Vitamin D supplementation to prevent asthma exacerbations: systematic review and meta-analysis of individual participant data

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Abstract

Background: Aggregate data meta-analysis of randomized controlled trials (RCTs) shows that vitamin D supplementation reduces the rate of moderate/severe asthma exacerbations, defined as those requiring treatment with systemic corticosteroids. It is not known whether this effect is restricted to patients with lower baseline vitamin D status.

Methods: We conducted a systematic review with one-step and two-step meta-analyses of individual participant data (IPD) from randomised controlled trials adjusting for age, sex, and clustering by study. Sub-group analyses were done to determine whether effects of vitamin D on risk of asthma exacerbation varied according to baseline 25-hydroxyvitamin D (25[OH]D) concentration, age, sex, ethnic / racial origin, body mass index, vitamin D dosing regimen, use of inhaled corticosteroids or study duration.

Findings: IPD were sought for eight eligible randomised controlled trials (total 1,078 participants), and obtained for 955/978 (97.6%) participants in seven studies. Vitamin D supplementation reduced the rate of moderate/severe asthma exacerbation among all participants (adjusted Incidence Rate Ratio [aIRR] 0.74, 95% CI 0.56 to 0.97, P=0.03, 955 participants in 7 studies). Sub-group analyses revealed that statistically significant protective effects were seen in participants with baseline 25(OH)D <25 nmol/L (aIRR 0.33, 95% CI 0.11 to 0.98, P=0.046, 92 participants in 3 studies) but not in those with higher baseline 25(OH)D levels (aIRR 0.77, 95% CI 0.58 to 1.03, P=0.08, 764

participants in 6 studies; P for interaction =0.25). P values for interaction were also >0.05 for all other sub-group analyses performed.

Interpretation: Vitamin D supplementation reduced the rate of moderate/severe asthma exacerbation overall. We did not find definitive evidence that effects of this intervention differed across sub-groups of patients.

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Research in Context

Evidence before this study

The major circulating vitamin D metabolite, 25-hydroxyvitamin D, supports innate immune responses to respiratory viruses, which are the major precipitant of asthma exacerbations. A Cochrane meta-analysis of aggregate data from double-blind placebo-controlled RCTs found that vitamin D supplementation reduced the rate of moderate/severe asthma exacerbations, defined as those requiring treatment with systemic corticosteroids (rate ratio 0.63, 95% CI 0.45 to 0.88). It is not known whether this effect is restricted to patients with lower baseline vitamin D status: individual participant data meta-analysis could resolve this issue, but this has not previously been performed.

Added value of this study

Our meta-analysis of individual participant data from 955 participants in 7 randomised controlled trials provides an updated pooled estimate of the protective effects of vitamin D against moderate/severe asthma exacerbation overall. Uniquely, it also investigates whether the effect of vitamin D on risk of asthma exacerbation varies according to baseline 25-hydroxyvitamin D concentrations.

Implications of all the available evidence

Overall, vitamin D reduced the rate of moderate/severe asthma exacerbations, as compared with placebo (0.30 vs. 0.43 events per person per year respectively, P=0.03). Sub-group analysis revealed that vitamin D reduced the rate of moderate/severe asthma exacerbations in people with baseline 25-hydroxyvitamin D <25 nmol/L (0.19 *vs.* 0.42 events per person per year, P=0.046) but the effect in those with higher baseline concentrations was not statistically significant (0.33 *vs.* 0.46 events per person per year, P=0.08). The P value for interaction for this sub-group analysis was non-significant (P=0.25), indicating that we have not formally demonstrated that effects of vitamin D supplementation differ according to baseline vitamin D status.

Introduction

Asthma affects more than 300 million people worldwide and is estimated to cause almost 400,000 deaths annually [1, 2]. Asthma mortality arises primarily during episodes of acute worsening of symptoms, termed exacerbations, which are commonly precipitated by viral upper respiratory infections [3]. Virus-induced asthma exacerbations are associated with increased production of pro-inflammatory cytokines such as interleukin-17A (IL-17A), which exacerbate allergic airway responses [4]. Vitamin D metabolites support antiviral responses in respiratory epithelial cells [5] and inhibit production of IL-17A in peripheral blood mononuclear cells isolated from patients with severe asthma [6]. Low circulating concentrations of the major circulating vitamin D metabolite, 25-hydroxyvitamin D (25[OH]D), associate with increased risk of asthma exacerbation in both children [7] and adults [8], and a total of 8 published double-blind placebo-controlled RCTs have investigated effects of vitamin D supplementation on the risk of asthma exacerbation [9-16]. To date, six meta-analyses incorporating data from trials of vitamin D for the management of asthma have been conducted: four report protective effects of vitamin D supplementation against asthma exacerbation [17-20], one reports no such effect [21] and one did not attempt meta-analysis for the outcome of exacerbation [22]. The most recent of these, a Cochrane systematic review and aggregate data meta-analysis including data from both children and adults and restricted to double-blind placebo-controlled RCTs, found that vitamin D supplementation reduced the rate of moderate/severe asthma exacerbations, defined as those requiring treatment with systemic corticosteroids, by 37% [20]. However, lack of individual participant data (IPD) precluded conduct of sub-group analyses to address the question of whether

protective effects of vitamin D supplementation against asthma exacerbation are stronger in those with lower baseline vitamin D status. This might be expected on the grounds that individuals with the lowest baseline levels of a micronutrient will derive the greatest benefit from its replacement. In keeping with this hypothesis, protective effects of vitamin D supplementation against acute respiratory infection [23] and acute exacerbations of COPD [24, 25] have been reported to be strongest in those with lower circulating 25(OH)D concentrations. We therefore set out to obtain IPD from double-blind placebo-controlled RCTs investigating effects of vitamin D supplementation on risk of asthma exacerbation, and meta-analyse them in order to obtain an updated estimate of overall effectiveness, and to determine whether effects of this intervention vary according to baseline vitamin D status.

Methods

Protocol and Registration

Methods were described in a protocol that was registered with the PROSPERO International Prospective Register of Systematic Reviews http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42014013953). Research Ethics Committee approval to conduct this meta-analysis was not required in the UK; local ethical permission to contribute de-identified IPD from primary RCTs was required and obtained for studies by Urashima *et al* [9] and Tachimoto *et al* [14] (Ethics committee of the Jikei University School of Medicine, ref 26-333: 7839). Findings are reported according to the PRISMA guidelines for IPD meta-analysis. [26]

Study Selection

Double-blind, placebo-controlled RCTs of supplementation with vitamin D_3 or vitamin D_2 in patients with asthma were eligible for inclusion if they had been approved by a Research Ethics Committee and if data on incidence of asthma exacerbation were reported.

Data Sources and Searches

Two investigators (DAJ and ARM) searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL) and Web of Science using the electronic search strategies described in the Appendix. Searches were regularly updated up to and including 26th October 2016. 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 RCTs. Three investigators (DAJ, CAC and ARM) determined which studies met the eligibility criteria.

Data Extraction and Quality Assurance

IPD were requested from the Principal Investigator for each eligible trial, and the terms of collaboration were specified in a data transfer agreement, signed by representatives of the data provider and the recipient (Queen Mary University of London). Data were de-identified at source prior to transfer *via* email. On receipt, three investigators (DAJ, RLH and LG) assessed data integrity by performing internal consistency checks and by attempting to replicate results of the analysis for incidence of asthma exacerbations where this was published in the trial report. Study authors were contacted to provide missing data and to resolve queries arising from these integrity checks. Once queries had been resolved, clean data were uploaded to the main study database, which was held in STATA IC v12 (College Station, TX).

Data relating to study characteristics were extracted for the following variables: setting, eligibility criteria, details of intervention and control regimens and study duration. IPD were extracted for the following variables, where available. Baseline data were requested for age, sex, racial/ethnic origin, weight, height, serum 25(OH)D concentration, study allocation (vitamin D *vs.* placebo) and details of stratification variables if applicable. Follow-up data were requested for the total number of asthma exacerbations a) requiring treatment with systemic corticosteroids, b) resulting in emergency department attendance and/or hospitalisation, and c) as defined in the trial protocol; time from first dose of study medication to first asthma exacerbation requiring treatment with systemic corticosteroids; occurrence of serious adverse events and potential adverse reactions to vitamin D supplementation (hypercalcemia or renal stones); serum 25(OH)D concentration at final follow-up; and duration of participant follow-up.

