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ORIGINAL ARTICLE

Double-blind randomised controlled trial of vitamin D_3 supplementation for the prevention of acute respiratory infection in older adults and their carers (ViDiFlu)

Adrian R Martineau,[1,2] Yasmeen Hanifa,[3] Karolina D Witt,[1] Neil C Barnes,[1] Richard L Hooper,[1] Mital Patel,[1] Natasha Stevens,[1] Zinat Enayat,[1] Zuhur Balayah,[1] Asmat Syed,[1] Aishah Knight,[1,4] David A Jolliffe,[1] Claire L Greiller,[1] David McLaughlin,[1] Timothy R Venton,[5] Marion Rowe,[5] Peter M Timms,[5] Duncan Clark,[6] Zia Sadique,[3] Sandra M Eldridge,[1] Christopher J Griffiths[1]

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For numbered affiliations see end of article.

Correspondence to
Professor Adrian R Martineau, Centre for Primary Care and Public Health, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 58 Turner Street, London E1 2AB, UK; a.martineau@qmul.ac.uk

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ABSTRACT

Rationale Low-dose vitamin D supplementation is already recommended in older adults for prevention of fractures and falls, but clinical trials investigating whether higher doses could provide additional protection against acute respiratory infection (ARI) are lacking.

Objective To conduct a clinical trial of high-dose versus low-dose vitamin D_3 supplementation for ARI prevention in residents of sheltered-accommodation housing blocks ('schemes') and their carers in London, UK.

Measurements and methods Fifty-four schemes (137 individual participants) were allocated to the active intervention (vitamin D_3 2.4 mg once every 2 months +10 μg daily for residents, 3 mg once every 2 months for carers), and 54 schemes with 103 participants were allocated to control (placebo once every 2 months +vitamin D_3 10 μg daily for residents, placebo once every 2 months for carers) for 1 year. Primary outcome was time to first ARI; secondary outcomes included time to first upper/lower respiratory infection (URI/LRI, analysed separately), and symptom duration.

Main results Inadequate vitamin D status was common at baseline: 220/240 (92%) participants had serum 25(OH) D concentration <75 nmol/L. The active intervention did not influence time to first ARI (adjusted HR (aHR) 1.18, 95% CI 0.80 to 1.74, p=0.42). When URI and LRI were analysed separately, allocation to the active intervention was associated with increased risk of URI (aHR 1.48, 95% CI 1.02 to 2.16, p=0.039) and increased duration of URI symptoms (median 7.0 vs 5.0 days for active vs control, adjusted ratio of geometric means 1.34, 95% CI 1.09 to 1.65, p=0.005), but not with altered risk or duration of LRI.

Conclusions Addition of intermittent bolus-dose vitamin D_3 supplementation to a daily low-dose regimen did not influence risk of ARI in older adults and their carers, but was associated with increased risk and duration of URI.

Trial registration number clinicaltrials.gov NCT01069874.

Key messages

What is the key question?

- Does addition of intermittent bolus-dose vitamin D_3 supplementation to a daily low-dose regimen enhance protection against acute respiratory infection in older adults and their carers?

What is the bottom line?

- This intervention did not influence risk of acute respiratory infection in the study population, but it was associated with increased risk and duration of upper respiratory infection.

Why read on?

- This clinical trial is the first study to evaluate the effect of vitamin D dosing interval on susceptibility to acute respiratory infection.

INTRODUCTION

Acute respiratory infections (ARIs) are the most common illnesses affecting humans. They have significant impact on health: upper respiratory infections (URIs, which primarily affect the respiratory tract above the vocal cords) are the most common cause of health-service consultation and productivity loss in industrialised countries,[1] while lower respiratory infections (LRIs, primarily affecting the respiratory tract below the vocal cords) caused an estimated 2.81 million deaths worldwide in 2010.[2] Existing prevention strategies focus on vaccination, but the effectiveness of this approach is limited as vaccines are lacking for many of the most common respiratory pathogens, and the immunogenicity of those in existence is impaired in older adults who are among the groups at highest risk.[3] The development of alternative strategies to boost innate immune response to a broad range of pathogens could offer significant public health benefits.

