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ORIGINAL ARTICLE

Double-blind randomised placebo-controlled trial of bolus-dose vitamin D_3 supplementation in adults with asthma (ViDiAs)

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ABSTRACT

Rationale Asthma exacerbations are commonly precipitated by viral upper respiratory infections (URIs). Vitamin D insufficiency associates with susceptibility to URI in patients with asthma. Trials of vitamin D in adults with asthma with incidence of exacerbation and URI as primary outcome are lacking.

Objective To conduct a randomised controlled trial of vitamin D_3 supplementation for the prevention of asthma exacerbation and URI (coprimary outcomes).

Measurements and methods 250 adults with asthma in London, UK were allocated to receive six 2-monthly oral doses of 3 mg vitamin D_3 (n=125) or placebo (n=125) over 1 year. Secondary outcomes included asthma control test and St George's Respiratory Questionnaire scores, fractional exhaled nitric oxide and concentrations of inflammatory markers in induced sputum. Subgroup analyses were performed to determine whether effects of supplementation were modified by baseline vitamin D status or genotype for 34 single nucleotide polymorphisms in 11 vitamin D pathway genes.

Main results 206/250 participants (82%) were vitamin D insufficient at baseline. Vitamin D_3 did not influence time to first severe exacerbation (adjusted HR 1.02, 95% CI 0.69 to 1.53, p=0.91) or first URI (adjusted HR 0.87, 95% CI 0.64 to 1.16, p=0.34). No clinically important effect of vitamin D_3 was seen on any of the secondary outcomes listed above. The influence of vitamin D_3 on coprimary outcomes was not modified by baseline vitamin D status or genotype.

Conclusions Bolus-dose vitamin D_3 supplementation did not influence time to exacerbation or URI in a population of adults with asthma with a high prevalence of baseline vitamin D insufficiency.

Trial registration number NCT00978315 (ClinicalTrials.gov).

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Key messages

What is the key question?

- Does vitamin D_3 supplementation prevent asthma exacerbation or upper respiratory infection (URI) in adults with inhaled corticosteroid-treated asthma?

What is the bottom line?

- In patients with a high prevalence of vitamin D insufficiency at baseline, vitamin D_3 supplementation did not influence time to exacerbation or URI or concentrations of inflammatory markers in induced sputum; effects of the intervention were not modified by baseline vitamin D status or by polymorphisms in the vitamin D pathway.

Why read on?

- This is the first clinical trial in adults to investigate the effects of vitamin D_3 supplementation on incidence of asthma exacerbation and URI as primary outcomes.

INTRODUCTION

Acute exacerbations are the major cause of morbidity and mortality in patients with asthma. Viral upper respiratory infections (URIs) are major precipitants, but interventions to prevent these are lacking.[1] Numerous observational studies have reported associations between inadequate vitamin D status (serum 25-hydroxyvitamin D (25(OH)D) concentration <75 nmol/L) and susceptibility to URI[2]: these are particularly strong in patients with asthma.[3] In vitro, vitamin D metabolites favourably modulate the immune response to respiratory viruses[4] and enhance responsiveness to corticosteroids for production of the anti-inflammatory cytokine interleukin-10.[5] Two small trials of vitamin D supplementation in children with asthma treated with inhaled corticosteroids (ICS) have reported reduced rates of exacerbation among participants randomised to the intervention arm.[6 7]

Recently, a trial of vitamin D supplementation conducted in adults reported a trend towards

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protection against asthma exacerbation in the intervention arm as a secondary outcome (adjusted HR 0.63, 95% CI 0.39 to 1.01).[8] Trials of vitamin D in adults with asthma with incidence of exacerbation and URI as primary outcome are still lacking however. Moreover, no studies have been conducted to determine the influence of vitamin D on FE_{NO} or concentrations of inflammatory mediators in induced sputum, or to investigate whether genetic factors influence responses to vitamin D supplementation in asthma, as suggested by an observational study.[9] We therefore conducted a double-blind randomised placebo-controlled trial of vitamin D_3 supplementation in adults with ICS-treated asthma to test the hypothesis that this intervention reduces incidence of URI and severe exacerbations in this population, including measures of lower airway inflammation as secondary outcomes. Prespecified subgroup analyses were performed to investigate whether 34 single nucleotide polymorphisms in 11 vitamin D pathway genes modified the effects of vitamin D supplementation on clinical outcomes. In the absence of a consensus on 25(OH)D thresholds required for protection against disease, we deliberately enrolled patients with a broad range of baseline 25(OH)D values so that prespecified interaction analyses could be conducted to determine whether the effects of supplementation varied according to baseline vitamin D status. A bolus dosing regimen was used to achieve rapid correction of vitamin D deficiency among participants in the intervention arm, and to allow supervised administration of trial medication to maximise adherence.

METHODS

Participants

Adult patients with a medical record diagnosis of asthma treated with ICS were identified by searching databases at 60 general practices and at asthma clinics in two Acute National Health Service Trusts in London, UK, and invited for screening. Principal exclusion criteria were age <16 years or >80 years; tobacco smoking history >15 pack-years; medical record diagnosis of COPD; and failure to exhibit significant variability/reversibility in airway obstruction. Written informed consent was obtained from all participants before enrolment.

Procedures

Participants attending screening visits completed the St George's Respiratory Questionnaire (SGRQ),[10] the EuroQoL-5D questionnaire[11] and the Asthma Control Test (ACT)[12] and underwent a baseline clinical assessment incorporating spirometry, measurement of FE_{NO} and collection of a blood sample. A subset of 50 participants was invited to undergo sputum induction with hypertonic saline. Participants fulfilling eligibility criteria entered a run-in period of at least 2 weeks, during which they were asked to complete a symptom diary on a daily basis (see online figure E1). Eligible patients were randomly assigned to receive six 2-monthly oral doses of 6 mL Vigantol oil (Merck Serono, Darmstadt, Germany) containing 3 mg (120 000 IU) vitamin D_3, or 6 mL organoleptically identical placebo (Miglyol oil, Caesar and Loretz, Hilden, Germany) with allocation ratio 1:1. Randomisation was assigned by permuted blocks of 10 and stratified according to (A) British Thoracic Society treatment step (2–3 vs 4–5) and (B) inclusion in versus exclusion from the induced sputum substudy.

Participants completed study diaries daily for 12 months. Five further doses of study medication were administered at 2-monthly intervals following the first dose. Face-to-face follow-up visits were performed at 2 months, 6 months and 12 months of follow-up.

Coprimary end points for the trial were time to first severe asthma exacerbation and time to first URI. Severe asthma exacerbation was defined as deterioration in asthma resulting in (A) treatment with oral corticosteroids, or (B) hospital admission or emergency department treatment, or (C) decrease in the morning peak expiratory flow rate (PEFR) to more than 25% below the mean run-in value on two or more consecutive days.[13] URI was defined as influenza-like illness[14] or as a cold with symptom scores meeting modified Jackson criteria.[15]

Secondary end points were peak values and areas under the curve for symptom scores during severe exacerbation/URI; proportion of days with poor asthma control; proportion of nights with awakenings due to asthma symptoms; time to unscheduled healthcare attendance and use of antibiotics for exacerbation/URI; ACT and SGRQ scores, FE_{NO} concentration, daily ICS doses, % predicted FEV_1, PEFR, use of inhaled relief medication and induced sputum differential cell count and supernatant inflammatory profiles at 2 months, 6 months and 12 months; trough serum concentrations of 25(OH)D and parathyroid hormone (PTH) at 2 months and 12 months; and health economic outcomes (costs of exacerbations and URI, quality-adjusted life years and incremental net benefit over 1 year).

Sample size and statistical analysis

Assuming a median time to event of 120 days[16] we calculated that 200 participants (100 in each group) would need to be randomised in order to detect a 60 day difference in median time to event between intervention and control groups with 80% power using a two-sided test at the 5% significance level.[17] A total of 250 participants were recruited in order to allow for 20% loss to follow-up.

Analysis was by intention-to-treat: all participants who took at least one dose of study medication were included in efficacy and safety analyses. Significance was tested at the 5% level. Time-to-event outcomes were analysed using Cox regression adjusted for stratification factors. Subgroup analyses were conducted to determine whether the effect of vitamin D_3 supplementation on coprimary outcomes was modified by baseline vitamin D status (using serum 25(OH)D thresholds of 50 nmol/L and 75 nmol/L) or genotype.

Further details of Methods are presented in online supplementary information.

RESULTS

Five hundred and ninety adults with a medical record diagnosis of asthma were invited to participate in the trial. Of these, 297 attended a screening visit and were assessed for eligibility between 27 August 2009 and 25 June 2012: 37 were ineligible to participate and 10 were eligible but declined randomisation. The remaining 250 participants were randomised to intervention versus control arms of the trial in equal numbers: all received at least one dose of study medication, and were included in the intention-to-treat analysis (figure 1). Clinical and demographic characteristics of randomised participants were comparable for intervention versus control groups (table 1). The majority (206/250, 82%) had inadequate vitamin D status (serum 25(OH)D<75 nmol/L) at baseline, and participants had a high rate of events requiring oral corticosteroids and/or an increase in ICS dose in the year preceding enrolment (median 2.0 episodes, IQR 1.0 to 3.0). The trial ended on the date of the final study visit of the final participant undergoing follow-up.

Allocation to vitamin D_3 versus placebo did not influence time to first severe asthma exacerbation (adjusted HR 1.02,

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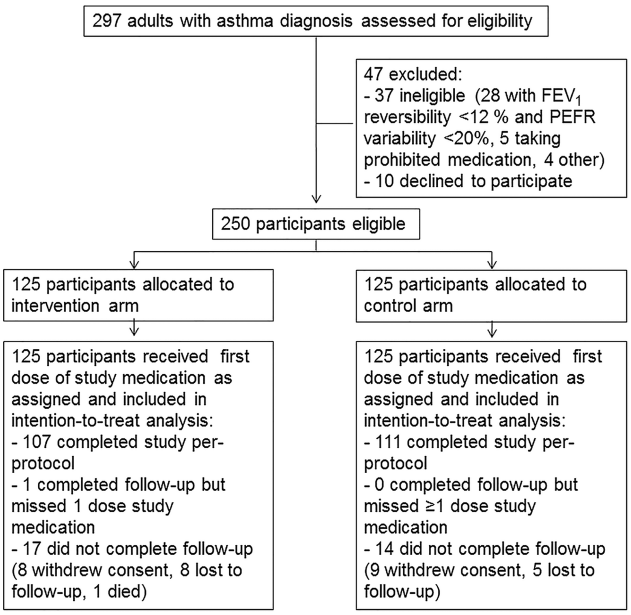

Figure 1 Trial profile.

95% CI 0.69 to 1.53, p=0.91) or time to first URI (adjusted HR 0.87, 95% CI 0.64 to 1.16, p=0.34; figure 2, table 2). Neither did it influence the annual rate of severe exacerbations or URI; the proportion of participants experiencing either outcome; or the peak severity or symptom score area under the curve for either event (table 2, see online figure E2, supplementary information). No effect of the intervention was seen on ACT scores, other measures of asthma symptom control, FEV_1, PEFR or FE_{NO} (table 2). Allocation to the intervention arm of the trial resulted in a significant increase in serum 25(OH)D concentration (mean 23.0 nmol/L increase at 12 months, p<0.001, table 2). This was associated with suppressed serum PTH concentration (mean 0.89 pmol/L decrease at 12 months, p=0.02), but not with a difference in serum corrected calcium concentration (see online table E5, supplementary information).

Administration of vitamin D_3 modestly improved respiratory quality of life as evidenced by adjusted interarm differences in total SGRQ score of −3.9 points at 2 months (p=0.005), −3.7 points at 6 months (p=0.038) and −3.3 points at 12 months (p=0.060; p for allocation-time interaction=0.026; table 2, see online figure E3, supplementary information). These reductions were associated with statistically significant decreases in component scores for the impacts dimension of the SGRQ at 2 months (p=0.05) and 6 months (p=0.005; p for allocation-time interaction=0.030, see online table E2, figure E3, supplementary information). No differences in EQ5D scores were observed between arms (see online table E3, supplementary information). Allocation to vitamin D_3 versus placebo did not influence differential white cell counts or inflammatory profile in induced sputum (see online table E4, supplementary information).