Risk of Bias Assessment for Individual Studies

We used the Cochrane Collaboration Risk of Bias tool [27] 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. Selectivity of reporting was assessed either by comparing study protocols against study reports, or by specifically asking study authors whether all pre-specified outcomes were reported. Study quality was assessed independently by two investigators (ARM and DAJ), except for the trial by Martineau and colleagues [12], which was assessed by CAC. Discrepancies were resolved by consensus.

Definition of Outcomes

The primary outcome of the meta-analysis was incidence of asthma exacerbation requiring treatment with systemic corticosteroids. This outcome was selected on the basis that requirement for systemic corticosteroids is a widely recognised indicator of exacerbation severity [28]. Secondary outcomes were incidence of exacerbations resulting in emergency department attendance and/or hospitalisation; incidence of exacerbations as defined in the protocol of the primary trial; incidence of serious adverse events; incidence of potential adverse reactions to vitamin D (hypercalcaemia and renal stones); and mortality (asthma-related and all-cause).

Data Synthesis and Analysis

Data were analysed by LG, DAJ and RLH. Our IPD meta-analysis approach followed published guidelines [29]. Initially, all studies were re-analysed separately; the original authors were asked to confirm accuracy of this re-analysis where it had been performed previously, and any discrepancies were resolved. Then, for each outcome separately, we performed both one-step and two-step IPD meta-analysis. In the one-step approach, IPD from all studies were modelled simultaneously while accounting for the clustering of participants within studies. Mixed models were used with a random effect for study and fixed effects for age and sex to obtain the pooled intervention effect with a 95% confidence interval. Event rates were analysed using mixed effect Poisson regression,

proportions were analysed using mixed effects logistic regression, additionally adjusted for duration of participant follow-up, and survival data were analysed using mixed effects parametric survival models. For the analysis of the proportion of participants experiencing at least one moderate/severe exacerbation, participants who were lost to follow-up before they experienced an exacerbation were classified as not having had such an exacerbation. We did not adjust for other covariates because missing values for some participants would have led to their exclusion from statistical analyses. In the two-step approach, IPD were first analysed for each separate study independently to produce an estimate of the treatment effect for that study; event rates were analysed using Poisson regression with adjustment for age and sex, proportions were analysed using logistic regression with adjustment for age, sex and duration of participant follow-up and survival data were analysed using parametric survival models with adjustment for age and sex. A weighted average of the individual treatment effect estimates was then calculated using the DerSimonian and Laird procedure for random effects meta-analysis [30]. For two-step IPD meta-analysis, heterogeneity was summarized using the I^2 statistic.

Exploration of Variation in Effects

In order to explore the causes of heterogeneity and identify factors modifying the effects of vitamin D supplementation, we performed pre-specified sub-group analyses by extending the one-step meta-analysis framework to include treatment-covariate interaction terms. Sub-groups were defined according to baseline vitamin D status (serum 25[OH]D <25 *vs.* ≥25 nmol/L), age (<16 vs. ≥16 years), ethnic / racial origin (African-American / Afro-Caribbean / Black African origin vs. Asian origin vs. White European / White European origin vs. other / mixed origin), body mass index (<25 kg/m^2 *vs.* ≥25 kg/m^2), vitamin D dosing regimen (daily or weekly administration without bolus dosing *vs.* administration of a regimen including at least one bolus dose of at least 30,000 IU vitamin D), dose size (daily equivalent <2,000 IU *vs.* ≥2,000 IU), and concomitant asthma treatment (use of inhaled corticosteroids vs. not). The 25 nmol/L cut-off for baseline 25(OH)D concentration in sub-group analyses was selected on the

grounds that it is the threshold for vitamin D deficiency defined by the UK Department of Health [31], and the level below which vitamin D supplementation protects most strongly against acute respiratory infection [23]. An exploratory analysis investigating effects in sub-groups defined using the 50 nmol/L and 75 nmol/L cut-offs for baseline circulating 25(OH)D concentration was also performed, because observational studies have reported that less profound states of vitamin D deficiency may associate independently with increased risk of asthma exacerbation [7, 8]. Exploratory sub-group analyses by sex and study duration (< 6 months vs. ≥ 6 months) were also performed in response to comments from reviewers. Statistical significance was inferred for sub-group effects where the P value for the treatment-covariate interaction terms was <0.05.

Quality Assessment Across Studies

For the primary analysis, the likelihood of publication bias was investigated through the construction of a contour-enhanced funnel plot [32]. We used the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) [33] to assess the quality of the body of evidence contributing to the principal analyses.

Additional Analysis

We conducted a responder analysis in participants randomized to the intervention arm of included studies for whom end-study 25(OH)D data were available, comparing risk of moderate/severe asthma exacerbation in those who attained a serum 25(OH)D ≥75 nmol/L *vs.* those who did not.

Role of the Funding Source

The National Institute of Health Research was not involved in study design; in the collection, analysis or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results

Study Selection and Individual Participant Data Obtained

The study selection process is illustrated in Figure 1. Our search identified 488 unique studies that were assessed for eligibility, of which 8 studies with a total of 1,078 randomized participants fulfilled eligibility criteria. IPD were sought for all studies, and obtained for 7/8 studies (total 978 participants); data were not obtained for 1 study (100 participants) as the corresponding author did not respond to invitations to contribute IPD to this meta-analysis. Outcome data were obtained for 955/978 (97.6%) randomized participants in these seven studies.

Study and Participant Characteristics

Characteristics of the seven studies and their participants for whom IPD were obtained are presented in Table 1. RCTs were conducted in 6 different countries on 3 continents, and enrolled participants of both sexes from 1.6 to 85.0 years of age; five RCTs with a total of 297 included participants enrolled children, and two RCTs with a total of 658 included participants enrolled adults. Baseline serum 25(OH)D concentrations were determined in 6/7 of these RCTs; they ranged from undetectable to 187.2 nmol/L (to convert to ng/ml, divide by 2.496). All studies administered oral vitamin D_3 to participants in the intervention arm: this was given as 2-monthly bolus doses in 1 study (100,000 IU per bolus); as a daily dose in 4 studies (ranging from 500 IU/day to 2000 IU

/day; and as a combination of bolus and daily doses in 2 studies (100,000 IU bolus then 400-4,000 IU/day). Study durations ranged from 15 weeks to 1 year. Details of the number of moderate/severe exacerbations and the proportion of participants experiencing at least one such event by arm and study are presented in Appendix Table 1. In two RCTs [9, 10] no moderate/severe exacerbations arose, and in one trial [14] only one moderate/severe exacerbation arose. Effects estimates could not be calculated for these three studies individually; accordingly they contributed data to one-step, but not two-step, meta-analyses.

IPD integrity was confirmed by replication of primary analyses in published papers where applicable. The process of checking IPD revealed two discrepancies with primary reports. In the trial by Urashima and colleagues, the relative risk for asthma exacerbation was calculated using denominators based on the study population as a whole, irrespective of whether or not they had asthma (n=334) [9]. By contrast, we calculated this figure using denominators based on the number of children with asthma for whom outcome data were available (n=99). In the trial by Castro and colleagues, IPD detailed 14 serious adverse events arising in participants randomized to placebo, as compared to 13 such events reported in the published manuscript [11].

Risk of Bias within Studies

Details of the risk of bias assessment are provided in Appendix Table 2. All RCTs but one were assessed as being at low risk of bias for all aspects considered. The trial by Kerley and colleagues [16] was assessed as being at unclear risk of bias due to its

relatively high rate of loss to follow-up (12/51 participants), although there was no evidence to suggest differential rates of loss to follow-up in intervention vs. control arms (7/24 vs. 5/27, respectively).

