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Vitamin D supplementation to prevent acute respiratory infections: systematic review and meta-analysis of aggregate data from randomised controlled trials

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Abstract

Background: A 2017 meta-analysis of data from 25 randomised controlled trials of vitamin D supplementation for the prevention of acute respiratory infections revealed a protective effect of the intervention. Since then, 21 new RCTs have been completed.

Methods: Systematic review and meta-analysis of data from randomised controlled trials (RCTs) of vitamin D for ARI prevention using a random effects model. Sub-group analyses were done to determine whether effects of vitamin D on risk of ARI varied according to baseline 25-hydroxyvitamin D (25[OH]D) concentration, dosing regimen or age. We searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science and the ClinicalTrials.gov registry from inception to 1st May 2020. Double-blind RCTs of supplementation with vitamin D or calcidiol, of any duration, were eligible if they were approved by a Research Ethics Committee and if ARI incidence was collected prospectively and pre-specified as an efficacy outcome. Aggregate data, stratified by baseline 25(OH)D concentration and age, were obtained from study authors. The study was registered with PROSPERO (no. CRD42020190633).

Findings: We identified 46 eligible RCTs (total 75,541 participants). Data were obtained for 48,488 (98.1%) of 49,419 participants in 43 studies, aged 0 to 95 years. For the primary comparison of vitamin D supplementation vs. placebo, the intervention reduced risk of ARI overall (Odds Ratio [OR] 0.92, 95% CI 0.86 to 0.99; 37 studies; I^2 35.6%; P for heterogeneity 0.02). No statistically significant effect of vitamin D was seen for any of the sub-groups defined by baseline 25(OH)D concentration. However, protective effects were seen in trials where vitamin D was given using a daily dosing regimen (OR 0.78, 95% CI 0.65 to 0.94; 19 studies; I^2 53.5%; P for heterogeneity 0.003), at daily dose equivalents of 400-1000 IU (OR 0.70, 95% CI 0.55 to 0.89; 10 studies; I^2 31.2%; P for heterogeneity 0.16), for a duration of ≤12 months (OR 0.82, 95% CI 0.72 to 0.93; 29 studies; I^2 38.1%; P for heterogeneity 0.02), and among participants aged 1.00 to 15.99 years at enrolment (OR 0.71, 95% CI 0.57 to 0.90; 15 studies; I^2 46.0%; P for heterogeneity 0.03). No significant interaction was seen between allocation to vitamin D vs. placebo and dose frequency, dose size, study duration or age. Vitamin D did not influence the proportion of participants experiencing at least one serious adverse event

(OR 0.97, 95% CI 0.86 to 1.07; 36 studies; I^2 0.0%; P for heterogeneity 0.99). Risk of bias within individual studies was assessed as being low for all but three trials. A funnel plot showed left-sided asymmetry (P=0.007, Egger's test).

Interpretation: Vitamin D supplementation was safe and reduced risk of ARI, despite evidence of significant heterogeneity across trials. Protection was associated with administration of daily doses of 400-1000 IU vitamin D for up to 12 months and age at enrolment of 1.00 to 15.99 years. The relevance of these findings to COVID-19 is not known and requires investigation.

Funding: None

Research in context

Evidence before this study

The active vitamin D metabolite, 1,25-dihydroxyvitamin D, induces innate immune responses to respiratory viruses and bacteria. A previous meta-analysis of individual participant data from 10,933 participants in 25 randomised controlled trials of vitamin D supplementation for the prevention of acute respiratory infection demonstrated an overall protective effect (adjusted Odds Ratio [aOR] 0.88, 95% confidence interval 0.81 to 0.96). Sub-group analysis revealed most benefit in those with the lowest vitamin D status at baseline who received daily or weekly supplementation (aOR 0.30, 0.17 to 0.53).

Added value of this study

Our meta-analysis of aggregate data from 48,488 participants in 43 randomised controlled trials, stratified by baseline 25(OH)D concentration, provides an updated estimate of the protective effects of vitamin D against acute respiratory infection overall, and in sub-groups defined by baseline vitamin D status and dosing frequency, amount and duration and age.

Implications of all the available evidence

Overall, vitamin D reduced the risk of having one or more acute respiratory infections (OR 0.92, 0.86 to 0.99), but there was evidence of significant heterogeneity across trials (P for heterogeneity 0.02). A funnel plot showed left-sided asymmetry, which may reflect publication bias and/or heterogeneity of effect across trials. No statistically significant effect of vitamin D was seen for any of the sub-groups defined by baseline 25(OH)D concentration. However, protective effects were seen in trials where vitamin D was given using a daily dosing regimen (OR 0.78, 0.65 to 0.94); at daily dose equivalents of 400-1000 IU (OR 0.70, 0.55 to 0.89); and for a duration of ≤12 months (OR 0.82, 0.72 to 0.93); and when vitamin D was given to children aged 1.00 to 15.99 years (OR 0.71, 95% CI 0.57 to 0.90). The relevance of these findings to COVID-19 is not known and requires investigation.

Introduction

Interest in the potential for vitamin D supplementation to reduce risk of acute respiratory infections (ARI) has increased since the emergence of the COVID-19 pandemic.[1] This stems from findings of laboratory studies, showing that vitamin D metabolites support innate immune responses to respiratory viruses,[2] together with observational studies reporting independent associations between low circulating levels of 25-hydroxyvitamin D (25[OH]D, the widely accepted biomarker of vitamin D status) and increased risk of ARI caused by other pathogens.[3,4] Randomised controlled trials (RCTs) of vitamin D for the prevention of ARI have produced heterogeneous results, with some showing protection, and others reporting null findings. We previously meta-analysed individual participant data from 10,933 participants in 25 RCTs[5-29] and showed a protective overall effect that was stronger in those with lower baseline 25(OH)D levels, and in trials where vitamin D was administered daily or weekly rather than in more widely spaced bolus doses.[30] Since the date of the final literature search performed for that study (December 2015), 21 RCTs with 64,220 participants fulfilling the same eligibility criteria have been completed.[31-51] We therefore sought data from these more recent studies for inclusion in an updated meta-analysis of stratified aggregate (trial-level) data to determine whether vitamin D reduced ARI risk overall, and to evaluate whether effects of vitamin D on ARI risk varied according to baseline 25(OH)D concentration, dosing regimen (frequency, dose size, and trial duration) or age at enrolment.

Methods

Protocol, Registration and Ethical Approvals

Methods were pre-specified in a protocol that was registered with the PROSPERO International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=190633). Research Ethics Committee approval to conduct this meta-analysis was not required in the UK; local ethical permission to contribute data from primary trials was required and obtained for studies by Camargo *et al*[13] (The Ethics Review Committee of the Mongolian Ministry of Health), Murdoch *et al*[14] (Southern Health and Disability Ethics Committee, ref. URB/09/10/050/AM02), Rees *et al*[17] (Committee for the Protection of Human Subjects, Dartmouth College, USA; Protocol # 24381), Tachimoto *et al*[28] (Ethics committee of the Jikei University School of Medicine, ref 26-333: 7839), Tran *et al*[18] (QIMR Berghofer Medical Research Institute Human Research Ethics Committee, P1570) and Urashima *et al*[6,20] (Ethics committee of the Jikei University School of Medicine, ref 26-333: 7839).

Eligibility Criteria

Randomised, double-blind, trials of supplementation with vitamin D_3, vitamin D_2 or 25(OH)D of any duration, with a placebo or low-dose vitamin D control, were eligible for inclusion if they had been approved by a Research Ethics Committee and if data on incidence of ARI were collected prospectively and pre-specified as an efficacy outcome. The latter requirement was imposed in order to minimise misclassification bias (prospectively designed instruments to capture ARI events were deemed more likely to be sensitive and specific for this outcome). Studies reporting results of long-term follow-up of primary RCTs were excluded.

Study Identification and Selection

Two investigators (ARM and DAJ) searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science and the ClinicalTrials.gov registry using the electronic search strategies described in the Methods Section of Supplementary Material. Searches were regularly updated up to and including 1st May

2020. No language restrictions were imposed. These searches were supplemented by searching review articles and reference lists of trial publications. Collaborators were asked if they knew of any additional trials. Three investigators (DAJ, CAC and ARM) determined which trials met the eligibility criteria.