Day-to-day values for PEFR, asthma symptom score, short-acting bronchodilator use or the percentage of participants woken at night or experiencing poor asthma control days did not fluctuate in relation to the timing of administration of intermittent bolus doses of study medication (see online figure E4, supplementary information). Allocation to vitamin D_3 versus placebo did not influence use of asthma medications or antimicrobials (see online table E6, supplementary information) or health service uptake (see online table E7, supplementary information). Neither did it influence work absence due to asthma symptoms or respiratory infections (see online table E8, supplementary information) or health economic outcomes (see

Table 1 Baseline characteristics by allocation

	Vitamin D_3 (n=125)*	Placebo (n=125)*
Age, years	49.4 (14.8)	46.4 (13.8)
Sex		
Male, n (%)	55 (44%)	54 (43%)
Female, n (%)	70 (56%)	71 (57%)
Ethnicity		
White, n (%)	103 (82%)	99 (79%)
Black/Black British, n (%)	10 (8%)	12 (10%)
Other, n (%)	12 (10%)	14 (11%)
Body mass index ≥30 kg/m^2	36 (29%)	29 (23%)
Current smoker, n (%)	8 (6%)	9 (7%)
BTS step of treatment at enrolment		
2: Regular preventer therapy	58 (46%)	56 (45%)
3: Initial add-on therapy	49 (39%)	54 (43%)
4: Persistent poor control	17 (14%)	15 (12%)
5: Continuous/frequent use of OCS	1 (1%)	0 (0%)
Medication use		
ICS dose at entry in betamethasone equivalents,† µg	668 (559)	783 (693)
Inhaled LABA use,‡ n (%)	63 (50%)	67 (54%)
Leukotriene antagonist use, n (%)	15 (12%)	14 (11%)
Vaccine uptake		
Influenza vaccine, n (%)	106 (85%)	109 (87%)
Pneumococcal vaccine, n (%)	50 (40%)	31 (25%)
Median no. of URI/exacerbations requiring OCS or increase in ICS in previous 12 months (IQR)	2.0 (1.0 to 3.0)	2.0 (1.0 to 3.0)
Median no. of days since last exacerbation/URI requiring OCS or increase in ICS (IQR)	122 (69 to 237)	132 (75 to 248)
Median asthma duration, years (IQR)	25 (14 to 36)	23 (14 to 32)
Managed exclusively in primary care, n (%)	110 (88%)	111 (89%)
ACT score (SD)	19.2 (3.9)	18.9 (3.9)
Median SGRQ score (IQR)	23.7 (14.4 to 37.4)	20.7 (11.1 to 35.3)
Eczema, n (%)	54 (43%)	48 (38%)
Allergic rhinitis, n (%)	89 (71%)	95 (76%)
Peripheral blood eosinophil count ×10^9/L	0.28 (0.22)	0.29 (0.23)
Peripheral blood eosinophil count >0.5×10^9/L, n (%)	11 (9%)	10 (8%)
Prebronchodilator FEV_1, % predicted	82.0 (18.7)	81.0 (20.4)
Morning PEFR during run-in period, L/min	383 (106)	379 (123)
FE_{NO}, ppb	38.1 (29.1)	37.0 (26.0)
Vitamin D status		
Serum 25(OH)D concentration, nmol/L	49.8 (25.2)	49.4 (24.2)
Serum 25(OH)D <75 nmol/L	105 (84%)	101 (81%)
Serum 25(OH)D <50 nmol/L	72 (58%)	72 (58%)
Serum 25(OH)D <25 nmol/L, n (%)	19 (15%)	17 (14%)
Randomised to induced sputum substudy	24 (19%)	26 (21%)

*Mean (SD) presented unless stated otherwise.
†1 µg betamethasone assumed equivalent to 1 µg budesonide, 0.5 mcg fluticasone dipropionate and 0.75 mcg ciclesonide.
‡Includes combinations of ICS/LABA and LABA.
25(OH)D, 25-hydroxyvitamin D; ACT, asthma control test; BTS, British Thoracic Society guidelines; ICS, inhaled corticosteroids; LABA, long-acting β-2 agonist; OCS, oral corticosteroids; PEFR, peak expiratory flow rate; ppb, parts per billion; SGRQ, St George's Respiratory Questionnaire; URI, upper respiratory infection.

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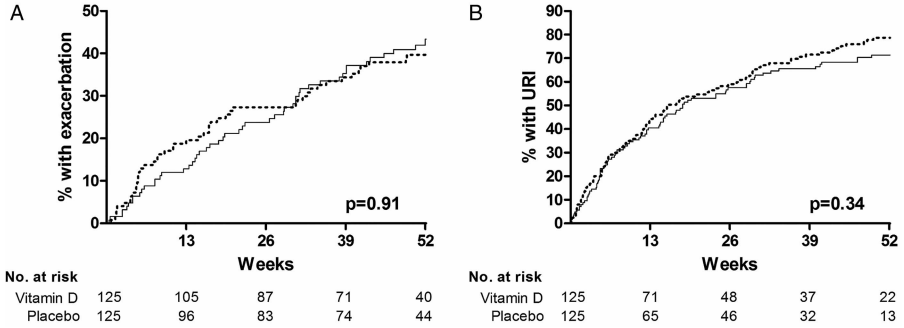

Figure 2 Coprimary outcomes by allocation. Time to first severe asthma exacerbation (A) and first upper respiratory infection (URI, B) by allocation. Numbers of participants yet to experience each outcome (number at risk) at 0 weeks, 13 weeks, 26 weeks, 39 weeks and 52 weeks are shown. Solid line, vitamin D_3; dotted line, placebo.

online table E9, supplementary information). The cost-effectiveness acceptability curve displayed in online figure E5, supplementary information, shows that the probability that vitamin D_3 versus placebo is cost-effective is around 70% at a realistic willingness to pay (£20 000) for a quality-adjusted life year gain. Prespecified subgroup analyses revealed no evidence of effect modification on coprimary outcomes according to baseline vitamin D status (see online table E10, supplementary information). After correction for multiple analyses, none of the single nucleotide polymorphism investigated were found to modify the effect of supplementation on risk of severe exacerbation or URI (see online table E11, supplementary information).

Twenty-three serious adverse events were reported in 20/250 participants receiving at least one dose of study medication; one participant died during the study, following a road traffic accident (see online table E12, supplementary information). No serious adverse event was attributed to study medication. A total of 1283 non-serious adverse events were reported in 240/250 participants: these were equally distributed between study arms (see online table E13, supplementary information). No hypercalcaemia was seen.

DISCUSSION

We report findings of the first trial of bolus dose vitamin D supplementation for the prevention of exacerbation and URI in adult patients with asthma. No effect of the intervention was seen on either coprimary outcome, despite a high prevalence of vitamin D insufficiency at baseline. Of 16 secondary outcomes investigated, only one—respiratory quality of life, as measured by the SGRQ—showed a statistically significant difference between arms, but this was just less than the 4-point minimum clinically important difference for this instrument.[18] Prespecified subgroup analyses demonstrated no statistically significant evidence of effect modification by clinical or genetic characteristics after correction for multiple analyses.

The results of our trial support and extend the findings of the VIDA trial, recently reported by Castro *et al*.[8] These investigators studied a population of patients with asthma with baseline 25(OH)D <75 nmol/L and reported no effect of vitamin D supplementation on a primary outcome of treatment failure. Subgroup analysis restricted to the 82% of vitamin D_3 supplementation in adults with asthma trial participants with baseline 25(OH)D <75 nmol/L revealed no effect of the intervention on either of our coprimary outcomes. Our trial may therefore be regarded as confirming the findings of the VIDA study.

Although primary outcomes for our trial were null, we did observe a modest but statistically significant improvement in respiratory quality of life among participants in the intervention arm, as measured by the total SGRQ score; this was associated with decreases in the impacts scores component of the SGRQ. One of the correlates of the impacts component of the SGRQ is exercise performance, and the favourable effects of vitamin D supplementation on muscle function in deficient subjects are well documented:[19] it may be, therefore, that positive effects of vitamin D_3 supplementation on participants' quality of life were mediated by enhancing muscle function; they were not mediated via improved control of asthma symptoms, as the intervention did not influence days in which asthma was poorly controlled, nights with awakenings or ACT scores. However, the possibility of a positive finding arising from type 1 error cannot be ruled out, given the large number of prespecified secondary outcomes in this study. This finding should therefore be regarded as exploratory.

Our trial has several strengths. Inadequate vitamin D status was highly prevalent among the study population at baseline, and participants had significant potential for improvement in asthma control at enrolment as evidenced by high FE_{NO} at baseline. We gave a generous dose of vitamin D_3, equivalent to 2000 IU (50 µg) per day—more than three times the recommended dietary allowance for adults proposed by the US Institute of Medicine.[20] The intermittent bolus dosing regimen we employed allowed us to achieve a high degree of compliance with the intervention (3/6 doses were directly observed and 3/6 were supervised telephonically), while the use of daily symptom diaries allowed us to characterise participants' symptoms in fine detail. Our trial complements the VIDA trial[8] by providing new data on efficacy of a bolus dosing regimen administered for a year to patients with a wide range of baseline serum 25(OH)D concentrations on additional outcomes including incidence of URI, other measures of airway inflammation (exhaled nitric oxide and induced sputum supernatant inflammatory profiles), potential genetic effect modifiers and health economic outcomes.

Our trial also has some limitations. Patients with URI symptoms were not sampled for detection of pathogens; we have previously validated the symptomatic definition of URI employed in this study against PCR in another trial however.[21] A minority (32%) of asthma exacerbations in our trial were associated with URI—a somewhat lower proportion than the 44% reported elsewhere.[22] Our trial was conducted in an urban setting, and participants had a relatively high prevalence of allergic rhinitis and eczema at baseline. Allergens and particulates may therefore have precipitated a significant number of exacerbations in our study population: if vitamin D only prevents exacerbation precipitated by URI, then this phenomenon could have contributed to our negative findings. Although 77% of participants in our trial experienced a URI, only 44% experienced a severe exacerbation; our study may therefore have lacked power to detect small or moderate effects of the intervention on exacerbation risk.

Another potential limitation relates to the intermittent bolus dosing regimen that we employed. Although this was reasonably effective in correcting vitamin D deficiency (80% of participants

Table 2 Outcomes by allocation

	Vitamin D_3 (n=125)	Placebo (n=125)	Adjusted HR/incidence rate ratio/odds ratio/mean difference/ratio of geometric means (95% CI)*	p Value
Severe asthma exacerbation				
Median time to first severe exacerbation, days (IQR)	– (192 to –)	– (136 to –)	1.02 (0.69 to 1.53)	0.91
Rate of severe exacerbations per participant-year	136/117.4=1.16	145/116.7=1.24	0.93 (0.57 to 1.51)	0.77
Proportion of participants with ≥1 severe exacerbation (%)†	50/108 (46%)	47/114 (41%)	1.20 (0.70 to 2.05)	0.52
Mean peak asthma symptom score per severe exacerbation‡	1.60 (1.22)	1.67 (1.13)	−0.13 (−0.52 to 0.26)	0.51
Mean area under the curve per severe exacerbation, asthma symptom score§	26.2 (23.7)	24.5 (18.2)	−0.44 (−8.65 to 7.76)	0.92
Proportion of exacerbations associated with URI (%)	45/136 (34%)	44/145 (30%)	1.04 (0.40 to 2.70)	0.96
URI				
Median time to first URI, days (IQR)	128 (42 to –)	112 (40 to 309)	0.87 (0.64 to 1.16)	0.34
Proportion of participants with ≥1 URI (%)†	85/115 (74%)	93/117 (79%)	0.71 (0.38 to 1.32)	0.28
Rate of URI per participant-year	234/117.4=1.99	250/116.7=2.14	0.89 (0.70 to 1.14)	0.35
Mean peak Jackson symptom score per URI¶	11.5 (5.1)	10.5 (4.7)	0.76 (−0.47 to 1.99)	0.23
Mean area under the curve per URI, Jackson symptom score**	105.0 (67.0)	90.6 (69.9)	5.1 (−13.5 to 23.8)	0.59
Days in which asthma is poorly controlled††				
Rate per participant-year	4486/117.4=38.2	4049/116.7=34.7	1.07 (0.75 to 1.51)	0.72
Nights with awakenings due to asthma symptoms				
Rate per participant-year	3166/117.4=27.0	2702/116.7=23.2	1.14 (0.69 to 1.86)	0.61
ACT score				
Mean (SD)				
2 months	20.1 (3.9)	19.5 (4.4)	0.52 (−0.27 to 1.31)	0.56‡‡
6 months	20.6 (3.5)	20.6 (3.8)	0.00 (−0.81 to 0.81)	
12 months	20.4 (4.0)	20.4 (4.2)	0.00 (−0.82 to 0.82)	
FEV_1 (% predicted)				
Mean (SD)				
2 months	81.5 (18.7)	81.6 (20.4)	−0.95 (−3.71 to 1.81)	0.53‡‡
6 months	80.6 (21.0)	81.0 (20.8)	−1.56 (−4.37 to 1.26)	
12 months	81.6 (18.5)	80.1 (22.8)	0.44 (−2.42 to 3.30)	
Morning PEFR (mean over period since preceding study visit)				
Mean (SD)				
2 months	380.6 (108.6)	375.2 (121.2)	−0.2 (−8.1 to 7.7)	0.38‡‡
6 months	385.0 (118.6)	382.9 (122.4)	−5.3 (−13.4 to 2.8)	
12 months	388.1 (116.8)	387.7 (122.9)	−5.4 (−13.6 to 2.8)	
Fractional exhaled nitric oxide, ppb				
Mean (SD)				
2 months	36.1 (25.7)	36.5 (31.7)	−1.3 (−6.4 to 3.8)	0.71‡‡
6 months	36.7 (28.8)	34.6 (32.4)	1.6 (−3.7 to 6.8)	
12 months	37.5 (26.9)	38.5 (36.9)	−1.4 (−6.8 to 3.9)	

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Table 2 Continued

	Vitamin D_3 (n=125)	Placebo (n=125)	Adjusted HR/incidence rate ratio/odds ratio/mean difference/ratio of geometric means (95% CI)*	p Value
SGRQ total score§§				
Median (IQR)				
2 months	16.0 (8.5 to 26.2)	17.3 (8.9 to 30.1)	0.87 (0.79 to 0.96)¶¶	0.005***
6 months[21]	14.7 (7.5 to 24.4)	14.6 (7.1 to 29.7)	0.90 (0.82 to 0.99)†††	0.038***
12 months[23]	13.6 (7.1 to 24.7)	13.9 (7.2 to 25.0)	0.91 (0.82 to 1.00)‡‡‡	0.060***
Serum 25(OH)D, nmol/L				
Mean (SD)				
2 months	61.2 (22.1)	48.5 (25.0)	12.5 (7.6 to 17.3)	<0.001§§§
12 months	69.4 (21.0)	46.5 (24.6)	23.0 (17.9 to 28.0)	<0.001§§§
Participants with 25(OH)D ≥50 nmol/L				
n (%)				
2 months	88/121 (73%)	52/119 (44%)	1.83 (1.04 to 2.62)	<0.001§§§
12 months	86/107 (80%)	43/110 (39%)	2.72 (1.83 to 3.61)	<0.001§§§