Overall Results: Incidence of Moderate/Severe Asthma Exacerbation

Results of one-step IPD meta-analysis testing effects of vitamin D on the rate of asthma exacerbations requiring treatment with systemic corticosteroids are presented in Table 2. Overall, vitamin D supplementation resulted in a statistically significant reduction in the rate of such exacerbations (adjusted Incidence Rate Ratio [aIRR] 0.74, 95% CI 0.56 to 0.97, P=0.03; 955 participants in 7 studies). In absolute terms, this represents a reduction from 0.43 events per person per year to 0.32 (95% CI 0.24 to 0.42) events per person per year (Appendix Table 3). Two-step IPD meta-analysis revealed a similar effect size (aIRR 0.69, 95% CI 0.52 to 0.92, P=0.01; P for heterogeneity =0.56; 719 participants in 4 studies; Figure 2). This evidence was assessed as being of high quality. Consistent trends were seen for analysis of the proportion of participants experiencing at least one moderate/severe asthma exacerbation in both one-step analysis (adjusted Odds Ratio [aOR] 0.75, 95% CI 0.51 to 1.10, , P=0.14; 955 participants in 7 studies) and two-step analysis (aOR 0.69, 95% CI 0.46 to 1.02, P=0.06; P for heterogeneity, 0.74; 719 participants in 4 studies; Appendix Figure 1). Similarly, trends towards a delay to first exacerbation with vitamin D vs. placebo were seen in both one-step analysis (adjusted Hazard Ratio [aHR] 0.78, 95% CI 0.55 to 1.10, P=0.15;

868 participants in 5 studies) and two-step analysis (aHR 0.74, 95% CI 0.52 to 1.05, P=0.09; P for heterogeneity 0.58; 680 participants in 3 studies; Appendix Figure 2).

Results of Sub-group Analyses: Incidence of Moderate/Severe Asthma Exacerbation

Sub-group analyses were conducted to investigate whether effects of vitamin D supplementation on rate of asthma exacerbations requiring treatment with systemic corticosteroids differed according to baseline vitamin D status, age, ethnic/racial origin, body mass index, administration of bolus-dose vitamin D, amount of vitamin D administered and concomitant use of inhaled corticosteroids. Results are presented in Table 2. Vitamin D supplementation resulted in a statistically significant reduction in the rate of moderate/severe asthma exacerbations among individuals with baseline circulating 25(OH)D <25 nmol/L (aIRR 0.33, 95% CI 0.11 to 0.98; 92 participants in 3 studies; within sub-group P=0.046). In absolute terms, this represents a reduction from 0.42 events per person per year to 0.14 (95% CI 0.05 to 0.42) events per person per year (Appendix Table 3). Vitamin D supplementation did not result in a statistically significant reduction in exacerbation rate in those with baseline 25(OH)D ≥25 nmol/L (aIRR 0.77, 95% CI 0.58 to 1.03; 764 participants in 6 studies; within sub-group P=0.08). The treatment-covariate interaction term (ratio of aIRRs) for this sub-group analysis was 0.56 (95% CI 0.20 to 1.52, P for interaction =0.25). Quality assessments of these within-subgroup effects were downgraded to moderate due to their relative imprecision (Appendix Table 3). An exploratory analysis testing effects of vitamin D supplementation in individuals with baseline 25(OH)D concentrations in the ranges 25-

49.9 nmol/L, 50-74.9 nmol/L and ≥75 nmol/L did not reveal evidence of effect modification (P for interaction 0.40) or statistically significant protective effects of vitamin D supplementation within these sub-groups (sub-group with baseline 25[OH]D 25.0-49.9 nmol/L, aIRR 0.79, 95% CI 0.50 to 1.23, 306 participants in 6 studies, within sub-group P=0.29; sub-group with baseline 25[OH]D 50.0-74.9 nmol/L, aIRR 0.76, 95% CI 0.48 to 1.22; 334 participants in 6 studies, within sub-group P=0.26; sub-group with baseline 25[OH]D ≥75 nmol/L at baseline, aIRR 0.79, 95% CI 0.37 to 1.69, 120 participants in 5 studies, within sub-group P=0.54; Figure 3). P values for interaction for all other sub-group analyses were also >0.05.

Secondary Outcomes: Efficacy

Results of one-step IPD meta-analysis of secondary efficacy outcomes are presented in Table 3. Vitamin D supplementation reduced the proportion of people experiencing at least one asthma exacerbation resulting in emergency department attendance and/or hospitalisation (aOR 0.46, 95% CI 0.24 to 0.91; 955 participants in 7 studies; P=0.03). In absolute terms, this represents a reduction from 58 per 1,000 people experiencing such an event to 28 per 1,000 people experiencing such an event (95% CI 15 to 53 per 1,000) (Appendix Table 3). No statistically significant effect of vitamin D supplementation was seen on risk of experiencing at least one asthma exacerbation as defined in the protocols of primary RCTs (aOR 0.81, 95% CI 0.58 to 1.11; 955 participants in 7 studies; P=0.19).

Secondary Outcomes: Safety

Results of one-step IPD meta-analysis of safety outcomes are also reported in Table 3. No participant experienced hypercalcaemia or renal stones. Vitamin D supplementation did not influence the risk of experiencing at least one serious adverse event of any cause (aOR 0.87, 95% CI 0.46 to 1.63; 955 participants in 7 studies; P=0.66). Only one death occurred in a trial participant; this was due to a road traffic accident.

Risk of Bias Across Studies

A funnel plot for the outcome of moderate/severe asthma exacerbation rate did not suggest publication bias in relation to this outcome, since the smaller RCTs showed equal spread of results on both sides of the overall adjusted rate ratio (Appendix Figure 3). Appendix Table 4 presents effect sizes for individual trials contributing to the two-step IPDMA ordered by study size: no relationship between effect size and study size was apparent.

Responder Analyses

Results of responder analyses are presented in Appendix Table 5. Among participants randomized to the intervention arm of studies for which end-study 25(OH)D data were available, no difference in risk of moderate/severe asthma exacerbation was observed between participants who attained a serum 25(OH)D ≥75 nmol/L *vs.* those who did not.

Discussion

We report results of the first IPD meta-analysis of RCTs of vitamin D to reduce the risk of asthma exacerbations. In the study population as a whole, one-step meta-analysis revealed that vitamin D supplementation reduced the rate of asthma exacerbations requiring treatment with systemic corticosteroids by 26%, and reduced the risk of experiencing at least one exacerbation requiring emergency department attendance and/or hospitalisation by 52%. Sub-group analyses revealed that reductions in exacerbation rate with vitamin D were statistically significant in those with baseline circulating 25(OH)D concentration <25 nmol/L, but not in those with higher baseline 25(OH)D levels.. Vitamin D supplementation was safe at the doses administered: no instances of hypercalcaemia or renal stones were seen, and serious adverse events were evenly distributed between participants randomized to vitamin D vs. placebo.

Our findings from analysis of the study population as a whole are consistent with those of our recent aggregate data meta-analysis of RCTs of vitamin D for the management of asthma, which reported protective effects against moderate/severe asthma exacerbations of similar magnitude (IRR 0.63, 95% CI 0.45 to 0.88) [20]. The current study represents a significant advance, as access to IPD has allowed conduct of sub-group analyses to evaluate whether certain factors modify effects of vitamin D supplementation on risk of asthma exacerbations. We hypothesized that protective effects of vitamin D supplementation against asthma exacerbation would be strongest in participants with the lowest baseline vitamin D status, as has been previously reported

for the outcome of acute respiratory infection [23]. We saw a statistically significant rate reduction in participants with baseline 25(OH)D <25 nmol/L, but not in those with higher baseline 25(OH)D levels. However, the P value for interaction for this sub-group analysis was non-significant (0.25);.formally, therefore, we have not demonstrated that effects are stronger in one group than in the other. P values for interaction were also >0.05 for sub-group analyses relating to age, sex, racial / ethnic origin, body habitus, vitamin D dosing regimen, use of inhaled corticosteroids and study duration. It may be that these factors do not modify the effects of vitamin D supplementation on exacerbation risk; alternatively, we may have lacked statistical power to detect the relevant interactions. Several additional RCTs are on-going (e.g. NCT01419262, NCT01728571, NCT02197702, NCT02424552), and, in due course, we hope to include IPD from these in an updated meta-analysis: this will provide increased power for sub-group analyses.