Data Collection Processes

Summary data from trials which contributed to our previous meta-analysis of individual participant data[30] were extracted from our central database, with permission from the Principal Investigators. Summary data relating to the primary outcome (overall and by sub-group) and secondary outcomes (overall only) from newly identified trials were requested from Principal Investigators. On receipt, they were assessed for consistency with associated publications. Study authors were contacted to provide missing data and to resolve any queries arising from these consistency checks. Once queries had been resolved, clean summary data were uploaded to the study database, which was held in STATA IC v14.2 (StataCorp, College Station, TX).

Data relating to study characteristics were extracted for the following variables: study setting, eligibility criteria, 25(OH)D assay and levels, details of intervention and control regimens, trial duration, case definitions for ARI and number entering primary analysis (after randomisation). Follow-up summary data were requested for the proportions of participants experiencing one or more ARI during the trial, both overall and stratified by baseline serum 25(OH)D concentration, where this was available. We also requested summary data on the proportions of participants who experienced one or more of the following events during the trial: upper respiratory infection (URI); lower respiratory infection (LRI); Emergency Department attendance and/or hospital admission for ARI; death due to ARI or respiratory failure; use of antibiotics to treat an ARI; absence from work or school due to ARI; a serious adverse event; death due to any cause; and potential adverse reactions to vitamin D (hypercalcaemia and renal stones).

Risk of Bias Assessment for Individual Studies

We used the Cochrane Collaboration Risk of Bias tool[52] to assess the following variables: sequence generation, allocation concealment, blinding of participants,

personnel and outcome assessors, completeness of outcome data, evidence of selective outcome reporting and other potential threats to validity. Study quality was assessed independently by two investigators (ARM and DAJ), except for the six trials for which DAJ and/or ARM were investigators, which were assessed by CAC and JDS. Discrepancies were resolved by consensus.

Definition of outcomes

The primary outcome of the meta-analysis was the proportion of participants experiencing one or more ARIs, with the definition of ARI encompassing events classified as URI, LRI and ARI of unclassified location (i.e. infection of the upper and/or lower respiratory tract). Secondary outcomes were incidence of URI and LRI, analysed separately; incidence of Emergency Department attendance and/or hospital admission for ARI; death due to ARI or respiratory failure; use of antibiotics to treat an ARI; absence from work or school due to ARI; incidence of serious adverse events; death due to any cause; and incidence of potential adverse reactions to vitamin D (hypercalcaemia and renal stones).

Synthesis Methods

Data were analysed by DAJ; results were checked and verified by JDS. Our meta-analysis approach followed published guidelines.[53] The primary comparison was of participants randomised to vitamin D vs. placebo: this was performed for all of the outcomes listed above. For trials that included higher-dose, lower-dose and placebo arms, data from higher-dose and lower-dose arms were pooled for analysis of the primary comparison. A secondary comparison of participants randomised to higher vs. lower doses of vitamin D was performed for the primary outcome only. A log odds ratio and its standard error was calculated for each outcome within each trial from the proportion of participants experiencing one or more events in the intervention vs. control arm. These were meta-analysed in a random effects model using the Metan package[54] within STATA IC v14.2 to obtain a pooled odds ratio with a 95% confidence interval and a measure of heterogeneity summarized by the I^2 statistic and its corresponding P value.

Exploration of variation in effects

To explore reasons for heterogeneity of effect of the intervention between trials we performed a stratified analysis according to baseline vitamin D status (serum 25[OH]D <25 *vs.* 25-49.9 *vs.* 50-74.9 *vs.* ≥75 nmol/L) and according to age at baseline (<1.00 *vs.* 1.00-15.99 *vs.* 16.00-64.99 *vs.* ≥65.00 years). We additionally conducted sub-group analyses according to vitamin D dosing regimen (administration of daily *vs.* weekly *vs.* monthly or less frequent doses), dose size (daily equivalent <400 IU *vs.* 400-1000 IU *vs.* 1001-2000 IU *vs.* >2,000 IU), trial duration (≤12 months *vs.* >12 months) and presence of airway disease (trial restricted to participants with asthma *vs.* those restricted to participants with COPD *vs.* those in which participants without airway disease were eligible). The thresholds for baseline 25(OH)D concentration used in sub-group analyses were selected *a priori* on the basis that they represent cut-offs that are commonly used to distinguish profound vitamin D deficiency (<25 nmol/L), moderate vitamin D deficiency (25-49.9 nmol/L) and sub-optimal vitamin D status (50-74.9 nmol/L).[55] An exploratory analysis restricted to studies with optimal dosing frequency, dose size and duration was also performed.

To investigate factors associated with heterogeneity of effect between subgroups of trials, we performed multivariable meta-regression analysis on trial-level characteristics, namely, dose frequency, dose size, trial duration and age at enrolment, to produce an adjusted odds ratio, a 95% confidence interval and a P value for interaction for each factor. Independent variables were dichotomised to create a more parsimonious model (baseline serum 25(OH)D of <25 vs. ≥25 nmol/L; administration of daily vs. non-daily doses; daily equivalent of ≤1000 IU vs. >1000 IU; trial duration of ≤12 vs. >12 months, and participant age of <16.00 vs. ≥16.00 years at enrolment). The meta-regression analysis excluded data from two placebo-controlled trials that included higher-dose, lower-dose and placebo arms[18,48] (since the higher-dose and lower-dose arms in these studies spanned the 1,000 IU/day cut-off), and four placebo-controlled trials that enrolled participants aged below and above the age cut-off of 16 years.[9,20,26,36] These factors rendered these trials unclassifiable for the purposes of the meta-regression analysis.

Quality Assessment Across Studies

For the primary analysis, the likelihood of publication bias was investigated through the construction of a contour-enhanced funnel plot.[56] We used the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias)[57] to assess the quality of the body of evidence contributing to analyses of the primary efficacy outcome and major secondary outcomes of our meta-analysis.

Sensitivity analyses

We conducted three exploratory sensitivity analyses for the primary comparison of the primary outcome: one excluded RCTs where risk of bias was assessed as being unclear; one excluded RCTs in which incidence of ARI was not the primary or co-primary outcome; and one substituted diary-defined ARI events (available for 2598 participants) for survey-defined ARI events (available for n=16,000 participants) from the trial by Pham et al.[45]

Role of the funding source

This study was conducted without external funding.

Results

Study selection and data obtained

The study selection process is illustrated in Figure 1. Our search identified a total of 1,528 unique studies that were assessed for eligibility, of which 46 studies with a total of 75,541 randomised participants fulfilled eligibility criteria. Studies for which full text was reviewed prior to exclusion due to ineligibility are listed in Table S1. Of the 46 eligible studies identified, 35 compared effects of a single vitamin D regimen vs. placebo only,[5-17,19,20,22,23,25-28,31,33,36,38,39,41-46,49-51] 5 compared effects of higher-dose, lower-dose and placebo arms,[18,21,24,40,48] and 6 compared effects of higher- vs. lower-dose regimens of vitamin D only.[29,32,34,35,37,47] Stratified aggregate data were sought and obtained for all but 3 eligible studies.[49-51] Data for the primary outcome (proportion of participants with one or more ARI) were obtained for 48,488 (98.1%) of 49,419 participants in 43 studies.[5-29,31-48]

Study and participant characteristics

Characteristics of the 43 studies contributing data to this meta-analysis and their participants are presented in Table 1. Trials were conducted in 23 different countries on 5 continents, and enrolled participants of both sexes from birth to 95 years of age. Baseline serum 25(OH)D concentrations were determined in 35 of 43 trials: mean baseline 25(OH)D concentration ranged from 18.9 to 90.9 nmol/L (to convert to ng/ml, divide by 2.496). Forty-two studies administered oral vitamin D_3 to participants in the intervention arm, while 1 study administered oral 25(OH)D. Vitamin D was given as monthly to 3-monthly bolus doses in 13 studies; as weekly doses in 6 studies; as daily doses in 22 studies; and as a combination of bolus and daily doses in 2 studies. Trial duration ranged from 8 weeks to 5 years. Incidence of ARI was primary or co-primary outcome for 23 studies, and a secondary outcome for 20 studies.

Risk of Bias Within Studies

Details of the risk of bias assessment are provided in supplementary Table S2. Four trials were assessed as being at unclear risk of bias due to high loss to follow-up. In the trial by Laaksi and colleagues,[8] 37% of randomised participants were lost to follow-up. In the trial by Dubnov-Raz and colleagues,[26] 52% of participants did not complete all symptom questionnaires. In the unpublished trial by Reyes and colleagues,[48] loss to follow-up ranged from 33% to 37% across the three study arms, and in the unpublished trial by Golan-Tripto and colleagues,[47] 50% of participants were lost to follow-up. All other trials were assessed as being at low risk of bias for all seven aspects assessed.