Vitamin D supplementation represents one such strategy. The major circulating metabolite, 25-hydroxyvitamin D (25(OH)D) supports innate responses to both viral and bacterial respiratory pathogens.[4 5] Older adults are at increased risk of suboptimal vitamin D status,[6 7] and serum 25(OH)

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D concentrations <75 nmol/L have been reported to associate independently with increased risk of ARI.[8] UK adults aged 65 years and older are advised to take a low-dose vitamin D supplement (10 μg=400 IU daily) for prevention of fractures and falls; no recommendation is made regarding vitamin D intake in younger adults who are widely perceived to be at low risk of vitamin D deficiency.[9] Although daily lower-dose regimens have been reported to offer protection against URI in children,[10 11] these regimens are insufficient to consistently elevate adults' 25(OH)D concentrations above the 75 nmol/L threshold associated with optimum protection against ARI. A question, therefore, remains as to whether offering high-dose vitamin D supplementation to adults might offer additional protection against ARI as compared with daily low-dose supplementation.

We therefore conducted a pragmatic double-blind randomised placebo-controlled clinical trial among residents and carers in sheltered accommodation schemes in the UK to evaluate whether the addition of intermittent bolus dose vitamin D_3 supplementation, administered once every 2 months for a period of 1 year, would offer superior protection against ARI as compared with standard of care (400 IU daily for residents and no supplementation for carers). 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. The trial was cluster-randomised, and carers were also enrolled, on the grounds that an intervention to prevent ARI in nursing home staff has previously been shown to reduce transmission of respiratory pathogens to those in their care.[12]

METHODS

An abbreviated Methods section follows: further details are provided in online supplementary material.

Participants

Sheltered accommodation schemes in London, UK, were identified by searching the online directory at http://www.housingcare.org/ and assessed for their eligibility to host the trial: schemes offering care exclusively for clients with dementia, learning disability, mental health needs and alcohol or drug dependency were excluded. Housing associations responsible for potentially eligible sheltered accommodation schemes were then approached for permission to conduct the trial on their premises. Individual residents and their carers at sheltered accommodation schemes managed by participating housing associations were sent a letter inviting them to attend a screening visit. Principal exclusion criteria were presence of cognitive impairment or a communication problem precluding informed consent, medical record diagnosis of asthma or COPD and ingestion of a dietary supplement or prescribed therapy containing >10 μg (400 IU) vitamin D per day up to 2 months before first dose of study medication. Further details of eligibility criteria are provided in online supplementary material. The trial was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/112) and written informed consent was obtained from all participants before enrolment. The trial was registered at ClinicalTrials.gov (NCT01069874); the protocol is available from the corresponding author.

Procedures

Screening visit

Participants attending the screening visit completed the EuroQoL EQ-5D questionnaire.[13] They also underwent a baseline clinical assessment, including measurement of height and weight and collection of blood sample for determination of serum concentrations of calcium, albumin and total 25(OH)D. A urine sample was collected from women of childbearing potential for a pregnancy test (SA Scientific, San Antonio, Texas, USA).

Run-in period

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 (see online supplementary figure S1) recorded the presence or absence of cough, cold or 'flu symptoms for each day of participation in the trial. When symptoms were present, participants were also asked to record the severity of the following symptoms, scored from 0 (no symptoms) to 3 (symptoms severe enough to interfere with activity or sleep): headache, sneezing, rhinorrhoea, nasal congestion, sore throat, dyspnoea, wheeze, chest pain, cough, sputum production, sensation of fever or chilliness, myalgia and general malaise. The diary also recorded details of time off work (for carers only), healthcare use, medication use and out-of-pocket expenses incurred as a result of ARIs.