While vitamin D reduced the risk of asthma exacerbations requiring treatment with systemic corticosteroids, no statistically significant effect was seen on the risk of asthma exacerbations as originally defined in the protocols of primary trials. In the majority of trials, the original definition of exacerbation was broader than the one pre-specified for this meta-analysis, encompassing for example events that resulted in dips in peak expiratory flow rate or forced expiratory volume in 1 second (FEV1) that were not treated with systemic corticosteroids[11, 12]. Differing efficacy of vitamin D supplementation for these two outcomes may indicate that this intervention specifically reduces risk of more serious exacerbations. Alternatively, it may be that less stringent definitions of exacerbation in primary trial protocols resulted in a degree of

misclassification, with a consequent increase in noise: signal ratio that may have obscured a real effect of vitamin D on exacerbation risk.

Our study has several strengths. Included studies were of high quality, and of sufficient duration for steady-state 25(OH)D concentrations to be attained among participants randomised to receive vitamin D_3. The proportion of randomized participants with missing outcome data was small (2.4%) and 25(OH)D levels were measured using validated assays in laboratories that participated in External Quality Assessment schemes. Participants with diverse characteristics in multiple settings were represented: we incorporated new data from a trial conducted in children with severe asthma [16] that was published after the date of the final literature search conducted for our previous aggregate data meta-analysis [20]. Our findings therefore have a high degree of internal and external validity.

Our study also has some limitations. We failed to obtain IPD for one eligible trial [13]; however, this study was relatively small (n=100), and has previously been assessed as being at high risk of bias [20]. Of note, this study reported strong protective effects of vitamin D against asthma exacerbation [13]; thus, if exclusion of its findings leads to a bias, it is likely to be a bias towards the null. Interpretation of the Funnel plot (Appendix Figure 3) is limited by the small number of studies included, but the fact that the smaller RCTs showed an equal spread of results on both sides of the overall adjusted rate ratio provides some reassurance that publication bias was not a major issue in our meta-analysis – an impression that is reinforced by the lack of a relationship between effect

size and study size (Appendix Table 4). Power for some sub-group analyses was limited: this is an inescapable problem, given the relatively small number of published RCTs in this field. Where confidence intervals for estimates of effect from sub-group analyses were wide, we downgraded our quality assessment of sub-group findings to 'moderate' (Appendix Table 3).

In conclusion, our IPD meta-analysis confirms results from previous aggregate data meta-analysis showing that vitamin D supplementation safely reduces risk of moderate/severe asthma exacerbation. We did not find definitive evidence that effects of this intervention differed across sub-groups of patients, however. Given the low cost of this intervention and the major economic burden associated with asthma exacerbations, vitamin D supplementation represents a potentially cost-effective strategy to reduce this important cause of morbidity and mortality.

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Competing Interests

The authors declared no conflicts of interest: all have completed the ICMJE uniform disclosure form: We declare financial support for this work from the National Institute for Health Research (NIHR) under its Health Technology Assessment (HTA) Program. 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

ARM is the manuscript's guarantor and he affirms that the manuscript is an honest, accurate, and transparent account of the study being reported.

Data Sharing Statement

Data sharing: a partial dataset, incorporating patient level data from RCTs for which the relevant permission for data sharing have been obtained, is available from the corresponding author at a.martineau@qmul.ac.uk.

Author Contributions

ARM led the funding application, with input from RLH, CJG and CAC who were co-applicants. DAJ, CAC and ARM assessed eligibility of studies for inclusion. DAJ, CJG, CAC, CPK, MEJ, DM, IS, MU and ARM were all directly involved in the acquisition of data for the work. RLH designed statistical analyses in consultation with authors contributing individual patient data. Statistical analyses were done by LG, DAJ and RLH. 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.

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Table 1: Characteristics of trials and participants included in individual participant data meta-analysis

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.) [range]	Baseline 25(OH)D <25 nmol/L (%)	Intervention: control	Oral dose of vitamin D_3, intervention arm	Proportion of participants with 25(OH)D ≥75 nmol/L at final follow-up (%)	Control	Study duration	N with available outcome data / N randomized (%)
Urashima 2010[9]	Japan	School children with asthma	9.5 (2.1) [6.0 to 15.0]	56:43	--	Not determined	--	43:56	1,200 IU daily	--	Placebo	4 mo	99/110 (90.0)
Majak 2011[10]	Poland	School children with asthma	10.9 (3.3) [6.0 to 17.0]	32:16	RIA (BioSource Europe), RIQAS	88.9 (38.2) [31.5 to 184.7]	0/48 (0.0)	24:24	500 IU daily	16/24 (66.7)	Placebo	6 mo	48/48 (100.0)
Castro 2014[11]	USA	Adults with asthma	39.2 (12.9) [18.0 to 85.0]	130:278	CLA (DiaSorin), VDSP	47.0 (16.9) [10.0 to 74.6]	55/408 (13.5)	201:207	100,000 IU bolus then 4,000 IU daily	143/174 (83.6)	Placebo	28 wk	408/408 (100.0)
Martineau 2015[12]	UK	Adults with asthma	47.9 (14.4) [16.0 to 78.0]	109:141	LC-MS/MS, DEQAS	49.6 (24.7) [0.0 to 139.0]	36/250 (14.4)	125:125	120,000 IU bolus 2-monthly	40/107 (37.4)	Placebo	1 yr	250/250 (100.0)
Tachimoto 2016[14]	Japan	School children with asthma	9.9 (2.3) [6.0 to 15.0]	50:39	RIA (DiaSorin), CAP	74.9 (24.6) [20.0 to 187.2]	1/89 (1.1)	54:35	800 IU daily, first 2 mo.	34/54 (63.0)	Placebo	6 mo	89/89 (100.0)
Kerley 2016[16]	Ireland	School children with asthma	8.6 (2.8) [5.0 to 15.0]	24:15	LC-MS/MS, DEQAS	54.4 (17.4) [26.0 to 92.0]	0/39 (0.0)	17:22	2,000 IU daily	13/17 (76.5)	Placebo	15 wk	39/51 (76.5)
Jensen 2016[15]	Canada	Pre-school children with asthma	2.9 (1.1) [1.6 to 5.5]	7:15	LC-MS/MS, DEQAS	64.2 (14.0) [42.0 to 87.0]	0/22 (0.0)	11:11	100,000 IU bolus then 400 IU daily	7/8 (87.5)	400 IU vitamin D_3 daily	6 mo	22/22 (100.0)

40 international units (IU) vitamin D_3 = 1 μg; 25(OH)D concentrations reported in ng/ml were converted to nmol/L by multiplying by 2.496. 25(OH)D, 25-hydroxyvitamin D; mo, month; yr, year; wk, week. CAP, College of American Pathologists, CLA, chemiluminescent assay; DEQAS, Vitamin D External Quality Assessment Scheme; EQA, external quality assessment; LC-MS/MS, liquid chromatography tandem-mass spectrometry, RIA, radio-immunoassay; RIQAS, Randox International Quality Assessment Scheme; VDSP, Vitamin D Standardisation Program of the Office of Dietary Supplements, National Institutes of Health, USA.

Table 2: One-step individual participant data meta-analysis, rate of asthma exacerbations requiring treatment with systemic corticosteroids: overall and by sub-group.