*Adjusted for stratification factors, that is, British Thoracic Society treatment step (2/3 vs 4/5) and inclusion in vs exclusion from sputum induction substudy.
†This analysis excludes participants who withdrew from the trial without experiencing this event prior to withdrawal.
‡Asthma symptoms scored from 0 (no symptoms) to 3 (severe symptoms).
§Area under the curve calculated for asthma symptom score from 7 days preonset of exacerbation to 20 days postonset.
¶Jackson symptoms (sneezing, sore throat, headache, subjective sensation of fever or chilliness, malaise, nasal discharge, nasal obstruction, cough) each scored from 0 (no symptoms) to 3 (severe symptoms) and summed for each day of the URI.
**Area under the curve calculated for total Jackson symptom score from 7 days preonset of URI to 20 days postonset.
††Poorly controlled asthma day defined as a 24-h period in which diary recorded wakening at night due to asthma symptoms, morning PEFR ≥20% below mean run-in value or ≥2 uses of reliever medication above median run-in value; days included in a severe exacerbation are excluded from the count of poorly controlled asthma days.
‡‡p Value for allocation-time interaction presented; p values for effect of allocation at individual time points are not reported where p for allocation-time interaction ≥0.05.
§§A small constant (1.0) was added to each value prior to log transforming for the regression analysis, to avoid taking logs of zero.
¶¶Ratio of geometric means is presented; adjusted mean difference=3.9 points.
***p For allocation-time interaction=0.026.
†††Ratio of geometric means is presented; adjusted mean difference=3.7 points.
‡‡‡Ratio of geometric means is presented; adjusted mean difference=3.3 points.
§§§p For allocation-time interaction <0.001.
25(OH)D, 25-hydroxyvitamin D; ACT, asthma control test; PEFR, peak expiratory flow rate; ppb, parts per billion; SGRQ, St George's Respiratory Questionnaire; URI, upper respiratory infection.

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in the intervention arm had 25(OH)D levels ≥50 nmol/L at 12 months, as compared with 39% of those in the placebo arm, p<0.001), the interarm difference in serum 25(OH)D concentrations at 12 months was modest (22 nmol/L). It should be noted, however, that serum 25(OH)D concentrations at this time point represent 'trough levels': pharmacokinetic studies of the 25(OH)D response to bolus-dose vitamin D supplementation indicate that concentrations peak at 1 week post dose and remain high for several weeks thereafter;[23] the difference in vitamin D status between arms in our trial will therefore have been significantly higher than 22 nmol/L for much of the inter-dosing period. This is reflected in the fact that serum PTH concentrations were significantly lower in the intervention versus the control arm of the trial at 1 year.

While intermittent bolus-dosing with vitamin D can prevent bone fractures,[24] some have proposed that it may be less effective than daily dosing for inducing non-classical actions of vitamin D.[25 26] However, we[27 28] and others[29] have previously shown favourable effects of bolus-dose vitamin D supplementation on outcomes relating to respiratory infection. Nevertheless, we cannot exclude the possibility that results of the vitamin D_3 supplementation in adults with asthma trial might have been different if vitamin D had been administered on a daily basis, and trials comparing the effects of daily versus intermittent dosing are needed to address this issue. Pending the conduct of such studies, pooling of existing trial data for individual patient data meta-analysis has potential to increase power to detect effects of vitamin D supplementation on exacerbation risk, and thereby to advance the field.

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Double-blind randomised placebo-controlled trial of bolus-dose vitamin D_3 supplementation in adults with asthma (ViDiAs)

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Double-blind randomised placebo-controlled trial of bolus-dose vitamin D_3 supplementation in adults with asthma (ViDiAs): Supplementary Information.

Methods

Participants

Adult patients with a medical record diagnosis of asthma treated with ICS were identified by searching databases at 60 general practices and at asthma clinics in 2 Acute National Health Service Trusts in London, UK, and sent a letter inviting them to attend a screening visit *via* mailshot. Further patients were invited for screening *via* an email and poster advertising campaign conducted at Queen Mary, University of London, and at participating General Practices. Respondents were excluded from participation in the trial if they were not currently taking daily ICS; if they had not experienced a worsening of asthma symptoms requiring an increase in inhaled asthma therapy or systemic corticosteroids within the 2 years prior to screening; if they were aged <16 or >80 years; if they had a tobacco smoking history >15 pack-years; if they had a medical record diagnosis of chronic obstructive pulmonary disease, sarcoidosis, hyperparathyroidism, nephrolithiasis, active tuberculosis, renal or hepatic failure, terminal illness or malignancy other than non-melanoma skin cancer not in remission for ≥ 3 years; if they were taking a dietary supplement containing >10 µg vitamin D per day up to 2 months before first dose of study medication; if they were taking a cardiac glycoside, carbamazepine, phenobarbital, phenytoin or primidone; if they were taking a benzothiadiazine derivative at a dose higher than recommended in the British National Formulary, or in combination with a calcium supplement; if they had been treated with any investigational medical product or device up to 4 months before the first dose of study medication; if they did not exhibit significant variability / reversibility in airway obstruction, defined as either a ≥12% increase in forced expiratory volume in 1 second (FEV1) after inhalation of 400 µg salbutamol at screening, or ≥ 20% diurnal variability in peak expiratory flow rate (PEFR) documented during a two-week post-screening run-in period, or previously recorded within the last 3 years; if serum corrected calcium was >2.65 mmol/L; if serum creatinine was >125 µmol/L; if they were breastfeeding, pregnant or planning a pregnancy; if they were unable to use a spirometer or a PEFR meter; or if

they failed to complete the symptom / PEFR diary during the run-in period. A sub-group of 50 participants were enrolled in the sputum induction study; additional exclusion criteria for this sub-study were treatment of asthma at British Thoracic Society treatment step (BTS step) 4 or 5, baseline FEV1 <50% predicted and tobacco smoking within the 6 months preceding enrolment. The trial was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/67) and written informed consent was obtained from all participants before enrolment. This trial is registered with ClinicalTrials.gov (NCT00978315).

Procedures

Screening visit

Participants attended screening visits at Clinical Research Centres at Barts Health NHS Trust and Queen's Hospital, Romford, UK. They completed the St George's Respiratory Questionnaire (SGRQ) (1), the EuroQoL EQ-5D questionnaire (2) and the Asthma Control Test (ACT) (3) and underwent a baseline clinical assessment including the following: spirometry before and after inhalation of 400 µg salbutamol *via* a spacer device, performed using a MicroLab ML3500 desktop spirometer (CareFusion GmbH, Hoechberg, Germany) according to American Thoracic Society (ATS) / European Respiratory Society (ERS) recommendations (4); measurement of fractional exhaled nitric oxide (FeNO), performed using a NIOX MINO 09-1100 (Aerocrine, Solna, Sweden) according to ATS / ERS recommendations (5); measurement of height, performed using a Seca 220 Telescopic Measuring Rod (Seca, Hamburg, Germany); measurement of weight, performed using Marsden MMPS-250 column scales (Marsden, Rotherham, UK); and collection of a blood sample for subsequent DNA extraction and determination of full blood count (FBC) and serum concentrations of calcium, albumin, total 25-hydroxyvitamin D (25[OH]D) and parathyroid hormone (PTH). A urine sample was collected from women of childbearing potential for a pregnancy test (SA Scientific, San Antonio, TX USA). A sub-set of 50 participants were invited to undergo sputum induction with hypertonic saline, and their samples were processed to make cytospin slides according to methods described by Pizzichini *et al* (6). Differential cell counts were performed by one operator (CLG) for all specimens throughout the study; a second operator

(WRM) repeated cell counts on a randomly selected sub-set of 20 slides: differential cell counts were highly correlated between operators (for eosinophil count, Pearson's r =0.95, 95% CI 0.87 to 0.98, P<0.001). Induced sputum supernatants were stored at -80ºC pending measurement of inflammatory mediators as described below.

Participants fulfilling eligibility criteria then entered a run-in period of at least 2 weeks, during which they were asked to complete a study diary on a daily basis. This diary (Figure E1) recorded severity of asthma symptoms, Jackson URI symptoms (sneezing, sore throat, headache, subjective sensation of fever or chilliness, malaise, nasal discharge, nasal obstruction, cough) (7) and one additional symptom (muscle pain), scored from 0 (no symptoms) to 3 (symptoms severe enough to interfere with activity or sleep). The diary also recorded PEFR, medication use, health care use, time off work and out-of-pocket expenses incurred as a result of symptoms of asthma or acute respiratory infection.

Randomisation

As soon as compliance with diary completion was demonstrated and the screening corrected calcium concentration was available, participants whose eligibility was confirmed were randomly assigned to receive six 2-monthly oral doses of 6 ml Vigantol® oil (Merck Serono, Darmstadt, Germany) containing 3 mg (120,000 IU) vitamin D_3, or 6 ml organoleptically identical placebo (Miglyol® oil, Caesar and Loretz, Hilden, Germany) with allocation ratio 1:1. Randomisation was assigned by permuted blocks of ten and stratified according to a) BTS treatment step (2-3 *vs.* 4-5) and b) inclusion in *vs.* exclusion from the induced sputum sub-study as follows. Nova Laboratories (Wigston, Leicestershire, UK) prepared 360 packs of study medication according to Good manufacturing Practice: 180 packs contained active study drug and 180 packs contained the placebo. They then generated a randomisation sequence using a computer program that assigned the term active or placebo to consecutive numbers from 2001 to 2360 by permuted block randomisation with blocks of ten. The packs were then assigned a randomisation number according to this computer-generated randomisation sequence. At enrolment, study staff

categorised participants into one of three groups: those participating in the induced sputum sub-study (all treated at BTS Step 2/3); those treated at BTS treatment step 2/3 who were not participating in the induced sputum sub-study; and those treated at BTS treatment step 4/5. Participants in each group were allocated consecutive randomisation numbers from different blocks of ten (1-10, 11-20 etc). Study staff who assigned patients to the active drug or placebo had no knowledge of the next assignment in the sequence, because they did not have access to the study code. Treatment allocation was concealed from patients and study staff. Those analysing the data after completion of the trial were not masked to group assignment. Vitamin D_3 content of a random sample of active medication was determined by high performance liquid chromatography at the end of the study; it was found to contain 99.2% of its original vitamin D_3 content. Randomised participants were invited to attend a second study visit, at which the first dose of study medication was administered under direct supervision, and a new symptom diary was provided.

Follow-up

Participants completed study diaries daily for the 12 months of study participation. Each diary accommodated up to 12 weeks of data; participants completing follow-up filled 6 diaries in total. Five further doses of study medication were administered at 2-monthly intervals following the first dose: those at 2 and 6 months were directly observed, and those at 4, 8 and 10 months were supervised by telephone. Face-to-face follow-up visits were performed at 2, 6 and 12 months of follow-up, at which spirometry, FeNO measurement, sputum induction and administration of questionnaires (ACT, EQ-5D, SGRQ) were all repeated. Returned diaries were checked for completeness, and new diaries were issued as necessary. Additionally, at 2 and 12 months blood samples were taken from all participants, and serum was separated by centrifugation and frozen for subsequent assay of concentrations of 25(OH)D, albumin, calcium and PTH. Serum from 12-month blood samples was also frozen for the same determinations. On completion of the 12 month visit, final diaries were collected and participants were discharged from the study. Details of adverse events arising during the course of the trial were recorded throughout.