Overall Results, Primary Outcome

For the primary comparison of vitamin D vs. placebo control, supplementation resulted in a statistically significant reduction in the proportion of participants experiencing at least one ARI (Odds Ratio [OR] 0.92, 95% Confidence Interval [CI] 0.86 to 0.99; 46,166 participants in 37 studies; Figure 2, Table 2; Cates Plot, Figure S1). Heterogeneity of effect was moderate (I^2 35.6%, P for heterogeneity 0.02).

For the secondary comparison of higher- vs. lower-dose vitamin D, we observed no statistically significant difference in the proportion of participants with at least one ARI (OR 0.87, 95% CI 0.73 to 1.04; 3,047 participants in 11 studies; I^2 0.0%, P for heterogeneity 0.50; Figure S2).

Sub-group analyses, Primary Outcome

To investigate reasons for the observed heterogeneity of effect for the primary comparison of vitamin D vs. placebo control, we stratified this analysis by two participant-level factors (baseline vitamin D status and age) and by four trial-level factors (dose frequency, dose size, trial duration, and airway disease comorbidity). Results are presented in Table 2 and Figures S3-S8. No statistically significant effect of vitamin D was seen for participants with baseline 25(OH)D <25 nmol/L (OR 0.81, 95% CI 0.57 to 1.15; 3,777 participants in 20 studies), 25-49.9 nmol/L (OR 1.04, 95% CI 0.94 to 1.15; 9,896 participants in 29 studies), 50-74.9 nmol (OR 0.88, 95% CI 0.76 to 1.02; 6,283 participants in 30 studies), or ≥75 nmol/L (OR 1.00, 95% CI 0.85 to 1.18; 3,416 participants in 26 studies; Figure S3). A statistically significant protective effect of vitamin

D was seen for participants aged 1.00-15.99 years (OR 0.71, 95% CI 0.57 to 0.90; 11,871 participants in 15 studies), but not in participants aged <1 year (OR 0.95, 95% CI 0.82 to 1.10; 5,697 participants in 5 studies), 16.00-64.99 years (OR 0.97, 95% CI 0.93 to 1.09; 9,603 participants in 21 studies), or ≥65.00 years (OR 0.96, 95% CI 0.90 to 1.02; 19,140 participants in 17 studies; Figure S7).

With regard to dosing frequency, a statistically significant protective effect was seen for trials where vitamin D was given daily (OR 0.78, 95% CI 0.65 to 0.94; 6,162 participants in 19 studies), but not for trials in which it was given weekly (OR 0.97, 95% CI 0.88 to 1.06; 12,756 participants in 6 studies), or monthly to 3-monthly (OR 0.98, 95% CI 0.93 to 1.03; 27,248 participants in 12 studies; Figure S4). Statistically significant protective effects of the intervention were also seen in trials where vitamin D was administered at daily equivalent doses of 400-1000 IU (OR 0.70, 95% CI 0.55 to 0.89; 2,305 participants in 10 studies), but not where the daily dose equivalent was <400 IU (OR 0.65, 95% CI 0.31 to 1.37; 2,308 participants in 2 studies), 1001-2000 IU (OR 0.97, 95% CI 0.93 to 1.02; 33,859 participants in 16 studies), or >2000 IU (OR 1.05, 95% CI 0.84 to 1.31; 6,906 participants in 7 studies; Figure S5). Statistically significant protective effects were also seen for trials with a duration of ≤12 months (OR 0.82, 95% CI 0.72 to 0.93; 9,255 participants in 29 studies) but not in those lasting >12 months (OR 0.99, 95% CI 0.95 to 1.04; 36,911 participants in 8 studies; Figure S6).

Finally, statistically significant protective effects were also seen for trials that were not restricted to participants with asthma or COPD (OR 0.92, 95% CI 0.86 to 0.99; 44,956 participants in 31 studies), but not in trials that exclusively enrolled participants with asthma (OR 0.73, 95% CI 0.36 to 1.49; 795 participants in 4 studies), or COPD (OR 1.01, 95% 0.68 to 1.51; 415 participants in 2 studies; Figure S8).

An exploratory analysis restricted to eight placebo-controlled trials investigating effects of daily dosing at doses of 400-1000 IU/day with duration ≤12 months (for which mean baseline 25(OH)D level ranged from 54.8 nmol/L to 88.9 nmol/L) showed a statistically significant reduction in the proportion of participants experiencing at least one ARI (OR 0.58, 95% CI 0.45 to 0.75; 1,232 participants in 8 studies; Figure S9; Cates Plot, Figure S1). Heterogeneity of effect for this exploratory analysis was low (I^2 0.0%, P for heterogeneity 0.67).

Multivariable Meta-Regression Analysis

Multivariable meta-regression analysis of trial-level sub-groups did not identify a statistically significant interaction between allocation to vitamin D vs. placebo and dose frequency, dose size, trial duration or participant age (Table S3).

Secondary outcomes

Meta-analysis of secondary outcomes was performed for results of placebo-controlled trials only; results are presented in Table 3. Overall, without consideration of participant- or trial-level factors, vitamin D supplementation did not have a statistically significant effect on the proportion of participants with one or more URI, LRI, courses of antimicrobials for ARI, work/school absences due to ARI, hospitalisations or emergency department attendances for ARI, serious adverse events of any cause, death due to ARI or respiratory failure, death due to any cause, or episodes of hypercalcaemia or renal stones.

Risk of bias across studies

A funnel plot for the proportion of participants experiencing at least one ARI (Figure S10) showed left-sided asymmetry, confirmed with an Egger's regression test[58] (P=0.007). This might reflect heterogeneity of effect across trials, or publication bias arising from omission of small trials showing non-protective effects of vitamin D from the meta-analysis.[59] Given the latter possibility, the quality of the body of evidence contributing to analyses of the primary efficacy outcome and major secondary outcomes was downgraded to moderate (Table S4).

Sensitivity Analyses

Results of exploratory sensitivity analyses are presented in Table S5. Meta-analysis of the proportion of participants in placebo-controlled trials experiencing at least one ARI, excluding 3 studies assessed as being at unclear risk of bias,[8,26,48] revealed protective

effects of vitamin D supplementation consistent with the main analysis (OR 0.93, 95% CI 0.87 to 1.00; 45,783 participants in 34 studies). Sensitivity analysis for the same outcome, excluding 18 placebo-controlled trials that investigated ARI as a secondary outcome, did not show a statistically significant protective effect (OR 0.92, 95% CI 0.82 to 1.03; 9,694 participants in 19 studies). A sensitivity analysis for the same outcome, substituting diary-defined ARI events (available for 2598 participants) for survey-defined ARI events (available for n=16,000 participants) in the trial by Pham et al[45] revealed protective effects of vitamin D supplementation consistent with the main analysis (OR 0.91, 95% CI 0.84 to 0.99; 32,764 participants in 37 studies).

Discussion

This updated meta-analysis of RCTs of vitamin D supplementation for the prevention of ARI includes data from an additional 18 studies completed since December 2015, when we performed the final literature search for our prior individual participant data meta-analysis.[30] For expediency during the COVID-19 pandemic, we used a trial-level approach for this update, which includes data from a total of 48,488 participants in 43 trials. Overall, we report a modest statistically significant protective effect of vitamin D supplementation, as compared with placebo (OR 0.92, 95% CI 0.86 to 0.99). As expected, there was significant heterogeneity (P=0.02) across trials, which might have led to an under-estimate of the protective effect, and contributed to the asymmetry observed in the funnel plot.[59] Alternatively, left-sided asymmetry in the funnel plot may reflect publication bias, which might have led to an over-estimate of the protective effect. In contrast to findings of our previous meta-analysis,[30] we did not observe enhanced protection in those with the lowest 25(OH)D levels at baseline. However, there was evidence that efficacy of vitamin D supplementation varied according to dosing regimen, trial duration and age, with protective effects associated with daily administration of doses of 400-1000 IU vitamin D given for ≤12 months, and age of 1.00 to 15.99 years at enrolment. An exploratory analysis restricted to data from 8 trials fulfilling these design criteria revealed a larger protective effect (OR 0.58, 95% CI 0.45 to 0.75) without significant heterogeneity across trials (P for heterogeneity 0.67).