	No. participants (no. trials)[1]	Event rate per participant-year, control group	Event rate per participant-year, intervention group	Adjusted incidence rate ratio (95% CI)[2]	P value	P value for Interaction
Overall	955 (7)	121/284.7 (0.43)	85/286.6 (0.30)	0.74 (0.56, 0.97)	0.03	--
Baseline 25(OH)D (nmol/L)						
<25	92 (3)	14/33.0 (0.42)	6/32.2 (0.19)	0.33 (0.11, 0.98)	0.046	0.25
≥25	764 (6)	107/233.8 (0.46)	79/240.2 (0.33)	0.77 (0.58, 1.03)	0.08	
Age, years						
<16	290 (5)	26/57.6 (0.45)	19/61.8 (0.31)	0.64 (0.34, 1.20)	0.16	0.56
≥16	665 (3)	95/227.2 (0.42)	66/224.7 (0.29)	0.70 (0.51, 0.97)	0.03	
Sex						
Female	547 (7)	80/163.6 (0.49)	47/167.7 (0.28)	0.61 (0.43, 0.88)	0.008	0.17
Male	408 (7)	41/121.1 (0.34)	38/118.9 (0.32)	0.91 (0.58, 1.42)	0.67	
Ethnic / racial origin						
African-American Afro-Caribbean/ Black African origin	154 (3)	28/46.4 (0.60)	14/43.4 (0.32)	0.54 (0.29, 1.03)	0.06	0.32
Asian origin	207 (5)	6/42.0 (0.14)	4/48.5 (0.08)	0.81 (0.19, 3.51)	0.78	
White European/White European origin	520 (5)	80/177.8 (0.45)	59/172.3 (0.34)	0.79 (0.56, 1.11)	0.17	
Other/Mixed	74 (3)	7/18.6 (0.38)	8/22.3 (0.36)	0.88 (0.31, 2.53)	0.81	
Body habitus						
Not overweight	381 (7)	38/110.5 (0.34)	26/104.5 (0.25)	0.91 (0.55, 1.51)	0.71	0.31
Overweight[3]	574 (7)	83/174.3 (0.48)	59/182.0 (0.32)	0.68 (0.49, 0.95)	0.02	
Bolus-dose vitamin D given						
No	275 (4)	13/53.8 (0.24)	10/58.9 (0.17)	0.65 (0.26, 1.63)	0.36	0.49
Yes	680 (3)	108/230.9 (0.47)	75/227.6 (0.33)	0.71 (0.52, 0.95)	0.02	
Daily Dose Equivalent, IU						
<2,000	258 (4)	13/52.1 (0.25)	10/58.6 (0.17)	0.62 (0.26, 1.44)	0.26	0.78
≥2,000	697 (3)	108/232.7 (0.46)	75/228.0 (0.33)	0.73 (0.54, 0.98)	0.03	
Inhaled corticosteroids						
No	92 (4)	1/18.8 (0.05)	4/26.1 (0.15)	1.11 (0.07, 18.40)	0.94	0.19
Yes	764 (5)	120/248.0 (0.48)	81/246.3 (0.33)	0.71 (0.54, 0.95)	0.02	
Study Duration						
<6 months	138 (2)	13/25.0 (0.52)	9/19.4 (0.46)	0.50 (0.18, 1.37)	0.18	0.62
≥6 months	816 (5)	108/259.8 (0.42)	76/267.2 (0.28)	0.72 (0.53, 0.96)	0.03	

1, some trials did not contribute data to a given sub-group, either because individuals within that sub-group were not represented, or because data relating to the potential effect modifier were not available; accordingly the number of trials represented varies between sub-groups. **2**, adjusted for age and sex. **3**, overweight defined as body mass index z-score ≥1.0 for participants aged <19 years and as body mass index ≥25 kg/m^2 for participants aged ≥19 years. **Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval.

Table 3: One-step individual participant data meta-analysis, secondary outcomes

	No. participants (no. trials)	**Proportion with ≥1 event, control group (%)**	**Proportion with ≥1 event, intervention group (%)**	**Adjusted odds ratio (95% CI)[1]**	**P value**
Asthma exacerbation resulting in ED attendance and/or hospitalisation	955 (7)	28/480 (5.8)	14/475 (2.9)	0.46 (0.24 to 0.91)	0.03
Asthma exacerbation as defined in primary trial	955 (7)	123/480 (25.6)	105/475 (22.1)	0.81 (0.58 to 1.11)	0.19
Serious adverse event of any cause	955 (7)	22/480 (4.6)	20/475 (4.2)	0.87 (0.46 to 1.63)	0.66
Hypercalcaemia	955 (7)	0/480 (0.0)	0/475 (0.0)	-	-
Renal stones	955 (7)	0/480 (0.0)	0/475 (0.0)	-	-
Death due to asthma exacerbation	955 (7)	0/480 (0.0)	0/475 (0.0)	-	-
Death due to any cause	955 (7)	0/480 (0.0)	1/475 (0.2)[2]	-	-

1, adjusted for age, sex and duration of participant follow-up. 2, death due to road traffic accident. Abbreviation: CI, confidence interval; ED, emergency department.