Data management and study definitions

All case report form (CRF) and diary data were entered into a database in Microsoft Access 2010. Entries for a 10% subset of participants were checked against source data: error rates of 0.075% and 0.097% were detected for data from diaries / CRFs respectively. Diary data were then imported into STATA and episodes of severe exacerbation and URI were identified using algorithms based on the following definitions: severe asthma exacerbation was defined as deterioration in asthma resulting in a) treatment with oral corticosteroids, or b) hospital admission or emergency department treatment, or c) decrease in the morning PEFR to more than 25% below the mean run-in value on 2 or more consecutive days (8). URI was defined as a) influenza-like illness, as indicated by the presence of cough, feeling of fever/chilliness and muscle pain (9), or b) a cold, defined as i) total Jackson symptom score of ≥14 together with the subjective impression of having a cold, or ii) total Jackson symptom score of ≥14 together with increased nasal discharge for at least 3 days, or iii) total Jackson symptom score <14 together with the subjective impression of having a cold and an increase in nasal discharge score above median run-in nasal discharge score for ≥ 3 days (7). We have previously validated this definition against polymerase chain reaction detection of 11 respiratory viruses in nasopharyngeal swabs in another trial (10). A poorly-controlled asthma day was defined as a 24-hour period in which a diary recorded either a) wakening at night due to asthma symptoms, or b) morning PEFR ≥ 20% below the mean value observed during the run-in period, or c) ≥ 2 uses of inhaled reliever medication above the median number of uses observed during the run-in period (8). Days included in a severe exacerbation were excluded from the count of poorly-controlled asthma days. Body mass index (BMI) was calculated using the formula: BMI = weight (kg) / [height (m)].^{2}

Statistical analysis

Co-primary end points for the trial were time to first severe asthma exacerbation and time to first URI. Assuming a median time to event of 120 days (11) we calculated that a total of 200 participants (100 in each group) would need to be randomised in order to detect a 60 day difference in median time to event between intervention and

control groups with 80% power using a 2-sided test at the 5% significance level, assuming a follow-up period for each participant of one year (12). Pre-specified secondary endpoints were peak values and areas under the curve for symptom scores during severe exacerbation / URI; proportion of days with poor asthma control; proportion of nights with awakenings due to asthma symptoms; time to unscheduled health care attendance and use of antibiotics for exacerbation / URI; ACT and SGRQ scores, FeNO, daily ICS doses, % predicted FEV1, PEFR and use of inhaled relief medication at 2, 6 and 12 months; serum concentrations of 25(OH)D and PTH at 2 and 12 months; and health economic outcomes (costs of exacerbations and acute respiratory infections, quality-adjusted life years [QALY] and incremental net benefit over one year). Sub-group analyses were conducted to determine whether the effect of vitamin D_3 supplementation on co-primary outcomes was modified by baseline vitamin D status (using serum 25(OH)D thresholds of both 50 nmol/L and 75 nmol/L) or vitamin D pathway genotype (single nucleotide polymorphisms investigated are listed in Table E1).

Analyses were performed using STATA/IC (versions 12.1, 2012 and 13, 2013), GraphPad Prism (version 4.03, 2005) and R (version 3.0.2, 2013) software packages. Analysis was by intention-to-treat: all participants who took at least one dose of study medication were included in both efficacy and safety analyses. Significance was tested at the 5% level. A single pre-specified interim efficacy analysis of time to co-primary outcomes was performed after enrolment of 125 participants. Interim safety analyses of fatal / life-threatening events were conducted at 6-monthly intervals throughout the course of the trial: 7 such analyses were conducted in total. Results of interim analyses were reviewed by the Data Monitoring Committee, who recommended continuation of the trial following each review. The study protocol specified P value thresholds of 0.001 and 0.018 to stop the trial for efficacy and safety interim analyses, respectively.

Time-to-event outcomes were analyzed using Cox regression adjusted for stratification factors; the assumption of proportional hazards was confirmed for all survival analyses using methods proposed by Grambsch and Therneau (13). Analyses of proportions used logistic regression adjusted for stratification factors. Analyses of event rates used negative binomial regression adjusted for stratification

factors, accounting for the appropriate length of follow-up. Quantitative outcomes assessed more than once in the same participant, but not at fixed times, were analyzed using linear regression adjusted for stratification factors with random effects of individual. Data for a given episode were considered missing if that episode was incomplete at the end of follow-up. Quantitative outcomes assessed more than once in the same participant at fixed time-points in addition to a baseline assessment were analyzed using linear regression adjusted for stratification factors with random effects of individual, constrained so that there was no treatment effect at baseline, and with a treatment effect estimated at each subsequent time-point. A P-value for allocation-time interaction was used to evaluate evidence for an effect of allocation; where evidence was found ($P<0.05$), P-values for the effect of allocation at individual time-points are reported. Outcomes with highly skewed distributions were log-transformed prior to analysis, following addition of a small constant. Analysis of inflammatory profile in induced sputum supernatants was restricted to those mediators whose median concentration was above the limit of detection at baseline. Sub-group analyses were performed by repeating primary efficacy analyses with the inclusion of the appropriate interaction term. Interaction effects were summarised as a ratio of hazard ratios with 95% confidence interval and P-value. The Benjamini-Hochberg procedure for multiple testing correction was applied to genetic analyses and analysis of inflammatory mediators in induced sputum to control the false discovery rate at 20% (14).

Analysis of health economic outcomes was undertaken from a societal perspective. Unit costs for general practitioner (GP) and nurse consultations, outpatient attendances and emergency department attendances were obtained from the Unit Costs of Health and Social Care (15). Unit costs for hospital admissions were obtained from the Reference Costs Database (16). Unit drug costs were calculated from the British National Formulary (17). Participants' costs were obtained from study diaries and included time lost from work due to asthma exacerbation or URI as well as travel expenses and out-of-pocket expenses on prescription drugs and over-the-counter medication incurred as a result of asthma exacerbation or URI. Time lost from work due to asthma exacerbation or URI was valued using age- and sex-adjusted average daily wage rates from the Office for National Statistics (18). Total health care costs calculated from diary data were validated against those calculated

from GP records for 25 randomly selected participants: good correlation between the two estimates was observed (Pearson's r 0.94, 95% CI 0.86 to 0.97, P<0.001).

EQ-5D quality of life data were combined with survival data to calculate QALY (2). Participants' EQ-5D profiles were combined with health state preference values from the UK general population (19) to derive EQ-5D utility index scores at 2, 6 and 12 months of follow-up on a scale anchored at 0 (death) and 1 (perfect health). QALY were calculated for each participant using the weighted average of time spent in the study and quality of life.

Cost effectiveness analysis (CEA) was undertaken to assess the relative cost effectiveness of vitamin D_3 supplementation *vs.* placebo for the prevention of asthma exacerbations and URI. The CEA used bivariate regression methods to allow for correlation between costs and outcomes to report mean values and 95% confidence intervals for incremental costs and QALY of vitamin D_3 supplementation *vs.* placebo at one year, adjusted for stratification factors.

Missing data for health economic analyses were addressed with multiple imputation. The imputation model included stratification factors, and baseline covariates (age, sex, ethnicity, ACT score, FeNO, BMI, baseline serum 25[OH]D concentration, current smoking) as predictors. We applied analytical methods in each imputed dataset (n=5) and combined the resultant estimates with Rubin's rules (20). Incremental net monetary benefits were estimated by valuing incremental QALY at a threshold of £20,000 per QALY and subtracting incremental costs. A cost-effectiveness acceptability curve was calculated by reporting the probability that vitamin D_3 supplementation was cost-effective at different levels of willingness to pay for a QALY gain (£0 to £50,000 per QALY gained) (21).

Laboratory analyses

Serum concentrations of 25(OH)D_2 and 25(OH)D_3 were determined by isotope-dilution liquid chromatography–tandem mass spectrometry (22) and summed to give values for total 25(OH)D concentration. Sensitivity for this assay was 10 nmol/l. PTH, albumin and total serum calcium concentrations were determined using an Architect ci8200 analyser (Abbott Diagnostics, Chicago, IL, USA). Calcium concentration was corrected for serum albumin concentration using the formula: corrected calcium (mmol/l) = total calcium (mmol/l) + 0.02 × (40 – albumin [g/l]). Concentrations of 30 inflammatory mediators (IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-α, IFN-γ, TNF, CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL5, CCL11, EGF, FGF-β, HGF and VEGF) were quantified in induced sputum supernatants obtained at baseline, 2 months and 12 months using a human 30-plex bead immunoassay panel (Invitrogen, Camarillo, CA, USA).

Fifty-four single nucleotide polymorphisms (SNP) in 11 genes in the vitamin D pathway having minor allele frequency ≥ 0.04 and associating with serum concentrations of vitamin D metabolites or disease risk were identified by systematic literature review. Twenty-four of these SNP were in high linkage disequilibrium (r^2 ≥ 0.8) in the HapMap database (release #27): for these variants, six tag SNP were selected as proxies using an algorithm developed by de Bakker *et al* (23) and typed (Table E1). An additional SNP in the class I MHC-restricted T cell-associated molecule (CRTAM) gene (rs2272094) reported to modify the influence of vitamin D status on asthma exacerbation risk (24) was also typed. DNA was extracted from whole blood using the salting-out method (25) on the Biomek FX robot (Beckman Coulter), quantified using the Nanodrop spectrophotometer and normalised to 5ng/µl. 10ng DNA was used as template for 2 µl TaqMan assays (Applied Biosystems, Foster City, CA, USA) performed on the ABI 7900HT platform in 384-well format and analysed with Autocaller software. Pre-developed assays were used to type 34/37 SNP. Customised assays were used to type the following polymorphisms: rs2740574 in CYP3A4 (forward primer sequence CCAGGCATAGGTAAAGATCTGTAGGT, reverse primer sequence CTCAAGTGGAGCCATTGGCATA, reporter sequences ACAAGGGCAAGAGAG and

ACAAGGGCAGGAGAG), rs3740165 in CUBN (forward primer sequence GCAATGAGATTAAATCTTCAGGAAACACA, reverse primer sequence CTGGAGGTATAGGAAGCAGTGAAG, reporter sequences CCGCCATATGGCCTG and CGCCATACGGCCTG) and rs7861779 in RXRA (forward primer sequence TGGCCCATGCACGAGTAG, reverse primer sequence ACCGAGACAGGCCAAACTC, reporter sequences CAGCAGAGGTGGCCGA and CAGCAGAGATGGCCGA). Alleles at all loci conformed to the Hardy-Weinberg equilibrium. Typing for two SNP (rs6127118 and rs11574010) failed.

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Table E1: Single nucleotide polymorphisms (SNP) identified as putative modifiers of the effects of vitamin D supplementation

Gene	Target SNP	Tag SNP[1]	r^2
CYP24A1	rs2762934	-	-
	rs6127118	-	-
	rs2248137	-	-
	rs2762939	-	-
CYP27B1	rs4646536	-	-
	rs10877012	rs4646536	1.00
	rs703842	rs4646536	1.00
	rs4646537	-	-
CYP2R1	rs2060793	-	-
	rs10741657	rs2060793	1.00
	rs1993116	rs2060793	1.00
	rs7116978	rs2060793	0.92
	rs10500804	-	-
	rs12794714	rs10500804	1.00
	rs10766197	-	-
CYP3A4	rs2740574	-	-
CYP27A1	rs17470271	-	-
VDR	rs1544410	-	-
	rs731236	-	-
	rs4516035	-	-
	rs4334089	-	-
	rs10783219	-	-
	rs7976091	-	-
	rs11574010	-	-
	rs2853559	-	-
	rs2238136	-	-
	rs7975232	-	-
	rs2228570	-	-
	rs7970314	-	-
	rs11568820	-	-
DBP	rs4588	-	-
	rs2282679	rs4588	1.00
	rs3755967	rs4588	1.00
	rs17467825	rs4588	1.00
	rs1155563	rs4588	0.83
	rs2298850	rs4588	0.95
	rs7041	-	-
	rs222035	rs7041	0.92
	rs842999	rs7041	0.96
	rs2298849	-	-
	rs16846876	-	-
	rs12512631	-	-
	rs2070741	-	-
DHCR7	rs12785878	-	-
	rs4944957	rs12785878	1.00
	rs4945008	rs12785878	0.95
	rs3794060	rs12785878	1.00
	rs7944926	rs12785878	1.00
	rs12800438	rs12785878	1.00
	rs3829251	-	-
CUBN	rs3740165	-	-
RXRA	rs9409929	-	-
	rs7861779	-	-
LRP2	rs3755166	-	-
CRTAM	rs2272094	-	-

1. Six tag SNP were selected as proxies for target SNP in high linkage disequilibrium ($r^2 \geq 0.8$) in the HapMap database (release #27) and typed

Table E2: St George's Respiratory Questionnaire component scores by allocation

			Vitamin D_3 (n=121 at 2 mo, n=114 at 6 mo, n=108 at 12 mo)	Placebo (n=119 at 2 mo, n=115 at 6 mo, n=111 at 12 mo)	Adjusted ratio of geometric means (95% CI)[1]	Overall P[2]	P for individual time points[3]
Symptoms score, SGRQ[4]	Median (IQR)	2 months	30.2 (15.4 to 45.9)	34.3 (21.4 to 57.9)	0.85 (0.67 to 1.08)	0.25	--
		6 months	25.2 (14.1 to 39.0)	27.5 (12.7 to 43.7)	1.04 (0.81 to 1.32)		--
		12 months	25.5 (11.0 to 45.2)	27.7 (15.7 to 46.3)	0.83 (0.65 to 1.06)		--
Activity score, SGRQ[4]	Median (IQR)	2 months	23.3 (11.2 to 41.5)	23.4 (11.2 to 41.8)	0.82 (0.65 to 1.03)	0.32	--
		6 months	18.5 (11.2 to 35.8)	18.5 (6.2 to 35.8)	0.88 (0.70 to 1.11)		--
		12 months	18.5 (11.2 to 35.7)	17.4 (6.0 to 35.8)	0.98 (0.77 to 1.25)		--
Impacts score, SGRQ[4]	Median (IQR)	2 months	7.9 (2.0 to 15.7)	8.6 (3.7 to 18.9)	0.82 (0.67 to 1.00)	0.030	0.050
		6 months	5.6 (1.6 to 14.5)	6.4 (2.5 to 19.1)	0.75 (0.61 to 0.92)		0.005
		12 months	5.8 (1.6 to 12.7)	5.9 (1.6 to 16.0)	0.84 (0.68 to 1.03)		0.099

Mo, months; CI, confidence interval; SGRQ, St George's Respiratory Questionnaire. IQR, inter-quartile range.

1, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. 2, P for allocation-time interaction. 3, presented where overall P<0.05. 4, a small constant (1.0) was added to each value prior to log transforming for the regression analysis to avoid taking logs of zero.