Randomisation

As soon as compliance with diary completion was demonstrated, and serum concentrations of corrected calcium and creatinine were available for at least one participant at a given sheltered accommodation scheme, this scheme was randomly assigned to active or control arms of the trial with a 1:1 ratio. Individual participants at randomised schemes then received one of the regimens detailed in table 1, according to (a) the allocation of the scheme at which they were enrolled, and (b) whether they were a resident or a carer at that scheme. All participants in the intervention arm received a total dose of 3 mg vitamin D_3 over a 2-month period: for carers, this was given as a single bolus of 3 mg once every 2 months, while for residents, this was given as a daily dose of 10 μg plus a bolus dose of 2.4 mg once every 2 months. This regimen was designed to accommodate recommendations from the Department of Health that adults aged 65 years or more should receive a daily dose of 10 μg vitamin D in order to meet their reference nutrient intake.[9] Details of the randomisation process are supplied in online supplementary material. Vitamin D_3 content of a random sample of active medication was determined at the end of the study. Treatment allocation was concealed from participants and study staff. Randomised participants were invited to attend a subsequent study visit, at which the first dose of study medication was administered under direct supervision, and a new symptom diary was provided.

Table 1 Active and control regimens

Type of participant	1-Year regimen	Equivalent daily dose of vitamin D_3
Active arm		
Carer	6×3 mg (120 000 IU) vitamin D_3 once every 2 months	50 μg (2000 IU)
Resident	6×2.4 mg (96 000 IU) vitamin D_3 once every 2 months +10 μg (400 IU) vitamin D_3 daily	50 μg (2000 IU)
Control arm		
Carer	6× placebo once every 2 months	Nil
Resident	6× placebo once every 2 months +10 μg (400 IU) vitamin D_3 daily	10 μg (400 IU)

Follow-up

Participants were asked to complete study diaries daily for the 12 months of study participation. Each diary accommodated up to 12 weeks of data; participants completing follow-up filled six diaries in total. Five further bolus doses of study medication were administered once every 2 months following the first dose under direct supervision. Repeat blood samples were taken at 2 and 12 months, and serum was separated by centrifugation and frozen for subsequent assay of concentrations of 25(OH)D, albumin and calcium. Completion of the EQ5D questionnaire was repeated at 2, 6 and 12 months of follow-up. 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 and use of concomitant medications were recorded throughout. All study visits were conducted at participating sheltered accommodation schemes.

Data management and study definitions

All case report form and diary data were entered into a database in Microsoft Access 2010. Diary data were then imported into Stata, and episodes of ARI (categorised as either URI or LRI) were identified using algorithms based on the following definitions. URI was defined as (a) influenza-like illness as indicated by the presence of cough, feeling of fever/chilliness and muscle pain[14] or (b) a cold, defined using the Jackson criteria.[15] LRI was defined according to the Macfarlane criteria as follows. Each of five Macfarlane symptoms (cough, sputum production, dyspnoea, wheeze, chest discomfort/pain) was scored from 0 to 3, and an LRI was defined as presence of cough with symptom score at least one point over that recorded during the run-in period plus at least one other Macfarlane symptom scoring at least one point over that recorded during the run-in period.[16] Further details of ARI definitions are supplied in online supplementary material.

Validation of ARI definition

In order to validate the diary definition for ARI, we performed paired nasopharyngeal and throat swabs on a subset of study participants during symptomatic events meeting ARI criteria and on occasions during which participants were asymptomatic.

Patients were sampled using flocked nasopharyngeal swabs (Copan Diagnostics, Murrieta, California, USA). Swabs were transferred to the laboratory in Universal Transport Medium (Copan Diagnostics), and tested for the presence of nucleic acids for ten respiratory pathogens (adenovirus; enterovirus; influenza A; influenza B; metapneumovirus; parainfluenza 1, 2 and 3; rhinovirus and respiratory syncytial virus) using real-time PCR.[17]