Figure 1: PRISMA Diagram of Study Selection

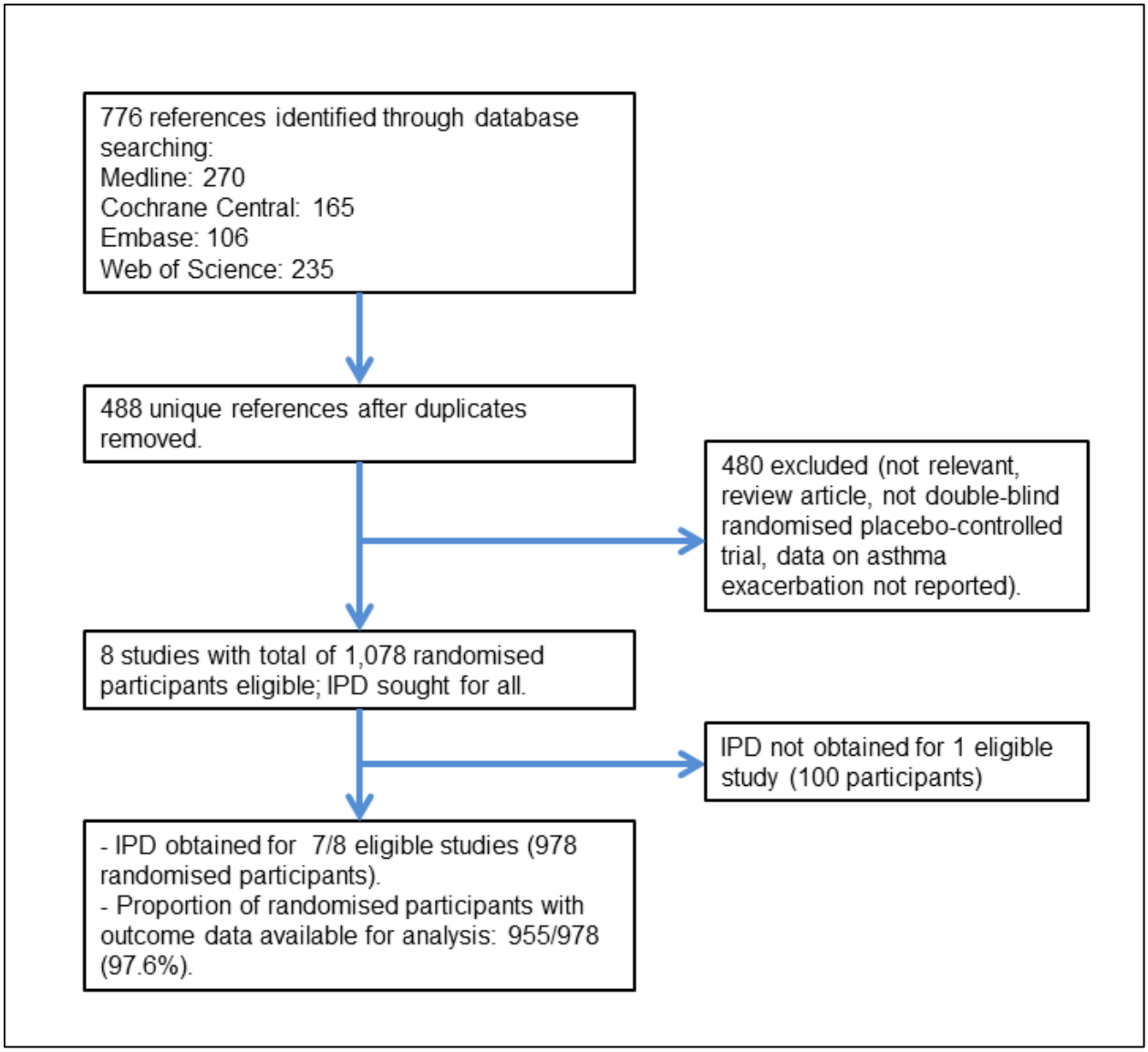
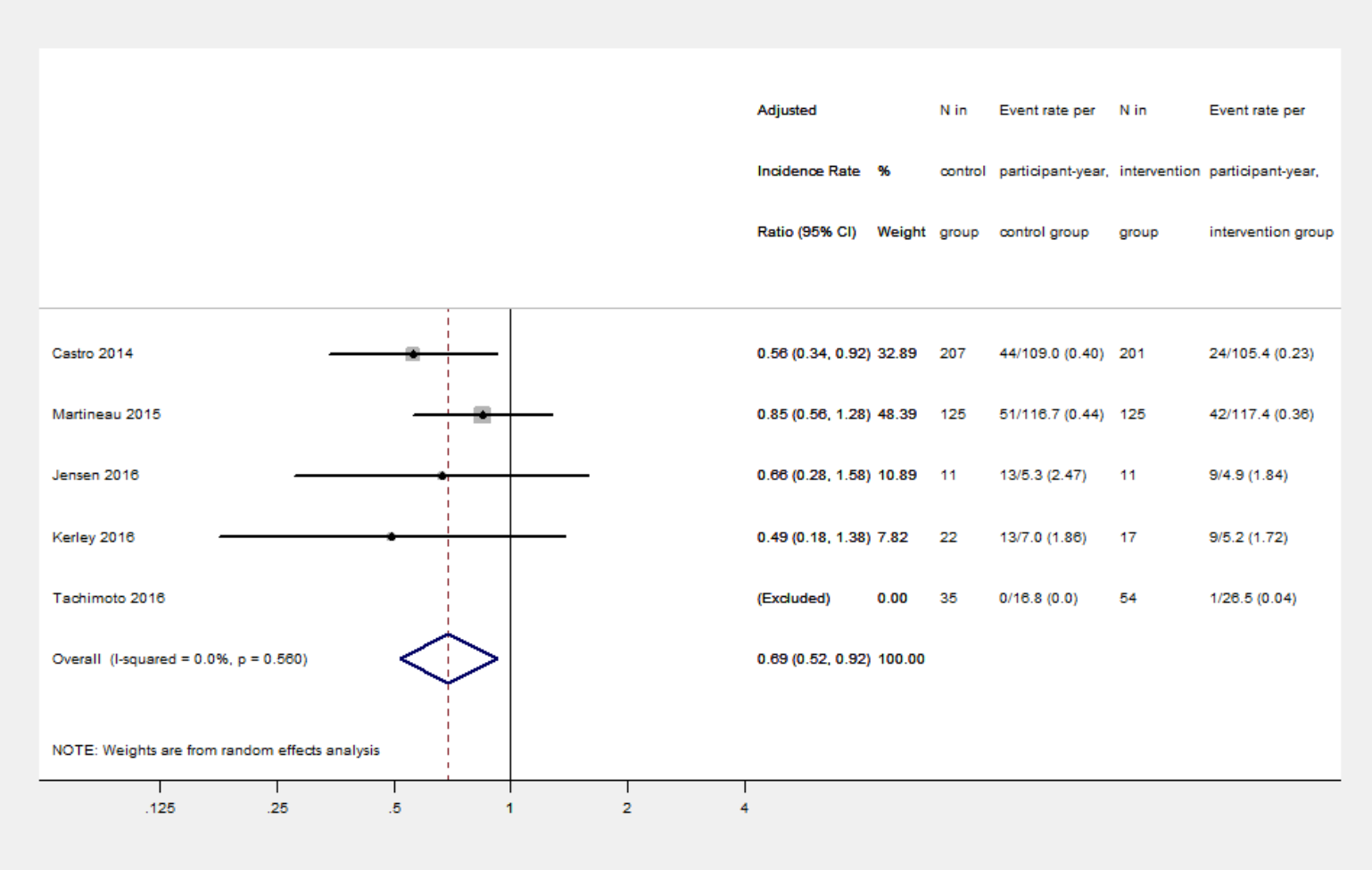
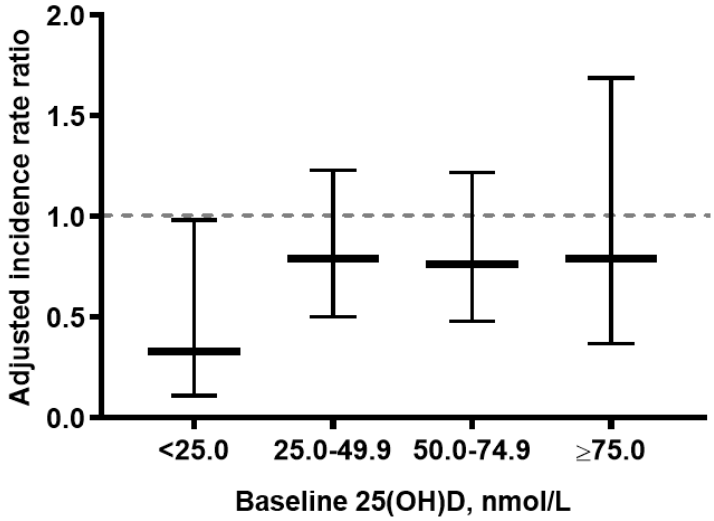


Figure 2: Two-step individual participant data meta-analysis, event rate for asthma exacerbations requiring treatment with systemic corticosteroids



Footnote: no asthma exacerbations requiring treatment with systemic corticosteroids arose in the trials by Urashima *et al* [9] and Majak *et al* [10]. Only one such event arose in the trial by Tachimoto *et al* [14]; thus an adjusted incidence rate ratio could not be calculated for this study.

Figure 3: Effects of vitamin D supplementation on asthma exacerbation rate by baseline circulating 25-hydroxyvitamin D concentration categorised by 25 nmol/L strata: results of one-step individual participant data meta-analysis.



Footnote: Incidence rate ratio adjusted for age and sex. Mean and 95% CI are presented. 25(OH)D, 25-hydroxyvitamin D.

Vitamin D supplementation to prevent asthma exacerbations: systematic review and meta-analysis of individual participant data

Appendix

Search Strategies

A. Medline

Cochrane Highly Sensitive Search Strategy for identifying randomized controlled trials

#1. randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]

#2. animals [mh] NOT humans [mh]

#3. #1 NOT #2

Terms specific to vitamin D

#4. Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Terms specific to asthma

#5 Asthma OR bronchial hyperreactivity OR bronchial hyper-reactivity OR respiratory hypersensitivity OR reactive airway

Combination of terms to identify randomized controlled trials of vitamin D conducted in patients with asthma

#3 AND #4 AND #5

B. RANDOMIZED

Terms for identifying randomized controlled trials

#1 ‘randomized controlled trial’/exp OR ‘single blind procedure’/exp OR ‘double blind procedure’/exp OR ’crossover procedure’/exp
#2 random*:ab,ti OR placebo*:ab,ti OR crossover*:ab,ti OR ’cross over’:ab,ti OR allocat*:ab,ti OR ((singl* OR doubl*) NEXT/1 blind*):ab,ti OR trial:ti
#3. #1 OR #2

Terms specific to vitamin D

#4. vitamin AND d OR vitamin AND d2 OR vitamin AND d3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Terms specific to asthma

#5 asthma OR (bronchus AND hyperreactivity) OR (respiratory AND tract AND allergy) OR (reactive AND airway*) OR asthma* OR (bronchial AND hyperreactivity) OR (bronchial AND 'hyper reactivity') OR (respiratory AND hypersensitivity)

Combination of terms to identify randomized controlled trials of vitamin D conducted in patients with asthma

#3 AND #4 AND #5

C. Cochrane Central

Terms specific to vitamin D

#1. Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Terms specific to asthma

#2. Asthma OR bronchial hyperreactivity OR bronchial hyper-reactivity OR respiratory hypersensitivity OR reactive airway

Combination of terms to identify randomised controlled trials of vitamin D conducted in patients with asthma

#1 AND #2

D. Web of Science

TS =(Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol) AND TS =(Asthma OR bronchial hyperreactivity OR bronchial hyper-reactivity OR respiratory hypersensitivity OR reactive airway) AND TS =(placebo* or random* or clinical trial* or double blind* or single blind* or rct)

Results

Appendix Table 1: Asthma exacerbations requiring treatment with systemic corticosteroids by study and allocation