The magnitude of the overall protective effect seen in the current analysis (OR 0.92, 95% CI 0.86 to 0.99) is modest, and similar to the value reported in our previous meta-

analysis of individual participant data (adjusted OR 0.88, 95% CI 0.81 to 0.96).[30] In keeping with our previous study, the point estimate for this effect was lower among those with baseline 25(OH)D <25 nmol/L than in those with higher baseline vitamin D status. However, in contrast to our previous finding, a statistically significant protective effect of vitamin D was not seen in those with the lowest 25(OH)D concentrations. This difference reflects the inclusion of null data from four new RCTs in which vitamin D was given in relatively high doses at weekly or monthly intervals over 2-5 years. [42,43,45,46] Null results of these studies contrast with protective effects reported from earlier trials in which smaller daily doses of vitamin D were given over shorter periods. [8,9,13,16] These differing findings suggest that the frequency, amount and duration of vitamin D supplementation may be key determinants of its protective efficacy. In keeping with this hypothesis, statistically significant protective effects of vitamin D were seen for meta-analysis of trials where vitamin D was given daily; where it was given at doses of 400-1000 IU/day; and where it was given for 12 months or less. When results of trials that investigated daily administration of 400-1000 IU over ≤12 months were pooled in an exploratory meta-analysis, a protective effect was seen (OR 0.58, 95% CI 0.45 to 0.75) with low heterogeneity (I^2 0.0%, P for heterogeneity 0.67). Greater protective efficacy of lower vs higher doses of vitamin D might reflect deleterious effects of higher-dose vitamin D on its own metabolism, or on host responses to respiratory pathogens: head-to-head mechanistic studies in individuals randomised to different regimens of vitamin D supplementation are needed to investigate this issue.

The current study has several strengths: it contains the very latest RCT data available in this fast-moving field, including findings from four large phase 3 trials published in 2020[41-43,45] as well as some as-yet unpublished studies.[47,48] The inclusion of additional studies allowed us to analyse results of placebo-controlled studies vs. high-dose / low-dose studies separately, and gave us the power to investigate reasons for heterogeneity of effect observed across trials. For example, we could distinguish the effects of daily vs. weekly dosing, which were previously pooled.[30]

Our work also has limitations. Given the need to generate a rapid update of our previous work in the context of the COVID-19 pandemic, we meta-analysed aggregate (trial-level) data, rather than individual participant data; this allowed us to proceed rapidly, without the delays introduced by the need to establish multiple data sharing agreements.

However, we did contact authors to get unpublished estimates of effect that were stratified by pre-defined baseline 25(OH)D levels, harmonised across studies: thus, we were able to provide accurate data for the major participant-level effect-modifier of interest. Despite the large number of trials overall, relatively few compared effects of lower- vs. higher-dose vitamin D: our power for this secondary comparison was therefore limited. We lacked the individual participant data to investigate race/ethnicity and obesity as potential effect-modifiers. We also could not account for other factors that might influence the efficacy of vitamin D supplements for ARI prevention (e.g., taking the supplement with or without food) or secular trends that would influence trials, such as the increased societal use of vitamin D supplements;[60] concurrent use of standard dose vitamin D supplements or multivitamins in the "placebo" group would effectively render these as high- vs. low-dose trials and potentially drive results toward the null. Another limitation relates to the funnel plot, which suggests that the overall effect size may have been over-estimated due to publication bias; we have mitigated this by inclusion of data from unpublished studies identified by searching clinicaltrials.gov where this was obtainable. Finally, we acknowledge that additional randomised controlled trials investigating the effects of vitamin D on risk of acute respiratory tract infection are ongoing or have not yet reported.[50,51] We hope to include data from these studies in future meta-analyses.

In summary, this updated meta-analysis of data from RCTs of vitamin D for the prevention of ARI showed a statistically significant overall protective effect of the intervention. The protective effect was heterogenous across trials; it also may have been over-estimated due to publication bias. In contrast to findings of our previous meta-analysis of individual participant data, we did not see a protective effect of vitamin D supplementation among those with the lowest baseline vitamin D status. The vitamin D dosing regimen of most benefit was daily and used standard doses (e.g., 400 to 1000 IU) for up to 12 months. The relevance of these findings to COVID-19 is not known and requires investigation.

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Author Contributions

DAJ and ARM wrote the study protocol and designed statistical analyses. DAJ, CAC and ARM assessed eligibility of studies for inclusion. DAJ, ARM, CAC and JDS performed risk of bias assessments. Statistical analyses were done by DAJ; results were checked and verified by JDS. DAJ and ARM wrote the first draft of the report. All authors revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Competing Interests

All authors have completed the ICMJE uniform disclosure form. No author has had any financial relationship with any organisations that might have an interest in the submitted work in the previous three years. No author has had any other relationship, or undertaken any activity, that could appear to have influenced the submitted work.

Transparency Declaration

DAJ and ARM are the manuscript's guarantors and they affirm that this is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted. All analyses were pre-specified in the study protocol, other than the exploratory analyses whose results are presented in Table 2 (sub-group analyses by age and presence of asthma/COPD, requested by reviewers), Table S5 and Figure S7.

Data Sharing: the study dataset is available from d.a.jolliffe@qmul.ac.uk.