Table E3: EQ5D outcomes by allocation

			Vitamin D_3 (n=121 at 2 mo, n=114 at 6 mo, n=108 at 12 mo)	Placebo (n=119 at 2 mo, n=115 at 6 mo, n=111 at 12 mo)	Adjusted odds ratio / mean difference (95% CI)[1]	P[2]
EQ5D index score	Mean (s.d.)	2 months	0.91 (0.16)	0.87 (0.22)	1.81 (0.75 to 4.36) [3]	0.14[3]
		6 months	0.91 (0.17)	0.88 (0.20)	2.81 (1.13 to 6.98) [3]	
		12 months	0.90 (0.17)	0.89 (0.19)	1.23 (0.49 to 3.05) [3]	
Reporting any mobility problem	n (%)	2 months	13/121 (11%)	16/119 (13%)	0.61 (0.16 to 2.31)	.74
		6 months	11/114 (10%)	14/115 (12%)	0.73 (0.18 to 2.98)	
		12 months	12/108 (11%)	17/111 (15%)	0.49 (0.13 to 1.92)	
Reporting any self-care problem	n (%)	2 months	3/121 (2%)	7/119 (6%)	0.49 (0.01 to 27.14)	.95
		6 months	3/114 (3%)	6/115 (5%)	1.17 (0.02 to 83.13)	
		12 months	2/108 (2%)	4/111 (4%)	0.17 (0.00 to 184.26)	
Reporting any usual activity problem	n (%)	2 months	18/121 (15%)	17/119 (14%)	0.64 (0.20 to 2.07)	0.23
		6 months	15/114 (13%)	18/115 (16%)	0.37 (0.11 to 1.26)	
		12 months	15/108 (14%)	19/111 (17%)	0.33 (0.10 to 1.12)	
Reporting any pain / discomfort	n (%)	2 months	24/121 (20%)	34/119 (29%)	0.33 (0.13 to 0.85)	0.13
		6 months	27/114 (24%)	31/115 (27%)	0.55 (0.21 to 1.43)	
		12 months	26/108 (24%)	29/111 (26%)	0.63 (0.24 to 1.66)	
Reporting any anxiety / depression	n (%)	2 months	17/121 (14%)	23/119 (19%)	0.52 (0.17 to 1.59)	0.22
		6 months	14/114 (12%)	24/115 (21%)	0.30 (0.10 to 0.96)	
		12 months	14/108 (13%)	18/111 (16%)	0.69 (0.21 to 2.29)	
EQ5D VAS score	Mean (s.d.)	2 months	78.5 (13.5)	76.4 (18.3)	3.7 (0.6 to 6.7)	0.098
		6 months	79.3 (13.7)	78.3 (16.2)	2.7 (-0.5 to 5.8)	
		12 months	80.2 (14.2)	80.6 (14.8)	1.2 (-2.0 to 4.4)	

CI, confidence interval; s.d., standard deviation.

1, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. 2, P for allocation-time interaction. 3, results show odds ratios and P-value from a logistic regression with (EQ5D = 1) as the outcome.

Table E4: Induced sputum outcomes by allocation

			Vitamin D (n=19 at 2 mo, n=18 at 12 mo)[1]	Placebo (n=16 at 2 mo, n=15 at 12 mo)[2]	Mean difference / ratio of geometric means (95% CI)[3]	P[4]
Differential white cell counts	**% eosinophils[5]**	Median (IQR) 2 mo	2.25 (0.33 to 4.17)	4.79 (1.25 to 8.92)	0.53 (0.27 to 1.06)	0.18
		12 mo	1.25 (0.42 to 5.25)	3.80 (0.42 to 5.42)	0.90 (0.45 to 1.80)	
	% lymphocytes	Median (IQR) 2 mo	0.55 (0.33 to 1.00)	0.50 (0.42 to 0.75)	0.92 (0.62 to 1.36)	0.052
		12 mo	0.50 (0.33 to 0.83)	0.82 (0.58 to 1.17)	0.61 (0.41 to 0.91)	
	% macrophages	Mean (s.d.) 2 mo	45.9 (15.8)	35.6 (22.8)	9.1 (-4.1 to 22.3)	0.30
		12 mo	41.1 (22.4)	42.3 (23.2)	-2.7 (-16.1 to 10.6)	
	% neutrophils	Mean (s.d.) 2 mo	49.0 (15.1)	53.1 (23.8)	-3.5 (-17.5 to 10.6)	0.45
		12 mo	54.4 (21.6)	47.2 (25.7)	7.7 (-6.5 to 21.9)	
Supernatant concentrations of inflammatory markers[6]	**IL-1RA, pg/ml[5]**	Median (IQR) 2 mo	90.9 (78.7 to 119.4)	100.3 (79.7 to 124.4)	1.1 (0.5 to 2.2)	0.83
		12 mo	73.7 (55.6 to 95.8)	112.4 (61.6 to 164.6)	0.8 (0.4 to 1.7)	
	IL-2, pg/ml [5]	Median (IQR) 2 mo	0.0 (0.0 to 0.7)	0.6 (0.0 to 0.8)	0.6 (0.3 to 1.4)	0.26
		12 mo	0.0 (0.0 to 0.5)	0.0 (0.0 to 1.0)	0.5 (0.2 to 1.2)	
	IL-2R, pg/ml [5]	Median (IQR) 2 mo	11.2 (0.0 to 18.6)	5.5 (0.0 to 20.2)	0.8 (0.1 to 4.9)	0.62
		12 mo	16.1 (0.0 to 22.8)	0.0 (0.0 to 18.9)	2.3 (0.4 to 15.4)	
	IL-4, pg/ml [5]	Median (IQR) 2 mo	7.3 (2.0 to 7.9)	2.6 (0.9 to 7.8)	1.6 (1.1 to 2.4)	0.054
		12 mo	7.6 (2.0 to 7.9)	2.2 (1.0 to 7.3)	1.3 (0.9 to 2.0)	
	IL-6, pg/ml [5]	Median (IQR) 2 mo	16.7 (6.5 to 28.4)	10.6 (4.7 to 40.7)	1.0 (0.4 to 2.7)	0.96
		12 mo	16.1 (6.9 to 23.4)	10.6 (5.6 to 36.9)	0.8 (0.3 to 2.3)	
	IL-10, pg/ml [5]	Median (IQR) 2 mo	0.0 (0.0 to 2.2)	1.2 (0.0 to 3.9)	0.8 (0.4 to 1.4)	0.40
		12 mo	0.0 (0.0 to 2.2)	1.6 (0.0 to 4.6)	1.2 (0.7 to 2.2)	
	IL-13, pg/ml [5]	Median (IQR) 2 mo	7.0 (3.9 to 8.8)	7.0 (5.5 to 11.5)	0.7 (0.4 to 1.1)	0.26
		12 mo	7.0 (6.8 to 9.6)	7.0 (5.0 to 13.2)	0.8 (0.5 to 1.3)	
	IL-15, pg/ml [5]	Median (IQR) 2 mo	5.1 (0.0 to 12.1)	0.0 (0.0 to 7.7)	2.2 (0.4 to 11.5)	0.52
		12 mo	6.5 (0.0 to 7.7	0.0 (0.0 to 8.5)	0.7 (0.1 to 4.0)	
	G-CSF, pg/ml [5]	Median (IQR) 2 mo	26.9 (0.0 to 47.8)	20.7 (12.7 to 51.9)	0.8 (0.1 to 5.3)	0.97
		12 mo	24.0 (0.0 to 32.8)	20.6 (13.7 to 30.8)	0.8 (0.1 to 5.7)	
	GM-CSF, pg/ml [5]	Median (IQR) 2 mo	4.0 (3.3 to 4.1)	3.4 (3.2 to 4.0)	1.0 (1.0 to 1.0)	0.11
		12 mo	4.0 (3.3 to 4.1)	3.6 (3.2 to 4.0)	1.0 (1.0 to 1.0)	
	IFN-γ, pg/ml [5]	Median (IQR) 2 mo	4.6 (0.0 to 4.7)	0.3 (0.0 to 1.4)	0.9 (0.6 to 1.4)	0.74
		12 mo	4.6 (0.0 to 4.7)	0.0 (0.0 to 1.0)	1.0 (0.7 to 1.6)	
	CCL2, pg/ml [5]	Median (IQR) 2 mo	14.8 (7.6 to 35.3)	6.2 (0.0 to 15.5)	2.4 (0.5 to 12.0)	0.56
		12 mo	10.8 (7.6 to 23.4)	10.4 (0.0 to 40.5)	1.4 (0.3 to 7.2)	
	CCL4, pg/ml [5]	Median (IQR) 2 mo	5.6 (0.0 to 14.9)	7.8 (0.0 to 17.4)	0.6 (0.1 to 3.1)	0.44
		12 mo	5.8 (0.0 to 13.1)	0.0 (0.0 to 18.2)	2.2 (0.4 to 13.1)	
	CXCL-8, pg/ml [5]	Median (IQR) 2 mo	224.4 (64.0 to 468.8)	152.7 (29.7 to 473.1)	0.9 (0.1 to 5.4)	0.49
		12 mo	66.3 (29.2 to 157.0)	119.9 (16.5 to 772.8)	0.3 (0.0 to 2.1)	
	CXCL-10, pg/ml [5]	Median (IQR) 2 mo	5.2 (0.0 to 14.3)	6.0 (0.0 to 11.4)	0.9 (0.1 to 6.3)	0.78
		12 mo	2.8 (0.0 to 8.3)	0.0 (0.0 to 12.1)	2.0 (0.3 to 13.9)	
	EGF, pg/ml [5]	Median (IQR) 2 mo	11.6 (6.4 to 21.0)	11.3 (2.8 to 23.5)	1.9 (0.5 to 7.4)	0.67
		12 mo	10.8 (7.7 to 13.7)	8.2 (0.0 to 15.5)	1.3 (0.3 to 5.5)	
	VEGF, pg/ml [5]	Median (IQR) 2 mo	9.5 (6.5 to 12.0)	11.3 (9.2 to 13.3)	0.9 (0.1 to 5.4)	0.24
		12 mo	8.8 (7.0 to 10.7)	8.5 (5.1 to 11.3)	0.3 (0.0 to 2.1)	

CI, confidence interval; IQR, interquartile range; mo, months; s.d., standard deviation;

1. Of 24 participants randomised to the vitamin D arm of the induced sputum sub-study, 2 were lost to follow-up and 3 were unable to produce a sputum sample at 2 mo; 3 were lost to follow-up and 3 were unable to produce a sputum sample at 12 mo. 2. Of 26 participants randomised to the placebo arm of the induced sputum sub-study, 4 were lost to follow-up and 6 were unable to produce a sputum sample at 2 mo; 5 were lost to follow-up and 6 were unable to produce a sputum sample at 12 mo. 3. Mean difference presented for variables summarized with mean and

standard deviations; ratio of geometric means presented for variables summarized with medians and IQRs; 4. P for allocation-time interaction; none of these P values were significant using a Benjamini & Hochberg procedure controlling the false discovery rate at 20%; 5. A small constant (0.05) was added to each value prior to log transforming for the regression analysis, to avoid taking logs of zero.6. Median concentrations of the following inflammatory mediators were undetectable at baseline and were therefore not analyzed: IL1-β, IL-5, IL-7, IL-12, IL-17, IFN-α A2, TNF, CCL3, CCL5, CCL11, CXCL9, HGF and FGF-β.

Table E5: Biochemical outcomes by allocation

			Vitamin D_3 (n=121 at 2 mo, n=107 at 12 mo)	Placebo (n=119 at 2 mo, n=110 at 12 mo)	Mean difference / odds ratio (95% CI)[1]	P[2]	P for individual time points[3]
Serum PTH, pmol/L	Mean (s.d.)	2 months	5.69 (2.51)	6.19 (2.56)	-0.62 (-1.35 to 0.12)	0.042	0.10
		12 months	5.50 (2.31)	6.31 (2.87)	-0.89 (-1.66 to -0.13)		0.022
Participants with PTH > 6.8 pmol/L	n (%)	2 months	28/120 (23%)	42/118 (36%)	0.32 (0.12 to 0.89)	0.014	0.029
		12 months	21/107 (20%)	39/110 (35%)	0.24 (0.08 to 0.71)		0.010
Serum corrected calcium, mmol/L	Mean (s.d.)	2 months	2.44 (0.09)	2.43 (0.09)	0.02 (0.00 to 0.04)	0.23	--
		12 months	2.41 (0.08)	2.40 (0.08)	0.01 (-0.01 to 0.03)		--

25(OH)D, 25-hydroxyvitamin D; s.d., standard deviation; PTH, parathyroid hormone.

1, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. **2**, P for allocation-time interaction. 3, presented where overall P <0.05 only.