Study first author & year	No. participants, control arm	No. participants with ≥1 exacerbation, control arm	No. exacerbations, control arm	Duration of follow-up, control arm (participant-years)	No. participants, intervention arm	No. participants with ≥1 exacerbation, intervention arm	No. exacerbations, intervention arm	Duration of follow-up, intervention arm (participant-years)
Urashima 2010[1]	56	0	0	18.0	43	0	0	14.1
Majak 2011[2]	24	0	0	11.9	24	0	0	13.1
Castro 2014[3]	207	36	44	109.0	201	24	24	105.4
Martineau 2015[4]	125	32	51	116.7	125	26	42	117.4
Tachimoto 2016[5]	35	0	0	16.8	54	1	1	26.5
Kerley 2016[6]	22	8	13	7.0	17	5	9	5.2
Jensen 2016[7]	11	5	13	5.3	11	7	9	4.9

Appendix Table 2: Risk of Bias Assessment

	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Urashima 2010[1]	✓	✓	✓	✓	✓	✓	✓
Majak 2011[2]	✓	✓	✓	✓	✓	✓	✓
Castro 2014[3]	✓	✓	✓	✓	✓	✓	✓
Martineau 2015[4]	✓	✓	✓	✓	✓	✓	✓
Tachimoto 2016[5]	✓	✓	✓	✓	✓	✓	✓
Kerley 2016[6]	✓	✓	✓	✓	?	✓	✓
Jensen 2016[7]	✓	✓	✓	✓	✓	✓	✓

✓ = low risk of bias; ? = unclear risk of bias;

Appendix Table 3: Summary of Findings Table

Summary of findings:

Vitamin D compared to placebo for prevention of severe asthma exacerbation

Patient or population: children and adults with predominantly mild to moderate asthma
Setting: primary and secondary care
Intervention: vitamin D_3 administered orally over study duration of 15 weeks to 1 year
Comparison: placebo

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo	Risk with vitamin D				
Rate ratio, exacerbations requiring systemic corticosteroids, overall.	0.43 events per person per year	0.32 events per person per year (0.24 to 0.42)	aIRR 0.74 (0.56 to 0.97)	955 (7 RCTs)	⊕⊕⊕⊕ HIGH	--
Rate ratio, exacerbations requiring systemic corticosteroids, sub-group with baseline 25(OH)D <25 nmol/L.	0.42 events per person per year	0.14 events per person per year (0.05 to 0.42)	aIRR 0.33 (0.11 to 0.98)	92 (3 RCTs)	⊕⊕⊕◯ MODERATE	Quality downgraded one level for imprecision
Rate ratio, exacerbations requiring systemic corticosteroids, sub-group with baseline 25(OH)D ≥25 nmol/L.	0.46 events per person per year	0.35 events per person per year (0.27 to 0.47)	aIRR 0.77 (0.58 to 1.03)	763 (6 RCTs)	⊕⊕⊕◯ MODERATE	Quality downgraded one level for imprecision
Proportion with ≥1 exacerbation requiring ED visit or hospitalisation or both.	58 per 1,000	28 per 1,000 (15 to 53)	aOR 0.46 (0.24 to 0.91)	955 (7 RCTs)	⊕⊕⊕⊕ HIGH	--
Proportion with ≥1 serious adverse event	46 per 1,000	40 per 1,000 (22 to 73)	aOR 0.87 (0.46 to 1.63)	955 (7 RCTs)	⊕⊕⊕◯ MODERATE	Quality downgraded one level for imprecision

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

aIRR: adjusted Incidence Rate Ratio; aOR: adjusted Odds ratio; CI: Confidence interval; RCT: randomised controlled trial.

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Appendix Table 4: Effect of vitamin D supplementation on rate of asthma exacerbations requiring treatment with systemic corticosteroids: individual trials listed by increasing study size

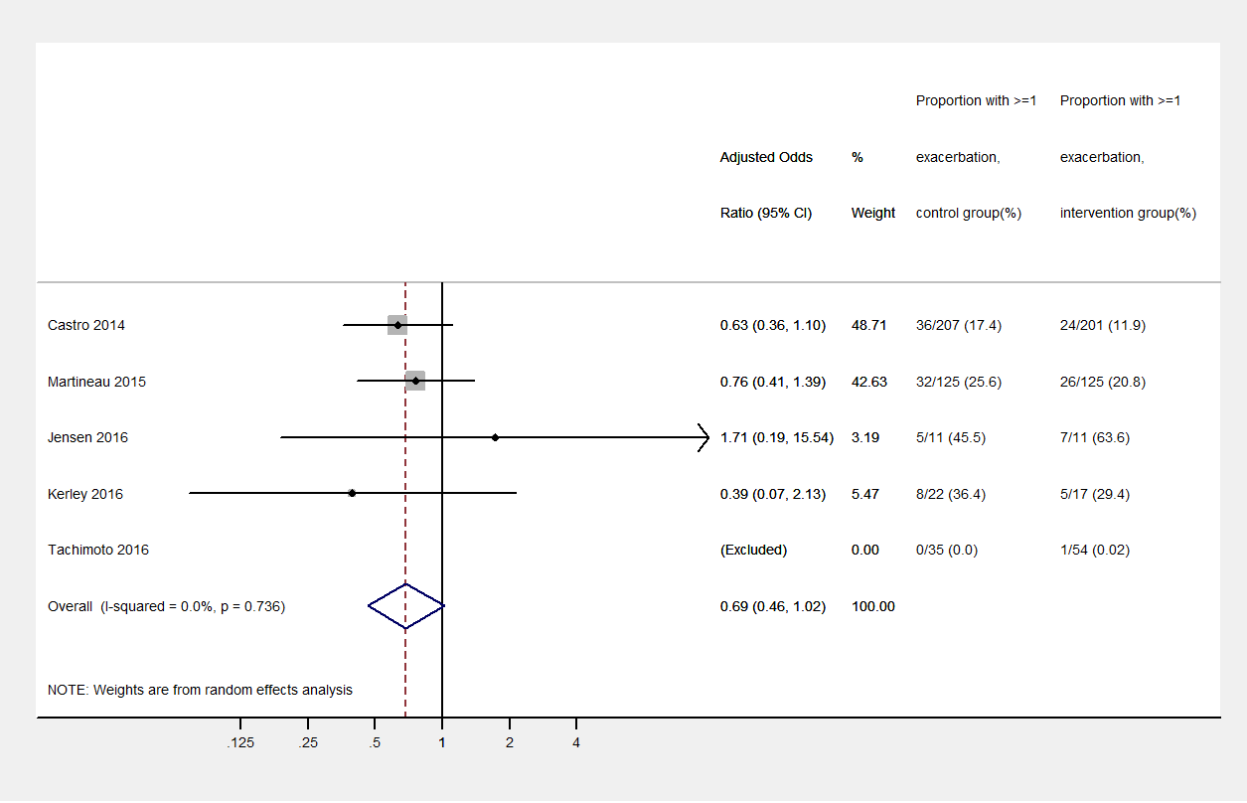
Reference	Sample size	Adjusted IRR (95% CI)
Jensen 2016 [7]	22	0.66 (0.28 to 1.58)
Kerley 2016 [6]	39	0.49 (0.18 to 1.38)
Martineau 2015 [4]	250	0.85 (0.56 to 1.28)
Castro 2014 [3]	408	0.56 (0.34 to 0.92)

Appendix Table 5: Responder analysis, one-step individual participant data meta-analysis