Table 1: Characteristics of the 42 eligible trials and their participants

Study first author, year	Setting	Participants	Mean age, years (s.d.) [range]	Male: Female	25(OH)D assay, EQA scheme	Mean baseline 25(OH)D, nmol/L (s.d.)	Baseline 25(OH)D <25 nmol/L (%)	Mean attained 25(OH)D, intervention arm, nmo/L (s.d.)	Intervention: Control (total)	Oral dose of vitamin D_3, intervention arm	Control	Trial duration	ARI definition	ARI primary or secondary outcome?	N contributing data / N randomised (%)
Li-Ng 2009[5]	USA	Healthy adults	57.9 (13.6) [21.4 - 80.6]	34:128	RIA (DiaSorin), DEQAS	63.7 (25.5)	3/150 (2.0)	88.5 (23.2)	84:78 (162)	50 µg daily	Placebo	3 mo	URI: ≥2 URI symptoms in absence of allergy symptoms	Primary	157/162 (96.9)
Urashima 2010[6]	Japan	Schoolchildren	10.2 (2.3) [6.0 – 15.0]	242:188	Not determined	Not determined	Not determined	Not determined	217:213 (430)	30 µg daily	Placebo	4 mo	URI: influenza A/B diagnosed by RIDT or RIDT-negative ILI	Primary	334/430 (77.7)
Manaseki-Holland 2010 [7]	Afghanistan	Pre-school children with pneumonia	1.1 (0.8) [0.1 – 3.3]	257:196	Not determined	Not determined	Not determined	Not determined	224:229 (453)	2.5 mg bolus once	Placebo	3 mo	LRI: repeat episode of pneumonia – age-specific tachypnoea without wheeze	Secondary	453/453 (100.0)
Laaksi 2010[8]	Finland	Military conscripts	19.1 (0.6) [18.0 – 21.0]	164:0	EIA (IDS OCTEIA)	75.9 (18.7)	0/73 (0.0)	71.6 (22.9)	80:84 (164)	10 µg daily	Placebo	6 mo	ARI: medical record diagnosis	Primary	164/164 (100.0)
Majak 2011 [9]	Poland	Children with asthma	10.9 (3.3) [6.0 – 17.0]	32:16	RIA (BioSource Europe), RIQAS	88.9 (38.2)	0/48 (0.0)	37.6 (13.1)	24:24 (48)	12.5 µg daily	Placebo	6 mo	ARI: self-report	Secondary	48/48 (100.0)
Trilok-Kumar 2011[10]	India	Low birthweight infants	0.1 (0.0) [0.0 – 0.3]	970:1109	--	Not determined	Not determined	55.0 (22.5)	1039:1040 (2079)	35 µg weekly	Placebo	6 mo	ARI: medical record diagnosis of events causing hospitalisation	Secondary	2064/2079 (99.3)
Lehouck 2012[11]	Belgium	Adults with COPD	67.9 (8.3) [48.0 – 86.0]	145:37	RIA (Diasorin), DEQAS	49.8 (29.2)	31/182 (17.0)	130.0 (44.7)	91:91 (182)	2.5 mg bolus monthly	Placebo	1 yr	URI: self-report	Secondary	175/182 (96.2)
Manaseki-Holland 2012[12]	Afghanistan	Infants	0.5 (0.3) [0.0 – 1.0]	1591:1455	--	Not determined	Not determined	32.7 (17.1)	1524:1522 (3046)	2.5 mg bolus 3-monthly	Placebo	1.5 yr	LRI: pneumonia confirmed by chest radiograph	Primary	3011/3046 (98.9)
Camargo 2012 [13]	Mongolia	3rd/4th grade schoolchildren	10.0 (0.9) [7.0 – 12.7]	129:118	LC-MS/MS, DEQAS	18.9 (9.7)	192/245 (78.4)	49.1 (15.1)	143:104 (247)	7.5 µg daily	Placebo	7 wk	ARI: parent-reported 'chest infections or colds'	Secondary	244/247 (98.8)
Murdoch 2012[14]	New Zealand	Healthy adults	48.1 (9.7) [18.0 – 67.6]	81:241	LC-MS/MS, DEQAS	72.1 (22.1)	5/322 (1.6)	123.6 (27.5)	161:161 (322)	2 x 5 mg bolus monthly then 2.5 mg bolus monthly	Placebo	1.5 yr	URI: assessed with symptom score	Primary	322/322 (100.0)
Bergman 2012[15]	Sweden	Adults with increased susceptibility to ARI	53.1 (13.1) [20.0 – 77.0]	38:102	CLA (DiaSorin), DEQAS	49.3 (23.2)	15/131 (11.45)	94.9 (38.1)	70:70 (140)	100 µg daily	Placebo	1 yr	URI: assessed with symptom score	Secondary	124/140 (88.6)
Marchisio 2013[16]	Italy	Children with recurrent acute otitis media	2.8 (1.0) [1.3 – 4.8]	64:52	CLA (DiaSorin), ISO9001	65.3 (17.3)	2/116 (1.7)	90.3 (21.1)]	58:58 (116)	25 µg daily	Placebo	6 mo	URI: doctor-diagnosed acute otitis media	Primary	116/116 (100.0)
Rees 2013[17]	USA	Adults with previous colorectal adenoma	61.2 (6.6) [47.1 – 77.9]	438:321[a]	RIA (IDS), DEQAS	62.5 (21.3)	0/759 (0.0)	186.9 (455.1)	399:360 (759)	25 µg daily	Placebo	13 mo (average)	URI: assessed from daily symptom diary	Secondary	759/759 (100.0)
Tran 2014[18]	Australia	Healthy older adults	71.7 (6.9) [60.3 – 85.2]	343:301	CLA (DiaSorin), DEQAS	41.7 (13.5)	66/643 (10.3)	71.0 (19.6)	430:214 (644)	0.75 mg bolus *vs.* 1.5 mg bolus monthly	Placebo	1 yr	URI: self-reported cold	Secondary	594/644 (92.2)
Goodall 2014[19]	Canada	Healthy university students	19.6 (2.2) [17.0 – 33.0]	218:382	Not determined	Not determined	Not determined	Not determined	300:300 (600)	0.25 mg weekly (2x2 factorial with gargling)	Placebo	8 wk	URI: self-reported cold	Primary	492/600 (82.0)
Urashima 2014[20]	Japan	High school students	16.5 (1.0) [15.0 – 18.0]	162:85	Not determined	Not determined	Not determined	Not determined	148:99 (247)	50 µg daily	Placebo	2 mo	URI: influenza A diagnosed by RIDT or RIDT-negative ILI	Primary	247/247 (100.0)
Grant 2014[21]	New Zealand	Pregnant women and offspring	Offspring unborn at baseline	0:260 (pregnant women) 121:128 (offspring)	LC-MS/MS, DEQAS	54.8 (25.8)	30/200 (15.0)	92.9 (41.6)	173:87 (pregnant women, 260) 164:85 (offspring, 249)	Pregnant women: 25 µg *vs.* 50 µg daily. Offspring: 10 µg *vs.* 20 µg daily	Placebo	9 mo (3 mo in pregnancy + 6 mo in infancy)	ARI: doctor-diagnosed ARI precipitating primary care consult	Secondary	236/260 (90.8)

Study first author, year	Setting	Participants	Mean age, years (s.d.) [range]	Male: Female	25(OH)D assay, EQA scheme	Mean baseline 25(OH)D, nmol/L (s.d.)	Baseline 25(OH)D <25 nmol/L (%)	Mean attained 25(OH)D, intervention arm, nmo/L (s.d.)	Intervention: Control (total)	Oral dose of vitamin D_3, intervention arm	Control	Trial duration	ARI definition	ARI primary or secondary outcome?	N contributing data / N randomised (%)
Martineau 2015a[22] [ViDiCO]	UK	Adults with COPD	64.7 (8.5) [40.0 – 85.0]	144:96	LC-MS/MS, DEQAS	46.1 (25.7)	50/240 (20.8)	67.3 (27.5)	122:118 (240)	3 mg bolus 2-monthly	Placebo	1 yr	URI: assessed from daily symptom diary	Co-primary	240/240 (100.0)
Martineau 2015b[23] [ViDiAs]	UK	Adults with asthma	47.9 (14.4) [16.0 – 78.0]	109:141	LC-MS/MS, DEQAS	49.6 (24.7)	36/250 (14.4)	69.4 (21.0)	125:125 (250)	3 mg bolus 2-monthly	Placebo	1 yr	URI: assessed from daily symptom diary	Co-primary	250/250 (100.0)
Martineau 2015c[24] [ViDiFlu]	UK	Older adults and their carers	67.1 (13.0) [21.4 – 94.0]	82:158	LC-MS/MS, DEQAS	42.9 (23.0)	60/240 (25.0)	84.8 (24.1)	137:103 (240)	Older adults: 2.4 mg bolus 2-monthly + 10 µg daily Carers: 3 mg 2-monthly	Older adults: placebo + 10 µg daily Carers: placebo	1 yr	URI & LRI, both assessed from daily symptom diary	Co-primary	240/240 (100.0)
Simpson 2015[25]	Australia	Healthy adults	32.2 (12.2) [18.0 – 52.0]	14:20	LC-MS/MS, DEQAS	67.9 (23.0)	0/33 (0.0)	Not determined	18:16 (34)	0.5 mg weekly	Placebo	17 wk	ARI assessed with symptom score	Primary	34/34 (100.0)
Dubnov-Raz 2015[26]	Israel	Adolescent swimmers with vitamin D insufficiency	15.2 (1.6) [12.9 – 18.6]	34:20	RIA (DiaSorin), DEQAS	60.4 (11.9)	0/54 (0.0)	73.7 (16.6)	27:27 (54)	50 µg daily	Placebo	12 wk	URI assessed with symptom score	Primary	25/54 (46.3)
Denlinger 2016[27]	USA	Adults with asthma	39.2 (12.9) [18.0 – 85.0]	130:278	CLA (DiaSorin), VDSP	47.0 (16.9)	55/408 (13.5)	104.3 (32.4)	201:207 (408)	2.5 mg bolus then 100 µg daily	Placebo	28 wk	URI assessed with symptom score	Secondary	408/408 (100.0)
Tachimoto 2016[28]	Japan	Children with asthma	9.9 (2.3) [6.0 – 15.0]	50:39	RIA (DiaSorin), CAP	74.9 (24.6)	1/89 (1.1)	85.7 (24.5)	54:35 (89)	20 µg daily, first 2 mo.	Placebo	6 mo	URI: assessed with symptom score	Secondary	89/89 (100.0)
Ginde, 2016[29]	USA	Institutionalised older adults	80.7 (9.9) [60.0 – 95.0]	45:62	LC-MS/MS, VDSP	57.3 (22.7)	12/107 (11.2)	Not determined	55:52 (107)	2.5 mg bolus monthly + ≤25 µg per day equivalent	Placebo + 10-25 µg per day equivalent	1 yr	ARI: medical record diagnosis	Primary	107/107 (100.0)
Gupta 2016[31]	India	Children with pneumonia	1.4 (1.1) [0.5 – 5.0]	226:98	RIA (Immunotech SAS/ DiaSorin)	43.9 (33.4)	104/312 (33.3)	64.1 (43.9)	162:162 (324)	2.5 mg bolus, single dose	Placebo	6 mo	Physician confirmed recurrent pneumonia	Co-primary	314/324 (96.9)
Aglipay 2017 [32]	Canada	Healthy children	2.7 (1.5) [1.0 – 5.0]	404:296	CLA (Roche ELECSYS)	90.9 (20.9)	1/703 (0.1)	High dose: 121.6 (2.2); Low dose: 91.9 (1.7)	349:354	50 µg daily	10 µg daily	4-8 mo (mean 6.3 mo)	URI: lab confirmed	Primary	699/703 (99.4)
Arihiro 2018[33]	Japan	Adults with diagnosis of inflammatory bowel disease	44.7 (1.3) [18.0 – 82.0]	136:87	RIA (Diasorin)	58.6 (22.0)	5/223 (2.2)	80.4 (21.5)	119:118 (237)	12.5 µg daily	Placebo	6 mo	Lab confirmed influenza	Primary	223/237 (94.1)
Hibbs 2018 [34]	USA	African American preterm infants	Offspring unborn at baseline	166:133[b]	RIA	55.4 (22.2)	0/300 (0.0)	95.0 (21.2)	153:147 (300)	10 µg daily, regardless of dietary intake	10 µg daily, only if dietary intake was <5 µg daily	1 yr	ARI: self-reported URI/LRI	Secondary	300/300 (100.0)
Lee 2018 [35]	USA	Children and young adults with sickle cell disease	9.9 (3.9) [3.0 – 20.0]	30:32	LC-MS/MS, DEQAS	35.7 (16.5)	18/62 (29.0)	92.4 (23.7)	31:31 (62)	2.5 mg bolus monthly	0.3 mg monthly	2 yrs	Self-reported respiratory events, including ARI	Primary	62/62 (100.0)
Loeb 2018 [36]	Vietnam	Healthy children and adolescents	8.5 (4.0) [3.0 – 17.0]	621:679	CLA (DiaSorin), DEQAS	65.5 (16.8)	5/1153 (43.4)	91.8 (23.6)	650:650 (1300)	0.35 mg weekly	Placebo	8 mo	RT-PCR confirmed influenza A or B	Primary	1153/1300 (88.7)
Rosendahl 2018 [37]	Finland	Healthy infants	Offspring unborn at baseline	495:492	CLA (IDS-iSYS) VDSP	81.5 (25.9)	0/879 (0.0)	117.7 (26.1)	492:495 (987)	30 µg daily	10 µg daily	2 yrs	Parent reported infections, including ARI	Co-primary	897/987 (90.9)
Shimizu 2018 [38]	Japan	Healthy adults	52.7 (6.5) [45.0 – 74.0]	66:149	RIA (DiaSorin)	48.9 (13.5)	1/214 (0.5)	114.6 (32.7)	126:126 (252)	10 µg daily (25[OH] D)[c]	Placebo	4 mo	URI: self-reported	Primary	215/252 (85.3)
Aloia 2019 [39]	USA	Healthy African American women aged over 60 years	69.0 (5.3) [65.4 – 72.5]	0:260	LC-MS/MS, NIST	54.4 (16.7)	9/258 (3.5)	117.0 (28.0)	130:130 (260)	50 µg daily	Placebo	3 mo	ARI: self-reported cold/flu	Secondary	260/260 (100.0)
Hauger 2019[40]	Denmark	Healthy children	6.6 (1.5) [4.0 – 8.0]	61:69	LC-MS/MS, DEQAS	56.8 (12.5)	0/118 (0.0)	20 µg arm: 75.8 (11.5) 10 ug arm: 61.8 (10.6)	43:44:43 (130)	20 µg / 10 µg daily	Placebo	5 mo	ARI: self-reported	Secondary	118/130 (90.8)