Table E6: Medication use by allocation

			Vitamin D (n=125)[1]	Placebo (n=125)[1]	Adjusted hazard ratio / odds ratio / incidence rate ratio / ratio of geometric means (95% CI)[2]	P
Use of antibiotics for acute exacerbation	Median time to first course of antibiotics, days (IQR)		-- (244 to --)	-- (204 to --)	0.80 (0.52 to 1.24)	0.32
	Proportion of participants taking ≥1 course of antibiotics (%)[3]		38/110 (35%)	44/113 (39%)	0.79 (0.45 to 1.38)	0.41
	Rate of antibiotic courses per participant-year		72/117.4 = 0.61	79/116.7 = 0.68	0.84 (0.53 to 1.32)	0.45
Use of oral corticosteroids for acute exacerbation	Median time to first course of oral corticosteroids, days (IQR)		390 (390 to --)	-- (314 to --)	0.74 (0.44 to 1.25)	0.27
	Proportion of participants taking ≥1 course of oral corticosteroids (%)[3]		26/109 (24%)	32/113 (28%)	0.73 (0.38 to 1.37)	0.32
	Rate of oral corticosteroid courses for exacerbation per participant-year		42/117.4 = 0.36	51/116.7 = 0.44	0.74 (0.43 to 1.26)	0.27
Use of over-the-counter (OTC) medication for URI	Median time to first course of OTC medication for URI, days (IQR)		131 (44 to --)	108 (39 to 285)	0.86 (0.64 to 1.15)	0.31
	Proportion of participants taking ≥1 course of OTC medication for URI (%)[3]		87/113 (77%)	95/118 (81%)	0.81 (0.43 to 1.52)	0.51
	Rate of courses of OTC medication for URI per participant-year		344/117.4 = 2.93	384/116.7 = 3.29	0.88 (0.67 to 1.16)	0.38
Mean number of uses of inhaled relief medication per 24 hours over period since preceding study visit	Median (IQR)	2 months	0.53 (0.07 to 1.72)	0.58 (0.08 to 1.94) [n=119]	0.85 (0.66 to 1.08)	0.54[5]
		6 months	0.47 (0.05 to 1.45) [n=112]	0.39 (0.07 to 1.74) [n=115]	0.94 (0.73 to 1.20)	
		12 months	0.43 (0.05 to 1.29) [n=106]	0.35 (0.05 to 1.49) [n=110]	1.00 (0.77 to 1.28)	
Mean daily dose of beclomethasone equivalent over previous 7 days	Median (IQR)	2 months	400 (200 to 1000) [n=119]	500 (300 to 1000) [n=119]	0.97 (0.87 to 1.09)	0.85[5]
		6 months	400 (200 to 1000) [n=113]	478 (213 to 1000) [n=110]	1.02 (0.91 to 1.14)	
		12 months	400 (200 to 1000) [n=105]	400 (400 to 1000) [n=108]	0.95 (0.85 to 1.07)	
Using any ICS	n (%)	2 months	119/121 (98%)	119/119 (100%)	--[6]	--
		6 months	113/114 (99%)	110/115 (96%)	--[6]	
		12 months	105/108 (97%)	108/111 (97%)	--[6]	
Using any LABA	n (%)	2 months	63/121 (52%)	63/119 (53%)	--[7]	--
		6 months	59/114 (52%)	60/115 (52%)	--[7]	
		12 months	56/108 (52%)	60/111 (54%)	--[7]	
Using any LTRA	n (%)	2 months	14/121 (12%^)	14/119 (12%)	--[7]	--
		6 months	11/114 (10%)	14/115 (12%)	--[7]	
		12 months	10/108 (9%)	12/111 (11%)	--[7]	

IQR, inter-quartile range; CI, confidence interval; URI, upper respiratory infection; OTC, over-the-counter; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; LTRA, leukotriene receptor antagonist.

1, n given separately if data missing. **2**, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. **3**, this analysis excludes participants who withdrew from the trial without taking this medication prior to date of withdrawal. **4**, this analysis excludes participants who withdrew from the trial without taking any oral corticosteroids for acute exacerbation prior to date of withdrawal. **5**, P value for allocation-time interaction presented; P values for effect of allocation at individual time points are not reported where P for allocation-time interaction ≥0.05. **6**, too few participants with no ICS to allow regression model to estimate odds ratios. **7**, results at different time points too closely associated to allow regression model to estimate odds ratios.

Table E7: Health service use by allocation

	Vitamin D (n=125)	Placebo (n=125)	Adjusted hazard ratio / odds ratio / incidence rate ratio (95% CI)[1]	P
Median time to first unscheduled health care attendance, days (IQR)	-- (211 to --)	-- (160 to --)	0.79 (0.53 to 1.18)	0.26
Proportion of participants with ≥1 unscheduled health care attendance (%)[2]	45/109 (41%)	53/114 (46%)	0.79 (0.46 to 1.35)	0.39
Rate of unscheduled health care attendances per participant-year	103/117.4 = 0.88	118/116.7 = 1.01	0.76 (0.50 to 1.16)	0.21

CI, confidence interval; URI, upper respiratory infection; IQR, inter-quartile range

1, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. 2, this analysis excludes participants who withdrew from the trial without having had an unscheduled health care attendance for exacerbation / URI

Table E8: Work absence by allocation

	Vitamin D (n=125)	Placebo (n=125)	Adjusted hazard ratio / incidence rate ratio / odds ratio (95% CI)[1]	P
Median time to first work absence due to URI or exacerbation (IQR)	-- (148 to --)	326 (77 to --)	0.77 (0.53 to 1.10)	0.15
Rate of days of missed work due to URI or exacerbation per participant-year	512/117.4 = 4.4	433/116.7 = 3.7	0.86 (0.50 to 1.46)	0.57
Proportion of participants missing ≥ 1 day of work due to URI or exacerbation (%)[2]	53/109 (49%)	64/117 (55%)	0.77 (0.45 to 1.30)	0.32

CI, confidence interval; URI, upper respiratory infection; IQR, inter-quartile range

1, adjusted for stratification factors, i.e. British Thoracic Society treatment step (2/3 *vs.* 4/5) and inclusion in *vs.* exclusion from sputum induction sub-study. 2, this analysis excludes participants who withdrew from the trial without having missed a day of work due to URI or exacerbation.

Table E9: Total one-year costs, quality-adjusted life years and incremental net benefit per participant by allocation

		Vitamin D_3 (n=125)[1]	**Placebo (n=125)[1]**	**Adjusted mean difference (95% CI)[2]**	**P**
Study medication, £		35.00 (0.00)	0.00 (0.00)	35.00[3]	-[3]
Asthma / URI-related healthcare use, £	Hospitalisation	19.03 (212.78)	5.86 (46.11)	11.19 (-26.75 to 49.13)	0.56
	A&E attendances	3.15 (19.95)	5.57 (24.15)	-2.64 (-8.14 to 2.87)	0.35
	Primary care consults	30.14 (65.52)	34.82 (63.64)	-6.15 (-21.53 to 9.23)	0.43
	OPD chest clinic	3.19 (26.51)	5.32 (42.73)	-2.27 (-11.17 to 6.63)	0.62
Asthma / URI-related prescribed medication use, £	Antimicrobials	1.14 (2.61)	1.35 (2.74)	-0.24 (-0.90 to 0.42)	0.47
	Inhaled bronchodilators / corticosteroids	1.56 (5.74)	1.80 (6.18)	-0.33 (-1.80 to 1.15)	0.66
	Other drugs[4]	0.65 (4.14)	0.37 (2.94)	0.26 (-0.64 to 1.15)	0.58
Out-of-pocket costs paid by participant, £	Travel	0.39 (2.94)	0.54 (3.08)	-0.18 (-0.93 to 0.57)	0.64
	Over-the-counter medication	5.07 (10.40)	6.59 (13.74)	-1.57 (-4.61 to 1.47)	0.31
	Prescriptions	3.27 (10.24)	3.51 (10.16)	-0.34 (-2.88 to 2.20)	0.79
Productivity loss, £		392.63 (1447.14)	343.21 (773.08)	34.05 (-250.36 to 318.46)	0.81
Total costs associated with asthma / URI over 12 months, £		**495.23 (1732.35)**	**408.93 (824.84)**	**66.78 (-263.47 to 397.03)**	**0.69**
QALY over 12 months		**0.85 (0.22)**	**0.83 (0.24)**	**0.021 (-0.04 to 0.08)**	**0.46**
Incremental net benefit, £[5]				**358.61 (-851.52 to 1568.75)**	**0.56**

CI, confidence interval; NA, not applicable; QALY, quality-adjusted life-years

1. Mean (standard deviation) are presented. 2, adjusted for stratification factors. 3, 95% CI and P value not presented as medication costs are constant across allocation groups. 4, leukotriene receptor antagonists and mucolytics. 5, incremental net benefit calculated by multiplying the mean QALY gain by £20,000 and subtracting the incremental cost.

Table E10: Time to co-primary outcomes by baseline vitamin D status

		Time to exacerbation			Time to URI		
Baseline serum 25(OH)D concentration	**N**	**HR (95% CI)[1]**	**HR ratio (95% CI)[2]**	**$P_{interaction}$[2]**	**HR (95% CI)[1]**	**HR ratio (95% CI)[2]**	**$P_{interaction}$[2]**
<50 nmol/L	144	0.77 (0.44 to 1.35)	Ref	-	0.88 (0.59 to 1.31)	Ref	-
≥ 50 nmol/L	106	1.43 (0.80 to 2.56)	1.92 (0.86 to 4.29)	0.11	0.90 (0.57 to 1.40)	1.00 (0.55 to 1.81)	0.99
<75 nmol/L	206	1.01 (0.65 to 1.59)	Ref	-	0.93 (0.67 to 1.28)	Ref	-
≥75 nmol/L	44	1.03 (0.41 to 2.56)	1.13 (0.41 to 3.10)	0.82	0.75 (0.35 to 1.60)	0.71 (0.32 to 1.56)	0.39

HR, hazard ratio; URI, upper respiratory infection; 25(OH)D, 25-hydroxyvitamin D; Ref, referent category.

1, from Cox regression in each subgroup separately, adjusting for stratification factors 2, from Cox regression using the whole sample and including an interaction between subgroup and allocation, adjusting for stratification factors