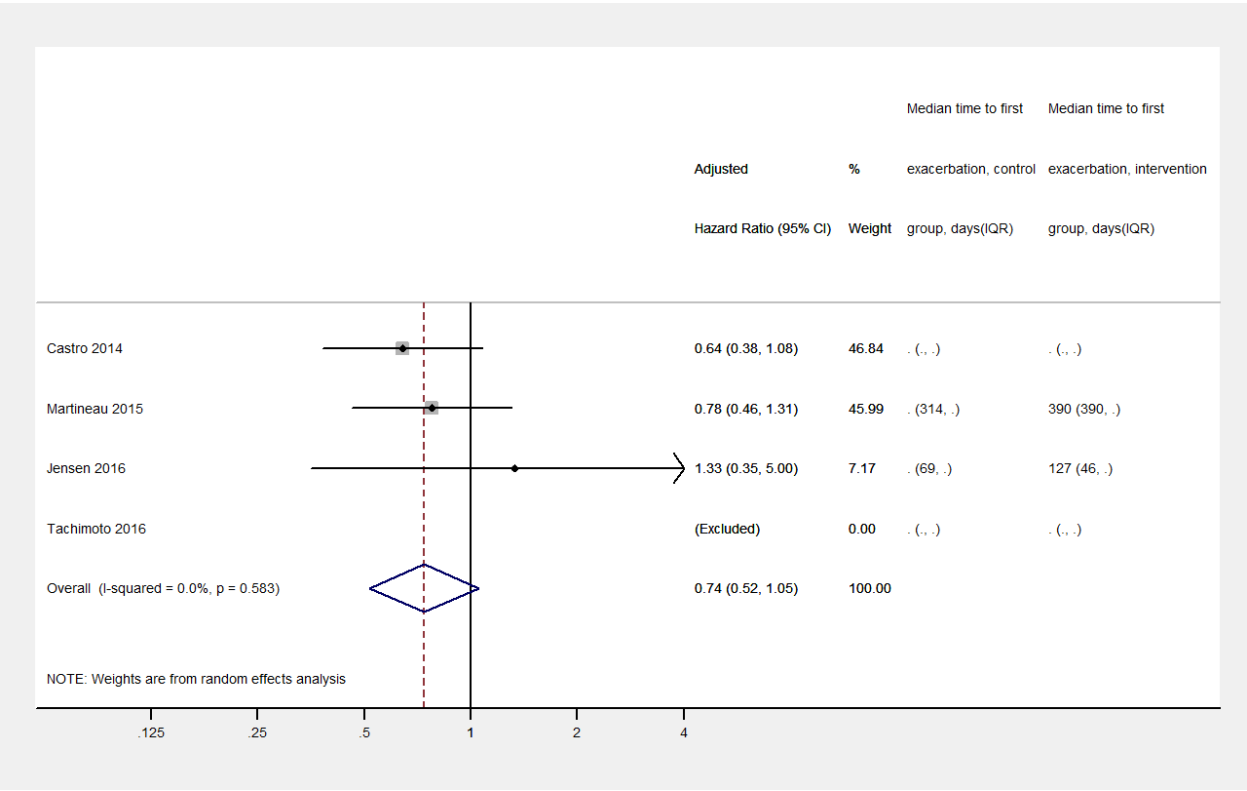
	No. participants (no. trials)	**Rate of asthma exacerbations requiring systemic corticosteroids per participant-year**	**Adjusted incidence rate ratio (95% CI)[1]**	**P value**
Intervention, end-study 25(OH)D < 75 nmol/L	131 (6)	35/100.5 (0.35)	1.00 (ref)	0.60
Intervention, end-study 25(OH)D ≥75 nmol/L	253 (6)	44/150.4 (0.29)	0.88 (0.53 to 1.44)	
	No. participants (no. trials)	**Proportion with ≥1 asthma exacerbation requiring systemic corticosteroids (%)**	**Adjusted odds ratio (95% CI)[2]**	**P value**
Intervention, end-study 25(OH)D < 75 nmol/L	131 (6)	23/131 (17.6)	1.00 (ref)	0.68
Intervention, end-study 25(OH)D ≥ 75 nmol/L	253 (6)	34/253 (13.4)	0.87 (0.44 to 1.71)	
	No. participants (no. trials)	**Median time to first asthma exacerbation requiring systemic corticosteroids, days (IQR)**	**Adjusted hazard ratio (95% CI)[1]**	**P value**
Intervention, end-study 25(OH)D < 75 nmol/L	118 (4)	-- (370 to --)[3]	1.00 (ref)	0.61
Intervention, end-study 25(OH)D ≥ 75 nmol/L	224 (4)	-- (318 to --)[3]	0.85 (0.46 to 1.59)	

1, adjusted for age and sex. 2, adjusted for age, sex and duration of participant follow-up. 3, medians and 75th centiles for time to first severe exacerbations in these groups cannot be defined. CI, confidence interval; IQR, inter-quartile range.

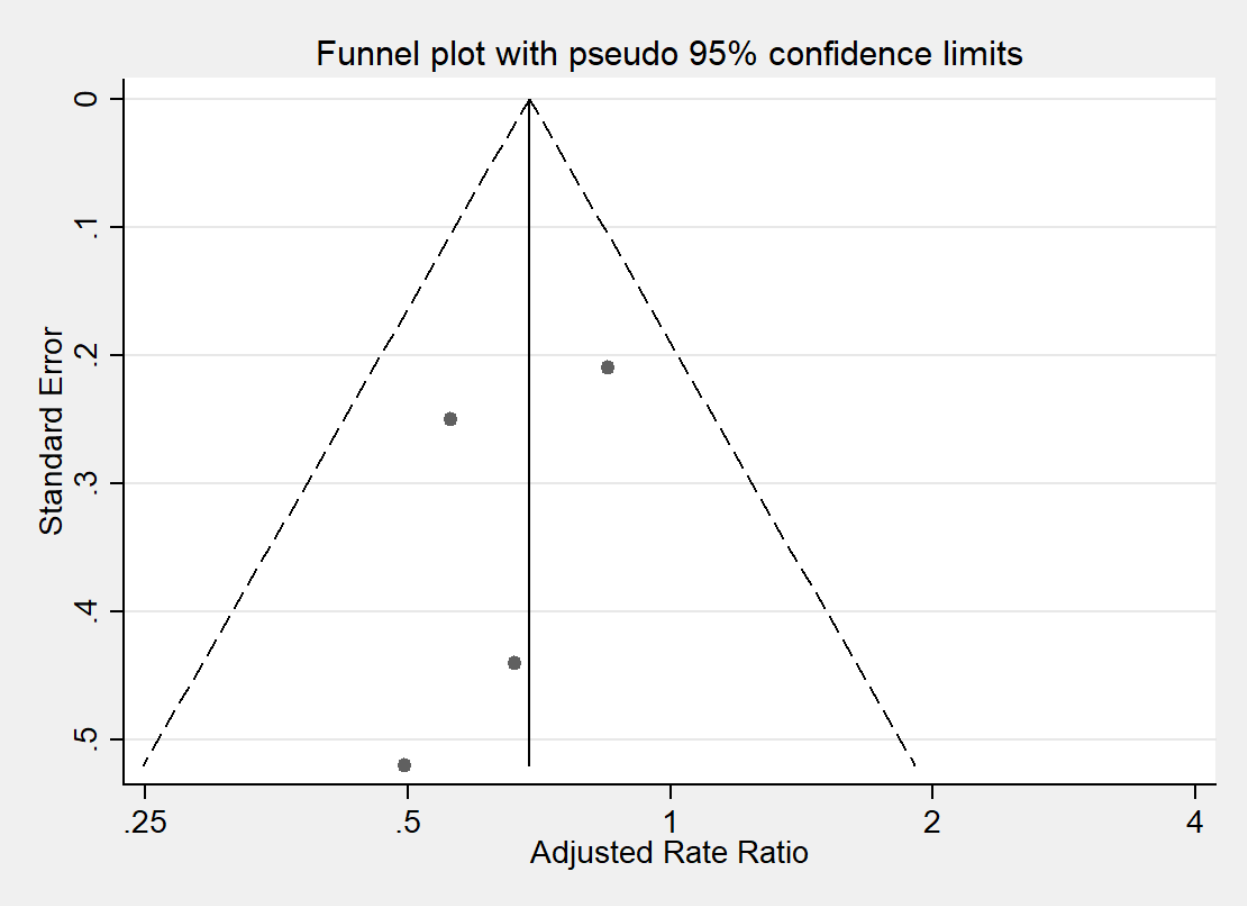
Appendix Figure 1: Two-step individual participant data meta-analysis, proportion of participants experiencing at least one asthma exacerbation requiring treatment with systemic corticosteroids



Appendix Figure 2: Two-step individual participant data meta-analysis, time to first asthma exacerbation requiring treatment with systemic corticosteroids



Appendix Figure 3: Funnel plot for individual patient data meta-analysis of severe asthma exacerbation rate



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