Study first author, year	Setting	Participants	Mean age, years (s.d.) [range]	Male: Female	25(OH)D assay, EQA scheme	Mean baseline 25(OH)D, nmol/L (s.d.)	Baseline 25(OH)D <25 nmol/L (%)	Mean attained 25(OH)D, intervention arm, nmo/L (s.d.)	Intervention: Control (total)	Oral dose of vitamin D_3, intervention arm	Control	Trial duration	ARI definition	ARI primary or secondary outcome?	N contributing data / N randomised (%)
Bischoff-Ferrari 2020 [41]	Switzerland, France, Germany, Portugal, and Austria	Older adults	74.9 (4.4) [70.0 – 95.0]	826:1331	LC-MS/MS, DEQAS	55.9 (21.0)	143/2140 (6.7)	93.8 (28.2)	1076:1081	50 µg daily (2x2x2 factorial with omega-3 fatty acid supplementation and strength-training exercise)	Placebo	3 yrs	ARI: self-reported and verified by independent physician	Co-primary	2157/2157 (100.0)
Camargo 2020[42]	New Zealand	Older adults	66.4 (8.3) [50.0 –84.0]	2935:2121	LC-MS/MS, DEQAS	63.4 (23.6)	89/5056 (1.8)	135.0 (39.9)	2558:2552 (5110)	5 mg bolus loading dose; then 2.5 mg bolus monthly	Placebo	3 yrs	ARI: self-reported cold/flu	Secondary	5056/5110 (98.9)
Ganmaa, 2020[43]	Mongolia	Healthy school children	9.4 (1.6) [6.0 – 13.0]	4485:4366	EIA (Biomerieux), DEQAS	29.7 (10.5)	2813/8851 (31.8)	77.4 (22.7)	4418:4433 (8851)	0.35 mg weekly	Placebo	3 yrs	ARI: self-reported	Secondary	8851/8851 (100.0)
Mandlik 2020 [44]	India	Healthy children	8.1 (1.2) [6.0 – 12.0]	158:127	EIA (DLD diagnostics)	58.9 (10.9)	0/237 (0.0)	80 (23.3)	135:150 (285)	25 µg daily + 500 mg calcium	Placebo	6 mo	URI: self-reported	Secondary	244/285 (85.6)
Pham 2020 [45]	Australia	Older adults	69.3 (5.5) [60.0 – 86.0]	8678:7322	LC-MS/MS, VDSP	Not determined	Not determined	114.8 (30.3)[d]	8000:8000 (16000)	1.5 mg bolus monthly	Placebo	5 yrs	ARI: self-reported	Secondary	16,000/16,000 (100.0)
Rake 2020 [46]	England	Healthy older adults	72.2 (4.9) [65.0 – 84.0]	408:379	CLA (Cobas 6000 Roche)	50.2 (27.1)	127/787 (16.1)	109.2 (33.9)	395:392 (787)	2.5 mg bolus monthly	Placebo	2 yrs	URI/LRI: GP recorded	Secondary	787/787 (100.0)
Golan-Tripto, unpublished [47]	Israel	Prematurely born infants	0 (0)	21:29	CLA (DiaSorin)	33.6 (29.7)	19/46 (41.3)	20 µg arm: 78.0 (75.0) 10 ug arm: 81.0 (73.0)	25:25 (50)	20 µg daily	10 µg daily	1 yr	ARI: GP recorded	Secondary	25/50 (50.0)
Reyes, unpublished [48]	Chile	Healthy pre-school children	2.2 (0.5) [1.3 – 3.3]	168:135	LC-MS/MS	62.2 (15.5)	1/194 (0.5)	0.14 mg arm: 82.4 (24.5) 0.28 mg arm: 104.6 (52.9)	99:103:101 (303)	0.14 mg / 0.28 mg weekly	Placebo	6 mo	ARI: self-reported	Primary	194/303 (64.0)

[a] Sex missing for two participants randomised to intervention arm and subsequently excluded from analysis due to lack of outcome data. [b] Sex missing for one participant. [c] equivalent to 30 ug vitamin D_3.[61] 1 µg vitamin D_3 = 40 international units (IU); 25(OH)D concentrations reported in ng/ml were converted to nmol/L by multiplying by 2.496. [d] from subset of participants randomised to intervention; for comparison, mean 25(OH)D at follow-up in subset of participants randomised to placebo was 77.5 nmol/L (sd 25.2 nmol/L); 25(OH)D, 25-hydroxyvitamin D; RIDT, rapid influenza diagnostic test; COPD, chronic obstructive pulmonary disease; D_3, vitamin D_3 (cholecalciferol); p.o., *per os* (orally); mo, month; yr, year; wk, week. ARI, acute respiratory infection; CAP, College of American Pathologists, CLA, chemiluminescent assay; DEQAS, Vitamin D External Quality Assessment Scheme; EIA, enzyme immunoassay; EQA, external quality assessment; GP, general practitioner; LC-MS/MS, liquid chromatography tandem-mass spectrometry, RIA, radio-immunoassay; URI, upper respiratory infection; LRI, lower respiratory infection; ILI, influenza-like illness; RIQAS, Randox International Quality Assessment Scheme; VDSP, Vitamin D Standardisation Program of the Office of Dietary Supplements, National Institutes of Health, USA

