-4

14 Louca P, Murray B, Klaser K, et al. Modest effects of dietary supplements during the COVID-19 pandemic: insights from 445 850 users of the COVID-19 Symptom Study app. *BMJ Nutr Prev Health* 2021;4:149-57. doi:10.1136/bmjnph-2021-000250

15 Chiodini I, Gatti D, Soranna D, et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. *Front Public Health* 2021;9:736665. doi:10.3389/fpubh.2021.736665

16 Dissanayake HA, de Silva NL, Sumanatilleke M, et al. Prognostic and Therapeutic Role of Vitamin D in COVID-19: Systematic Review and Meta-analysis. *J Clin Endocrinol Metab* 2022;107:1484-502. doi:10.1210/clinem/dgab892

17 Camargo CAJr, Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. *Pediatrics* 2012;130:e561-7. doi:10.1542/peds.2011-3029

18 Bergman P, Norlin AC, Hansen S, et al. Vitamin D3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study. *BMJ Open* 2012;2:e001663. doi:10.1136/bmjopen-2012-001663

19 Martineau AR, Hanifa Y, Witt KD, et al. Double-blind randomised controlled trial of vitamin D3 supplementation for the prevention of acute respiratory infection in older adults and their carers (ViDiFlu). *Thorax* 2015;70:953-60. doi:10.1136/thoraxjnl-2015-206996

20 Ganmaa D, Uyanga B, Zhou X, et al. Vitamin D Supplements for Prevention of Tuberculosis Infection and Disease. *N Engl J Med* 2020;383:359-68. doi:10.1056/NEJMoa1915176

21 Pham H, Waterhouse M, Baxter C, et al. The effect of vitamin D supplementation on acute respiratory tract infection in older Australian adults: an analysis of data from the D-Health Trial. *Lancet Diabetes Endocrinol* 2021;9:69-81. doi:10.1016/S2213-8587(20)30380-6

22 Jolliffe DA, Camargo CAJr, Sluyter JD, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. *Lancet Diabetes Endocrinol* 2021;9:276-92. doi:10.1016/S2213-8587(21)00051-6

23 Villasis-Keever MA, López-Alarcón MG, Miranda-Novales G, et al. Efficacy and Safety of Vitamin D Supplementation to Prevent COVID-19 in Frontline Healthcare Workers. A Randomized Clinical Trial. *Arch Med Res* 2022;53:423-30. doi:10.1016/j.arcmed.2022.04.003

24 Relton C, Torgerson D, O'Cathain A, Nicholl J. Rethinking pragmatic randomised controlled trials: introducing the "cohort multiple randomised controlled trial" design. *BMJ* 2010;340:c1066. doi:10.1136/bmj.c1066

25 Williamson AE, Tydeman F, Miners A, et al. Acute and long-term impacts of COVID-19 on economic vulnerability: a population-based longitudinal study (COVIDENCE UK).*MedRxiv* 2022. doi:10.1101/2022.03.03.22271835

26 Holt H, Relton C, Talaei M, et al. Cohort Profile: Longitudinal population-based study of COVID-19 in UK adults (COVIDENCE UK). *medRxiv* 2022 https://doi.org/10.1101/2022.06.20.22276205

27 Shea RL, Berg JD. Self-administration of vitamin D supplements in the general public may be associated with high 25-hydroxyvitamin D concentrations. *Ann Clin Biochem* 2017;54:355-61. doi:10.1177/0004563216662073

28 Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005;16:713-6. doi:10.1007/s00198-005-1867-7

29 Vieth R. What is the optimal vitamin D status for health?*Prog Biophys Mol Biol* 2006;92:26-32. doi:10.1016/j.pbiomolbio.2006.02.003

30 Bischoff-Ferrari HA. The 25-hydroxyvitamin D threshold for better health. *J Steroid Biochem Mol Biol* 2007;103:614-9. doi:10.1016/j.jsbmb.2006.12.016

31 Fletcher CM. Standardised questionnaire on respiratory symptoms: a statement prepared and approved by the MRC Committee on the Aetiology of Chronic Bronchitis (MRC breathlessness score). *BMJ* 1960;2:1665.

32 Yellen SB, Cella DF, Webster K, Blendowski C, Kaplan E. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. *J Pain Symptom Manage* 1997;13:63-74. doi:10.1016/S0885-3924(96)00274-6

33 Taylor RR, Trivedi B, Patel N, et al. Post-COVID symptoms reported at asynchronous virtual review and stratified follow-up after COVID-19 pneumonia. *Clin Med (Lond)* 2021;21:e384-91. doi:10.7861/clinmed.2021-0037

34 Grayling MJ, Wason JM. A web application for the design of multi-arm clinical trials. *BMC Cancer* 2020;20:80. doi:10.1186/s12885-020-6525-0

35 Vickerstaff V, Omar RZ, Ambler G. Methods to adjust for multiple comparisons in the analysis and sample size calculation of randomised controlled trials with multiple primary outcomes. *BMC Med Res Methodol* 2019;19:129. doi:10.1186/s12874-019-0754-4

36 Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. *J Am Stat Assoc* 1955;50:1096-121. doi:10.1080/01621459.1955.10501294.

37 Liu E, Meigs JB, Pittas AG, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. *Am J Clin Nutr* 2010;91:1627-33. doi:10.3945/ajcn.2009.28441

38 Breiman L. Random Forests. *Mach Learn* 2001;45:5-32. doi:10.1023/A:1010933404324.

39 Camargo CA, Sluyter J, Stewart AW, et al. Effect of monthly high-dose vitamin D supplementation on acute respiratory infections in older adults: A randomized controlled trial. *Clin Infect Dis* 2020;71:311-7. doi:10.1093/cid/ciz801

40 Butler-Laporte G, Nakanishi T, Mooser V, et al. Vitamin D and COVID-19 susceptibility and severity in the COVID-19 Host Genetics Initiative: A Mendelian randomization study. *PLoS Med* 2021;18:e1003605. doi:10.1371/journal.pmed.1003605

41 Ilahi M, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. *Am J Clin Nutr* 2008;87:688-91. doi:10.1093/ajcn/87.3.688

42 Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;356:i6583. doi:10.1136/bmj.i6583

43 Redlberger-Fritz M, Kundi M, Aberle SW, Puchhammer-Stöckl E. Significant impact of nationwide SARS-CoV-2 lockdown measures on the circulation of other respiratory virus infections in Austria. *J Clin Virol* 2021;137:104795. doi:10.1016/j.jcv.2021.104795

44 Howarth A, Munro M, Theodorou A, Mills PR. Trends in healthcare utilisation during COVID-19: a longitudinal study from the UK. *BMJ Open* 2021;11:e048151. doi:10.1136/bmjopen-2020-048151

45 Moynihan R, Sanders S, Michaleff ZA, et al. Impact of COVID-19 pandemic on utilisation of healthcare services: a systematic review. *BMJ Open* 2021;11:e045343. doi:10.1136/bmjopen-2020-045343

46 Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol* 2021;9:837-46. doi:10.1016/S2213-8587(21)00263-1

47 Office for National Statistics. Overview of the UK population: August 2019. 2019

48 Bhatnagar P, Wickramasinghe K, Wilkins E, Townsend N. Trends in the epidemiology of cardiovascular disease in the UK. *Heart* 2016;102:1945-52. doi:10.1136/heartjnl-2016-309573.

Supplementary information: study staff, additional tables 1-8, and additional figures 1 and 2