Table E11: Time to co-primary outcomes by allocation and genetic sub-group

				Time to exacerbation			Time to URI		
Gene	**SNP**	**Genotype**	**N**	**HR (95% CI)[1]**	**HR, interaction term (95% CI)[2]**	**$P_{interaction}$[2,3]**	**HR (95% CI)[1]**	**HR, interaction term (95% CI)[2]**	**$P_{interaction}$[2,3]**
CRTAM	rs2272094	AA	27	0.81 (0.19 to 3.49)	0.83 (0.47 to 1.50)	0.55	1.24 (0.45 to 3.40)	1.27 (0.81 to 1.99)	0.29
		AG	97	0.83 (0.45 to 1.51)			1.05 (0.65 to 1.71)		
		GG	120	1.08 (0.58 to 1.98)			0.66 (0.42 to 1.03)		
CUBULIN	rs3740165	TC	22	0.45 (0.13 to 1.62)	0.42 (0.11 to 1.61)	0.21	1.03 (0.38 to 2.74)	1.13 (0.43 to 3.01)	0.81
		TT	221	1.09 (0.71 to 1.68)			0.83 (0.60 to 1.13)		
CYP24A1	rs2762939	CC	17	0.79 (0.14 to 4.38)[4]	0.91 (0.47 to 1.76)	0.78	0.68 (0.16 to 2.94)	1.22 (0.74 to 2.01)	0.43
		CG	91	1.03 (0.53 to 2.01)			1.19 (0.71 to 2.00)		
		GG	135	1.01 (0.58 to 1.74)			0.72 (0.49 to 1.08)		
	rs2248137	GG	49	0.73 (0.26 to 2.02)[4]	1.04 (0.59 to 1.82)	0.91	0.93 (0.45 to 1.95)	1.05 (0.70 to 1.59)	0.81
		CG	110	1.79 (0.99 to 3.23)			0.88 (0.56 to 1.39)		
		CC	84	0.75 (0.38 to 1.51)			0.85 (0.51 to 1.42)		
	rs2762934	AA	6	[5]	0.37 (0.16 to 0.86)	0.021	1.73 (0.11 to 27.89)[4]	0.96 (0.52 to 1.77)	0.90
		AG	65	0.65 (0.27 to 1.56)			0.81 (0.44 to 1.49)		
		GG	175	1.24 (0.78 to 2.00)			0.87 (0.61 to 1.22)		
	rs6013897	AA	13	[5]	0.60 (0.30 to 1.18)	0.14	0.23 (0.02 to 2.21)[4]	0.97 (0.58 to 1.64)	0.92
		AT	82	1.08 (0.54 to 2.18)			1.01 (0.60 to 1.69)		
		TT	148	1.15 (0.68 to 1.96)			0.82 (0.55 to 1.21)		
CYP27A1	rs17470271	TT	37	0.93 (0.30 to 2.92)	0.82 (0.45 to 1.47)	0.50	0.88 (0.39 to 1.98)	0.87 (0.57 to 1.34)	0.53
		TA	113	0.91 (0.49 to 1.68)			0.73 (0.47 to 1.14)		
		AA	97	1.16 (0.62 to 2.14)			1.02 (0.64 to 1.63)		
CYP27B1	rs4646537	GT	22	3.06 (0.76 to 12.28)	2.05 (0.58 to 7.29)	0.27	1.02 (0.29 to 3.52)	1.44 (0.46 to 4.51)	0.53
		TT	223	0.94 (0.61 to 1.45)			0.82 (0.60 to 1.11)		
	rs4646536	GG	26	2.19 (0.33 to 14.50)[4]	0.88 (0.46 to 1.68)	0.69	0.39 (0.08 to 1.93)[4]	0.72 (0.45 to 1.16)	0.18
		GA	100	0.73 (0.40 to 1.32)			0.96 (0.61 to 1.52)		
		AA	114	1.50 (0.77 to 2.92)			1.00 (0.63 to 1.59)		
CYP2R1	rs10500804	GG	36	2.22 (0.59 to 8.31)	1.23 (0.67 to 2.25)	0.50	0.56 (0.25 to 1.29)	0.91 (0.58 to 1.43)	0.68
		GT	126	0.87 (0.50 to 1.51)			0.96 (0.63 to 1.46)		
		TT	84	0.99 (0.52 to 1.92)			0.82 (0.49 to 1.38)		
	rs2060793	AA	33	2.14 (0.63 to 7.26)	1.02 (0.58 to 1.81)	0.94	0.59 (0.24 to 1.45)	0.91 (0.58 to 1.42)	0.67
		AG	112	0.79 (0.45 to 1.37)			0.90 (0.58 to 1.41)		
		GG	92	1.15 (0.55 to 2.38)			0.90 (0.56 to 1.46)		
	rs10766197	AA	35	2.36 (0.71 to 7.80)	1.33 (0.71 to 2.50)	0.37	0.64 (0.28 to 1.48)	0.89 (0.56 to 1.42)	0.62
		AG	126	0.87 (0.49 to 1.54)			0.96 (0.63 to 1.46)		
		GG	72	1.02 (0.51 to 2.01)			0.86 (0.48 to 1.51)		
CYP3A4	rs2740574	GG	9	1.44 (0.09 to 23.24)[4]	0.65 (0.26 to 1.61)	0.35	1.73 (0.33 to 9.15)[4]	0.91 (0.51 to 1.63)	0.75
		AG	25	0.43 (0.11 to 1.58)[4]			0.34 (0.13 to 0.94)		
		AA	213	1.14 (0.74 to 1.76)			0.92 (0.67 to 1.28)		
DBP	rs7041	AA	54	1.51 (0.61 to 3.74)	1.47 (0.81 to 2.66)	0.20	0.86 (0.43 to 1.74)	1.11 (0.72 to 1.72)	0.63
		AC	113	1.09 (0.56 to 2.12)			0.97 (0.62 to 1.51)		
		CC	75	0.67 (0.33 to 1.36)			0.78 (0.45 to 1.35)		
	rs12512631	CC	34	0.52 (0.17 to 1.60)	0.49 (0.28 to 0.87)	0.016	0.68 (0.31 to 1.51)	0.80 (0.51 to 1.26)	0.34
		CT	117	0.78 (0.45 to 1.37)			0.79 (0.51 to 1.21)		
		TT	95	1.88 (0.92 to 3.86)			1.01 (0.61 to 1.67)		
	rs4588	TT	15	1.34 (0.20 to 8.90)[4]	1.50 (0.76 to 2.96)	0.25	1.38 (0.24 to 7.84)	0.90 (0.54 to 1.50)	0.68
		TG	97	1.54 (0.80 to 2.96)			0.73 (0.45 to 1.20)		
		GG	133	0.82 (0.47 to 1.41)			0.92 (0.62 to 1.36)		
	rs2070741	TG	39	3.24 (0.83 to 12.60)	3.56 (0.93 to 13.63)	0.064	1.03 (0.46 to 2.32)	1.53 (0.66 to 3.53)	0.32
		TT	204	0.81 (0.52 to 1.27)			0.76 (0.54 to 1.05)		
	rs2298849	GG	8	4.24 (0.25 to 70.72)[4]	1.55 (0.76 to 3.19)	0.23	10.81 (0.67 to 174.44)[4]	1.41 (0.83 to 2.39)	0.21
		GA	83	1.02 (0.52 to 2.02)			0.79 (0.48 to 1.31)		
		AA	154	0.86 (0.51 to 1.43)			0.82 (0.56 to 1.19)		
	rs16846876	TT	14	1.78 (0.24 to 13.33)[4]	1.29 (0.65 to 2.56)	0.48	0.73 (0.13 to 4.11)	0.84 (0.50 to 1.42)	0.52
		TA	111	1.13 (0.62 to 2.06)			0.77 (0.49 to 1.21)		
		AA	116	0.87 (0.49 to 1.56)			0.86 (0.55 to 1.33)		
DHCR7	rs3829251	AA	2	[5]	1.37 (0.58 to 3.24)	0.48	[5]	1.19 (0.62 to 2.29)	0.60
		AG	58	1.41 (0.63 to 3.13)			0.90 (0.49 to 1.67)		
		GG	186	0.92 (0.57 to 1.49)			0.85 (0.60 to 1.21)		
	rs12785878	GG	31	0.46 (0.14 to 1.54)	0.66 (0.37 to 1.15)	0.14	0.53 (0.21 to 1.31)	1.02 (0.66 to 1.57)	0.93
		GT	84	0.76 (0.39 to 1.48)			1.27 (0.76 to 2.13)		
		TT	131	1.25 (0.71 to 2.21)			0.75 (0.49 to 1.15)		
LRP2	rs3755166	AA	36	3.41 (0.86 to 13.54)	1.54 (0.85 to 2.80)	0.16	0.65 (0.27 to 1.53)	0.99 (0.64 to 1.53)	0.95
		AG	126	0.92 (0.56 to 1.53)			0.92 (0.61 to 1.39)		
		GG	84	0.77 (0.34 to 1.77)			0.77 (0.45 to 1.32)		
RXRA	rs7861779	AA	15	0.76 (0.12 to 4.62)[4]	0.73 (0.38 to 1.40)	0.35	0.95 (0.26 to 3.41)	1.21 (0.72 to 2.02)	0.48
		AG	75	0.74 (0.37 to 1.49)			1.03 (0.60 to 1.78)		
		GG	154	1.15 (0.68 to 1.92)			0.76 (0.52 to 1.10)		
	rs9409929	AA	28	1.17 (0.39 to 3.53)[4]	1.06 (0.58 to 1.93)	0.86	0.77 (0.30 to 1.99)	0.86 (0.55 to 1.36)	0.53
		AG	111	1.09 (0.58 to 2.04)			0.74 (0.47 to 1.17)		
		GG	106	0.97 (0.53 to 1.75)			0.98 (0.62 to 1.54)		

Table E11 continued: Time to co-primary outcomes by allocation and genetic sub-group

				Time to exacerbation			Time to URI		
Gene	**SNP**	**Genotype**	**N**	**HR (95% CI)[1]**	**HR, interaction term (95% CI)[2]**	**$P_{interaction}$[2,3]**	**HR (95% CI)[1]**	**HR, interaction term (95% CI)[2]**	**$P_{interaction}$[2,3]**
VDR	rs4334089	AA	31	1.70 (0.48 to 6.00)[4]	1.37 (0.75 to 2.48)	0.30	0.87 (0.37 to 2.07)	1.26 (0.82 to 1.95)	0.30
		AG	90	1.16 (0.59 to 2.29)			1.25 (0.75 to 2.06)		
		GG	124	0.89 (0.51 to 1.54)			0.67 (0.43 to 1.04)		
	rs10783219	TT	31	0.64 (0.23 to 1.73)	0.55 (0.30 to 1.00)	0.049	0.66 (0.28 to 1.55)	1.09 (1.69 to 1.72)	0.70
		AT	100	0.65 (0.33 to 1.29)			1.04 (0.63 to 1.71)		
		AA	113	1.67 (0.91 to 3.05)			0.72 (0.47 to 1.10)		
	rs4516035	CC	43	1.31 (0.47 to 3.64)	1.20 (0.69 to 2.08)	0.53	0.49 (0.24 to 1.02)	0.70 (0.46 to 1.08)	0.11
		CT	101	1.00 (0.52 to 1.91)			0.92 (0.57 to 1.48)		
		TT	99	0.87 (0.47 to 1.63)			1.12 (0.70 to 1.77)		
	rs11568820	TT	32	1.27 (0.40 to 4.03)	1.37 (0.77 to 2.44)	0.28	0.81 (0.38 to 1.74)	1.27 (0.83 to 1.93)	0.28
		CT	71	1.29 (0.61 to 2.76)			1.12 (0.64 to 1.97)		
		CC	136	0.76 (0.44 to 1.31)			0.70 (0.46 to 1.07)		
	rs7976091	TT	31	1.17 (0.35 to 3.91)	1.20 (0.67 to 2.15)	0.53	1.14 (0.52 to 2.49)	1.42 (0.93 to 2.17)	0.11
		CT	72	1.24 (0.58 to 2.63)			1.18 (0.67 to 2.06)		
		CC	138	0.89 (0.52 to 1.50)			0.69 (0.46 to 1.04)		
	rs2238136	TT	19	1.68 (0.38 to 7.36)	1.16 (0.60 to 2.24)	0.67	0.36 (0.13 to 1.03)	0.64 (0.39 to 1.06)	0.083
		CT	85	0.84 (0.37 to 1.87)			0.69 (0.40 to 1.18)		
		CC	142	0.98 (0.59 to 1.63)			1.04 (0.71 to 1.52)		
	rs1544410	TT	42	1.63 (0.65 to 4.07)	1.23 (0.69 to 2.20)	0.49	1.35 (0.65 to 2.79)	1.58 (1.04 to 2.41)	0.034
		CT	116	0.91 (0.50 to 1.66)			1.07 (0.70 to 1.64)		
		CC	85	0.82 (0.40 to 1.72)			0.50 (0.29 to 0.87)		
	rs2228570	AA	34	1.23 (0.35 to 4.31)	0.77 (0.43 to 1.37)	0.38	0.64 (0.27 to 1.49)	1.00 (0.65 to 1.54)	0.99
		AG	111	0.64 (0.35 to 1.17)			1.13 (0.72 to 1.77)		
		GG	102	1.49 (0.79 to 2.82)			0.76 (0.48 to 1.20)		
	rs2853559	AA	35	0.93 (0.30 to 2.84)	0.85 (0.47 to 1.55)	0.60	0.52 (0.21 to 1.30)	0.70 (0.46 to 1.08)	0.11
		AG	108	0.88 (0.46 to 1.70)			0.79 (0.51 to 1.23)		
		GG	103	1.15 (0.64 to 2.08)			1.00 (0.62 to 1.60)		
	rs7975232	CC	51	0.60 (0.25 to 1.45)	0.57 (0.32 to 1.04)	0.067	0.26 (0.12 to 0.56)	0.56 (0.36 to 0.86)	0.008
		AC	111	0.84 (0.44 to 1.61)			1.17 (0.75 to 1.82)		
		AA	75	1.76 (0.86 to 3.59)			1.22 (0.70 to 2.12)		
	rs7970314	GG	34	1.51 (0.49 to 4.65)	1.46 (0.83 to 2.57)	0.19	1.16 (0.54 to 2.49)	1.42 (0.93 to 2.14)	0.10
		AG	74	1.28 (0.61 to 2.67)			1.14 (0.66 to 1.96)		
		AA	138	0.78 (0.46 to 1.34)			0.69 (0.46 to 1.04)		
	rs731236	GG	34	1.38 (0.49 to 3.84)	1.22 (0.67 to 2.23)	0.51	1.28 (0.56 to 2.92)	1.72 (1.11 to 2.66)	0.015
		AG	114	1.10 (0.60 to 2.03)			1.18 (0.77 to 1.82)		
		AA	95	0.89 (0.46 to 1.72)			0.50 (0.30 to 0.82)		

URI, upper respiratory infection; SNP, single nucleotide polymorphism; HR, hazard ratio; CI, confidence interval.
1, from Cox regression in each subgroup separately, adjusting for stratification factors. **2**, from Cox regression using the whole sample and including an interaction between subgroup and allocation, adjusting for stratification factors. **3**, none of this column of P-values are significant when controlling the false discovery rate at 20% using a Benjamini-Hochberg procedure. 4, only possible to estimate by removing one or both of the stratification factors from the regression model, because of small cell counts. **5**, not possible to estimate because of small cell counts.

Table E12: Serious adverse events by allocation[1]

	Vitamin D_3 (n=125)	**Placebo (n=125)**
Cancer diagnosis		
Malignant melanoma recurrence	1	0
Cholangiocarcinoma	1	0
Emergency surgical admission		
Acute appendicitis	1	0
Back pain investigation (gall bladder polyps)	1	0
Plating and pinning, right wrist fractures	0	1
Internal fixation, right hip fracture	1	0
Elective surgery		
Knee replacement	1	0
Hip replacement	1	0
Nasal septoplasty and polypectomy	1	0
Tympanoplasty	1	0
Sinus surgery	0	1
Surgical correction of deformity, right great toe	0	1
Elective medical admission		
Colonoscopy	0	1
Emergency medical admission		
Acute myocardial infarction	0	1
Acute asthma exacerbation	2	3
Complete heart block	0	1
Depression	0	1
Upper gastrointestinal bleed (gastric ulcer)	1	0
Death		
Death due to road traffic accident	1	0
Total number of serious adverse events	13	10
Number of participants experiencing any serious adverse event (%)	12 (10%)	8 (6%)

1. Adverse events were classified as serious if they caused death or were life-threatening, or if they necessitated hospital admission or prolongation of hospital stay.