Table 2: Placebo controlled RCTs: Proportion of participants experiencing at least one acute respiratory infection, overall and stratified by potential effect-modifiers

Potential effect-modifier	No of trials	Proportion with ≥1 ARI, intervention group (%)	Proportion with ≥1 ARI, control group (%)	Odds ratio (95% CI)	I^2 %	P for heterogeneity
Overall	37	14332/23364 (61.3)	14217/22802 (62.3)	0.92 (0.86 to 0.99)	35.6	0.02
Baseline 25(OH)D, nmol/L[a]						
<25	20	1395/1879 (74.2)	1433/1898 (75.5)	0.81 (0.57 to 1.15)	44.5	0.02
25 – 49.9	29	3662/5022 (72.9)	3569/4874 (73.2)	1.04 (0.94 to 1.15)	0.0	0.49
50 – 74.9	30	1929/3279 (58.8)	1829/3004 (60.9)	0.88 (0.76 to 1.02)	9.3	0.32
≥75	26	1072/1742 (61.5)	1029/1674 (61.5)	1.00 (0.85 to 1.18)	0.0	0.78
Dosing frequency						
Daily	19	1703/3210 (53.1)	1672/2952 (56.6)	0.78 (0.65 to 0.94)	53.5	0.003
Weekly	6	4482/6421 (69.8)	4447/6335 (70.2)	0.97 (0.88 to 1.06)	0.0	0.48
Monthly or less frequently	12	8147/13733 (59.3)	8098/13515 (59.9)	0.98 (0.93 to 1.03)	0.0	0.57
Daily dose equivalent, IU[b]						
<400	2	482/1175 (41.0)	511/1133 (45.1)	0.65 (0.31 to 1.37)	86.3	0.007
400-1000	10	656/1236 (53.1)	627/1069 (58.7)	0.70 (0.55 to 0.89)	31.2	0.16
1001-2000	16	10593/16961 (62.5)	10674/16898 (63.2)	0.97 (0.93 to 1.02)	0.0	0.51
>2000	7	2291/3462 (66.2)	2250/3444 (65.3)	1.05 (0.84 to 1.31)	37.1	0.15
Trial duration, months						
≤12	29	1977/4887 (40.5)	1866/4368 (42.7)	0.82 (0.72 to 0.93)	38.1	0.02
>12	8	12355/18477 (66.9)	12351/18434 (67.0)	0.99 (0.95 to 1.04)	0.0	0.95
Age, yrs[a]						
<1.00	5	875/2901 (30.2)	839/2796 (30.0)	0.95 (0.82 to 1.10)	18.7	0.30
1.00-15.99	15	4297/5994 (71.7)	4303/5877 (73.2)	0.71 (0.57 to 0.90)	46.0	0.03
16.00-64.99	21	3137/4876 (64.3)	3087/4727 (65.3)	0.97 (0.93 to 1.09)	11.5	0.31
≥65.00	17	6023/9665 (62.3)	6004/9475 (63.4)	0.96 (0.90 to 1.02)	0.0	0.73
Airway disease						
Asthma only	4	203/404 (50.2)	202/391 (51.7)	0.73 (0.36 to 1.49)	71.7	0.01
COPD only	2	106/208 (51.0)	104/207 (50.2)	1.01 (0.68 to 1.51)	0.0	0.71
Unrestricted	31	14023/22752 (61.6)	13911/22204 (62.7)	0.92 (0.86 to 0.99)	33.0	0.04

[a] The number of trials in each category for this variable adds up to more than 36, since this is a participant-level variable, i.e. some trials contributed data from participants who fell into more than one category
[b] Data from two trials that included higher-dose, lower-dose and placebo arms[18,48] are excluded from this sub-group analysis, since the higher-dose and lower-dose arms spanned the 1,000 IU/day cut-off, rendering them unclassifiable

Table 3: Placebo-controlled studies: Secondary outcomes

Variables	No of trials	Proportion with ≥1 event, intervention group (%)	Proportion with ≥1 event, control group (%)	Odds ratio (95% CI)	I^2 %	P for heterogeneity
Efficacy outcomes						
Upper respiratory infection*	29	8578/14569 (58.9)	8475/14115 (60.0)	0.96 (0.91 to 1.02)	1.2	0.45
Lower respiratory infection*	15	3930/13243 (29.7)	3956/13108 (30.2)	0.98 (0.93 to 1.04)	0.0	0.63
Emergency department attendance and/or hospital admission due to ARI	19	139/10963 (1.3)	149/10850 (1.4)	0.90 (0.71 to 1.14)	0.0	1.00
Death due to ARI or respiratory failure	34	14/14688 (0.1)	11/14139 (0.1)	1.04 (0.61 to 1.77)	0.0	1.00
Use of antibiotics to treat an ARI*	14	2056/8638 (23.8)	2109/8504 (24.8)	0.92 (0.83 to 1.01)	9.0	0.35
Absence from work or school due to ARI	10	378/1527 (24.7)	364/1044 (34.9)	0.91 (0.69 to 1.20)	35.3	0.13
Safety outcomes						
Serious adverse event of any cause*	36	567/14937 (3.8)	585/14407 (4.1)	0.97 (0.86 to 1.07)	0.0	0.99
Death due to any cause	35	129/14930 (0.9)	110/14374 (0.8)	1.13 (0.88 to 1.44)	0.0	1.00
Hypercalcaemia	22	51/10370 (0.5)	41/10000 (0.4)	1.18 (0.80 to 1.74)	0.0	1.00
Renal stones	21	117/12616 (0.9)	136/12219 (1.1)	0.85 (0.67 to 1.11)	0.0	1.00

* This analysis includes a subset of participants in the trial by Pham et al, who completed symptom diaries.