Table E13: Non-serious adverse events by allocation

	Vitamin D_3 (n=125)	Placebo (n=125)
Number of AE by system		
Acute respiratory infection (self-reported)	383	410
Asthma exacerbation / worsening of symptoms	22	25
Allergy symptoms	7	9
Other ENT AE	12	11
Hypercalcaemia	0	0
Other biochemical abnormality	4	3
Haematological abnormality	2	0
Cardiovascular AE	8	11
CNS / Psychiatric AE	14	19
Dermatological AE	24	26
Fall	4	5
Fracture	6	3
Other musculoskeletal AE	40	36
Gastrointestinal AE	37	49
Genitourinary AE	13	11
Ophthalmic AE	8	7
Oral / dental AE	8	28
Endocrine / metabolic AE	2	1
Other AE	13	22
Total number of non-serious adverse events	607	676
Number of AE by relatedness to IMP:		
Not related / Doubtful	598	647
Possible	9	18
Probable	0	11[1]
Number of participants discontinuing IMP due to AE	2[2]	2[3]
Number of participants experiencing any non-serious adverse event (%)	119 (95%)	121 (97%)

1. 5 reports of abdominal discomfort, 4 reports of nausea, 1 report of sweating and 1 report of diarrhoea, all after taking IMP
2. Two participants diagnosed with vitamin D deficiency, discontinued IMP to take vitamin D supplementation
3. One participant with symptoms of anaphylaxis after taking IMP, one participant with nausea, abdominal pain and diarrhoea after taking IMP

Figure E1: **Study diary**

	DAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Write number	1. Date (day / month / year)							
	2. Peak flow (best of 3 before morning inhalers)							
	3. Ventolin - Number of times used in last 24 hours							
Circle No or Yes	4. Were you woken by asthma symptoms last night?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	5. Cold or flu symptoms yesterday?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	6. Day off yesterday for cold, flu or asthma symptoms?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	7. Doctor yesterday for cold, flu or asthma symptoms?	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26	No Yes If yes see p26
	8. Steroid tablets or other medication yesterday?	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27	No Yes If yes see p27
	9. Any costs of cold, flu or asthma symptoms yesterday?	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28	No Yes If yes see p28
Symtoms over last 24 hours. Circle • 0 for no symptoms • 1 for mild symtoms • 2 for moderate symptoms • 3 for severe symptoms (interfering with activity or sleep)	10. Asthma symptoms	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	11. Sneezing	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	12. Sore throat	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	13. Headache	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	14. Chills or fever	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	15. Feeling generally unwell	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	16. Blocked nose	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	17. Runny nose	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	18. Cough	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
	19. Muscle aches	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3

Figure E2: **Symptom scores by allocation**

Area under the curve for mean asthma symptom score (A) and mean Jackson symptom score (B) by allocation. Data for 224 severe exacerbations and 372 URI with complete symptom scores from 7 days pre-onset to 20 days post-onset are shown, respectively. Solid line, vitamin D_3; dotted line, placebo.

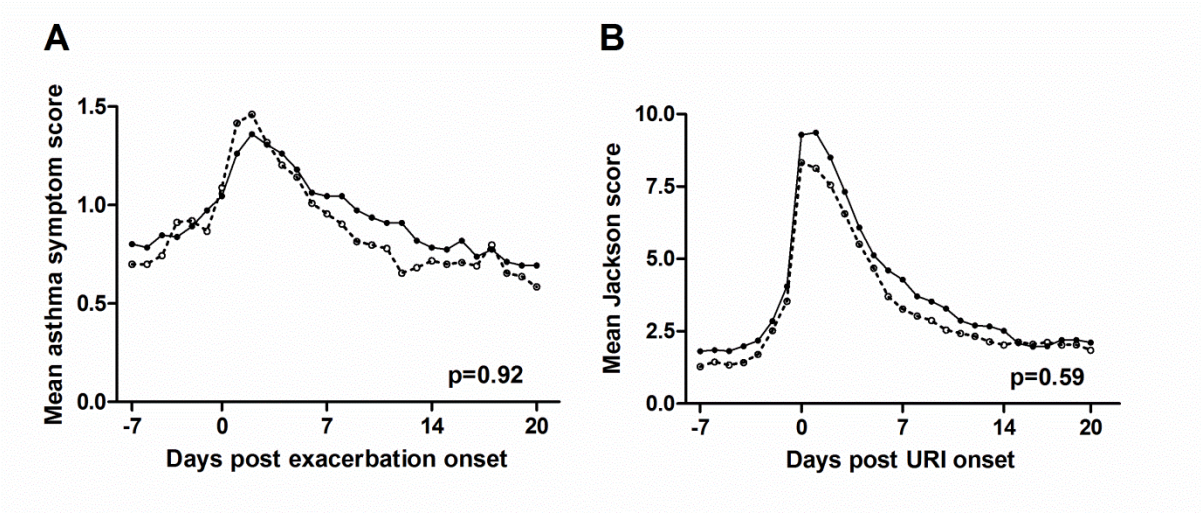


Figure E3: **St George's Respiratory Questionnaire scores by allocation**
Mean change (Δ) in total score (A), symptom score (B), activity score (C) and impacts score (D) for the St George's Respiratory Questionnaire from baseline (0 months) by allocation and duration of follow-up. Overall P values (i.e. P for allocation-time interaction) were calculated by linear regression of log-transformed data adjusted for stratification factors; a small constant (1.0) was added to each value prior to log transformation to avoid taking logs of zero. Where overall P<0.05, P values for individual time points are also presented. Error bars, standard error of the mean. Dotted line placebo, solid line vitamin D.

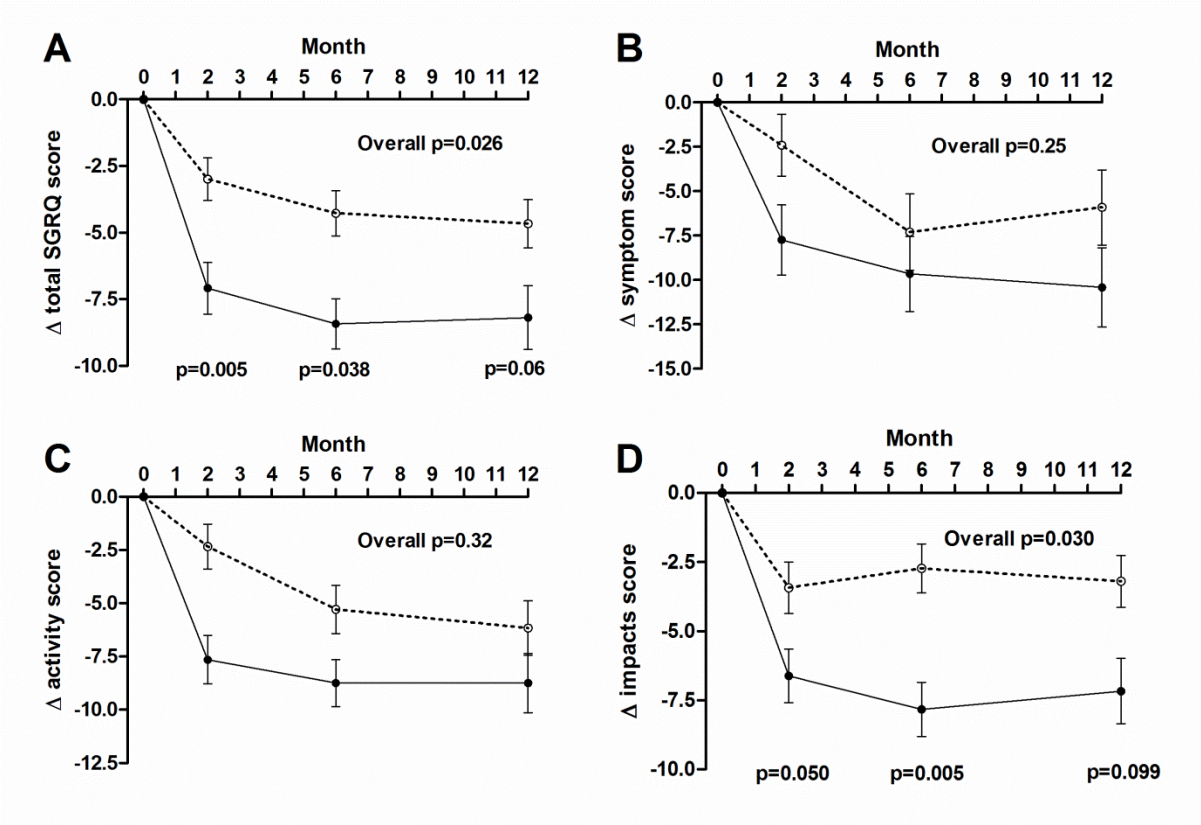


Figure E4: Day-to-day symptom diary data by allocation, presented from the start of the run-in period to the end of the trial, 12 months after the first dose of study medication. A, mean morning Peak Expiratory Flow Rate (PEFR); B, mean number of uses of short-acting bronchodilator per 24 hours; C, mean asthma symptom score; D, % participants with poor symptom control in the day; E, % participants woken by asthma symptoms at night. Solid line, vitamin D_3; dotted line, placebo. Arrows indicate timing of administration of study medication. Seven-day moving averages are presented.

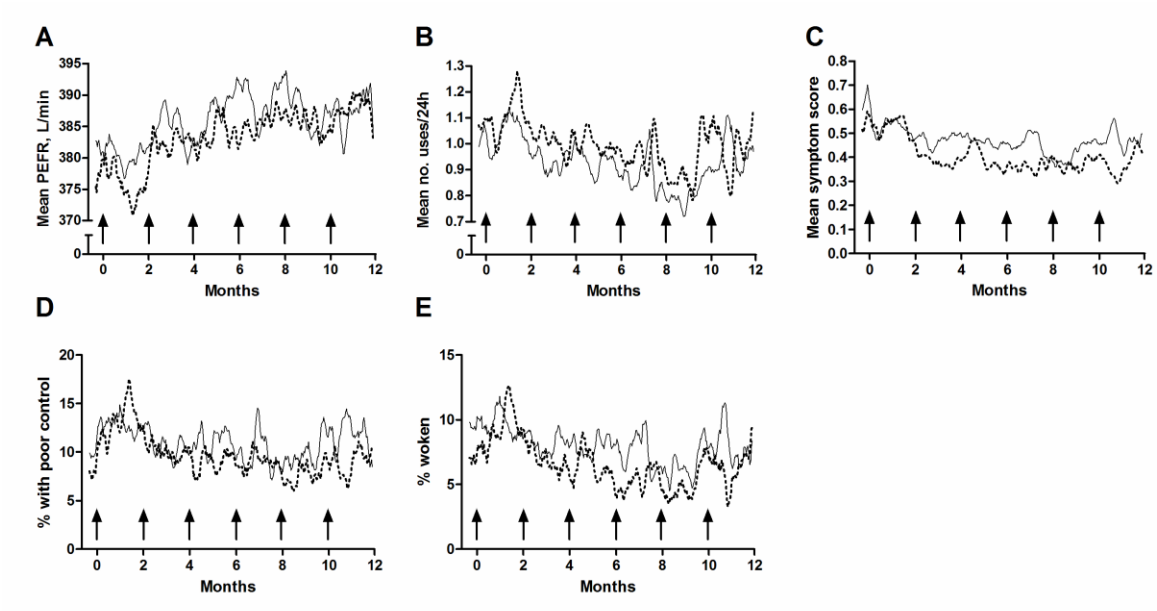
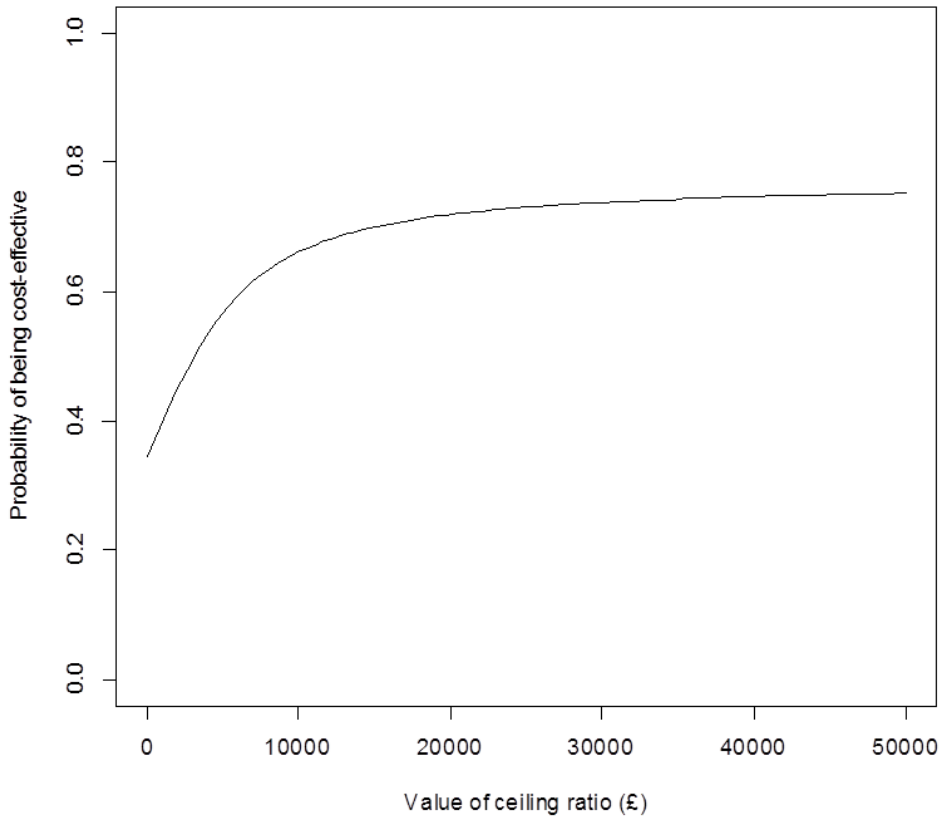


Figure E5: **Probability that vitamin D_3 supplementation is cost effective at alternative levels of willingness to pay for a quality-adjusted life-year (QALY) gain**



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