Figure 1: Flow chart of study selection

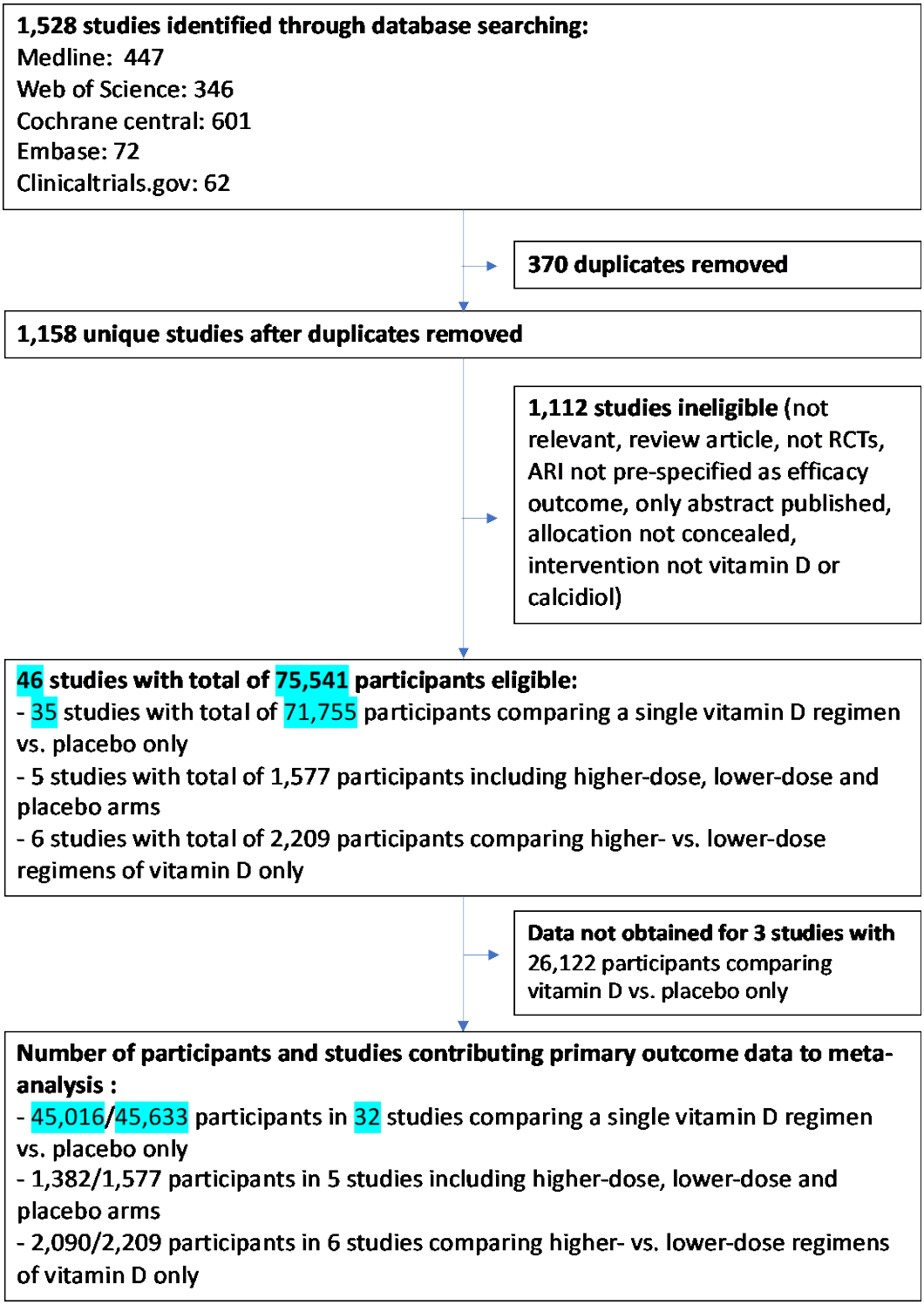
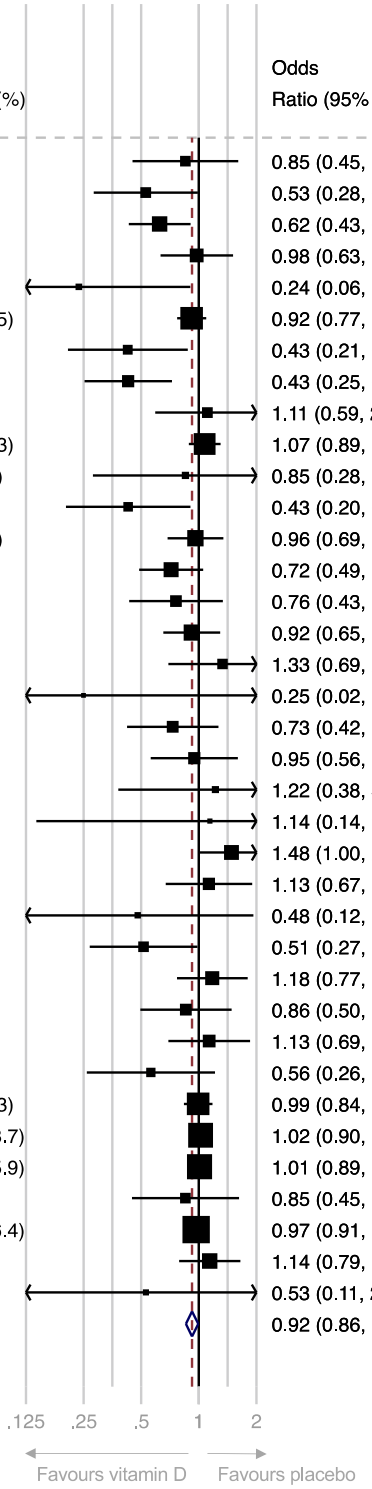


Figure 2: Forest plot of placebo-controlled RCTs reporting proportion of participants experiencing 1 or more acute respiratory infection.

1st Author (trial)	Year	Proportion with ≥1 ARI, intervention group(%)	Proportion with ≥1 ARI, control group(%)	Odds Ratio (95% CI)	% Weight
Li-Ng	2009	32/81(39.5)	33/76(43.4)	0.85 (0.45, 1.61)	1.25
Laaksi	2010	39/80(48.8)	54/84(64.3)	0.53 (0.28, 0.99)	1.28
Manaseki-Holland	2010	97/224(43.3)	126/229(55)	0.62 (0.43, 0.90)	3.07
Urashima	2010	68/167(40.7)	69/167(41.3)	0.98 (0.63, 1.51)	2.38
Majak	2011	4/24(16.7)	11/24(45.8)	0.24 (0.06, 0.90)	0.30
Trilok-Kumar	2011	438/1034(42.4)	458/1030(44.5)	0.92 (0.77, 1.09)	7.47
Bergman	2012	26/62(41.9)	39/62(62.9)	0.43 (0.21, 0.88)	0.99
Camargo	2012	44/141(31.2)	53/103(51.5)	0.43 (0.25, 0.72)	1.74
Lehouck	2012	30/86(34.9)	29/89(32.6)	1.11 (0.59, 2.07)	1.28
Manaseki-Holland	2012	260/1506(17.3)	245/1505(16.3)	1.07 (0.89, 1.30)	6.89
Murdoch	2012	154/161(95.7)	155/161(96.3)	0.85 (0.28, 2.59)	0.44
Marchisio	2013	26/58(44.8)	38/58(65.5)	0.43 (0.20, 0.90)	0.92
Rees	2013	303/399(75.9)	276/360(76.7)	0.96 (0.69, 1.34)	3.56
Goodall	2014	70/258(27.1)	80/234(34.2)	0.72 (0.49, 1.05)	2.90
Grant	2014	94/157(59.9)	53/80(66.3)	0.76 (0.43, 1.33)	1.55
Tran	2014	185/397(46.6)	96/197(48.7)	0.92 (0.65, 1.29)	3.46
Urashima	2014	32/148(21.6)	17/99(17.2)	1.33 (0.69, 2.56)	1.19
Dubnov-Raz	2015	10/14(71.4)	10/11(90.9)	0.25 (0.02, 2.65)	0.10
Martineau (ViDiAs)	2015	85/125(68)	93/125(74.4)	0.73 (0.42, 1.27)	1.61
Martineau (ViDiCO)	2015	76/122(62.3)	75/118(63.6)	0.95 (0.56, 1.60)	1.75
Martineau (ViDiFlu)*	2015	13/22(59.1)	13/24(54.2)	1.22 (0.38, 3.93)	0.40
Simpson	2015	16/18(88.9)	14/16(87.5)	1.14 (0.14, 9.21)	0.13
Denlinger	2016	110/201(54.7)	93/207(44.9)	1.48 (1.00, 2.19)	2.84
Gupta	2016	39/156(25)	36/158(22.8)	1.13 (0.67, 1.90)	1.78
Tachimoto	2016	4/54(7.4)	5/35(14.3)	0.48 (0.12, 1.93)	0.28
Arihiro	2018	19/115(16.5)	30/108(27.8)	0.51 (0.27, 0.98)	1.20
Loeb	2018	50/577(8.7)	43/576(7.5)	1.18 (0.77, 1.80)	2.48
Shimizu	2018	41/110(37.3)	43/105(41)	0.86 (0.50, 1.48)	1.62
Aloia	2019	76/130(58.5)	72/130(55.4)	1.13 (0.69, 1.85)	1.96
Hauger	2019	36/77(46.8)	25/41(61)	0.56 (0.26, 1.21)	0.87
Bischoff-Ferrari	2020	647/1076(60.1)	652/1081(60.3)	0.99 (0.84, 1.18)	7.53
Camargo	2020	1882/2539(74.1)	1855/2517(73.7)	1.02 (0.90, 1.16)	9.28
Ganmaa	2020	3783/4401(86)	3793/4418(85.9)	1.01 (0.89, 1.14)	9.49
Mandlik	2020	92/116(79.3)	99/121(81.8)	0.85 (0.45, 1.62)	1.22
Pham**	2020	5253/8000(65.7)	5310/8000(66.4)	0.97 (0.91, 1.03)	11.46
Rake	2020	73/395(18.5)	65/392 (16.6)	1.14 (0.79, 1.65)	3.11
Reyes	Unpublished	125/133 (94)	59/61(96.7)	0.53 (0.11, 2.57)	0.22
Overall (I-squared = 35.6%, p = 0.018)				0.92 (0.86, 0.99)	100.00

NOTE: Weights are from random effects analysis



*This analysis includes data from the subset of ViDiFlu trial participants who were randomised to vitamin D vs. placebo control. **For this trial, participants were asked to report the occurrence of ARI during the one month prior to completing each annual survey (max surveys=5). The numerator is the number of participants who reported an ARI on at least one survey. The ARI outcomes for participants who completed fewer than 5 surveys and who did not report an ARI (N=2239; 14%) were estimated based on the % affected among those who completed all 5 surveys (N=12152; 76%).

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