Sample size and statistical analysis

This trial was powered to detect a clinically significant difference in time to first ARI among participants enrolled in sheltered accommodation schemes allocated to active versus control arms of the trial (primary outcome). The proportion of the population experiencing at least one ARI per year is variously reported to be between 68% and 92%.[1 16 18] Employing the Xie and Waksman formula for sample size estimation in clinical trials, with clustered survival times as the primary endpoint[19] and assuming an average of three participants per unit, with intracluster coefficient of 0.05, equal numbers of units allocated to active and control arms of the study and 25% loss to follow-up of units, we calculated that a total of 108 units would need to be randomised to demonstrate a 20% reduction in proportion of participants experiencing at least one ARI in 1 year from 80% to 64%, with 80% power at the two-sided 5% significance level. This calculation was revised from the original power calculation, which indicated that we would need to randomise a total of 36 sheltered accommodation schemes, based on the assumption that 15 participants would be recruited in each scheme.

Prespecified secondary endpoints were the time to first URI and first LRI, the median duration of symptoms per episode, the proportion of participants experiencing at least one such episode, the rate of these episodes, the peak symptom score per episode, mean serum concentrations of 25(OH)D and corrected calcium at 2 and 12 months, unscheduled healthcare attendance for ARI, use of antibiotics and over-the-counter medications for treatment of ARI, quality of life as indicated by EQ5D scores; work absence (carers only), health economic outcomes (costs of ARI, quality-adjusted life years (QALY) and incremental net benefit over 1 year) and incidence of adverse events. Prespecified subgroup analyses were conducted to determine whether the effect of vitamin D_3 supplementation on risk of ARI, URI and LRI was modified by the type of participant (resident vs carer).

Analyses were performed using Stata/IC (V.12.1, 2012, and V.13, 2013), GraphPad Prism (V.4.03, 2005) and R (V.3.0.2, 2013) software packages. Analysis was by intention-to-treat (ITT), and significance was tested at the 5% level. Time-to-event outcomes were analysed using Cox regression adjusted for minimisation variables (level of care, size of scheme and season of randomisation) and participant study group (resident vs carer), allowing for a shared frailty within the same unit with frailty following a gamma distribution. Effects of allocation on time-to-event outcomes are presented as HRs, with the numerator being the hazard or chance of the outcome occurring in the intervention arm and the denominator being the hazard or chance of the outcome occurring in the control arm; thus, a HR>1 represents an increased risk of the outcome occurring in the intervention arm and vice versa. Further details of statistical analyses, including health economic analyses, are provided in online supplementary material.

Laboratory analyses

Serum concentrations of $25(OH)D_2$ and $25(OH)D_3$ were determined by isotope-dilution liquid chromatography–tandem mass spectrometry[20] and summed to give values for total 25(OH)D concentration. Sensitivity for this assay was 10 nmol/L. Albumin and total serum calcium concentrations were determined using an Architect ci8200 analyser (Abbott Diagnostics, Chicago, Illinois, 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)). Vitamin D_3 content of active medication was determined by high-performance liquid chromatography.

RESULTS

Two hundred and seventy-seven adults, resident or working in 116 sheltered accommodation schemes were assessed for eligibility to participate in the trial between 29 March 2010 and 22 March 2012: 29 were ineligible to participate, and 8 were eligible, but declined randomisation. The remaining 240 participants were either residents or carers at 1 of 108 schemes, which were then randomised to active versus control arms of the trial in equal numbers: 54 schemes with a total of 137 individual participants (115 residents, 22 carers) were assigned to the

active arm of the trial, and 54 schemes with a total of 103 individual participants (79 residents, 24 carers) were assigned to the control arm. All participants received at least one dose of study medication, and were included in the ITT analysis (figure 1). Clinical and demographic characteristics of randomised participants were comparable for active versus control groups (table 2). The majority of participants (220/240, 92%) had inadequate vitamin D status (serum 25(OH)D <75 nmol/L) at baseline. The trial ended on the date of the final study visit of the final participant undergoing follow-up.

Allocation to the active versus the control arm of the trial did not significantly influence time to first ARI (adjusted HR (aHR) 1.18, 95% CI 0.84 to 1.66, p=0.34, figure 2), the proportion of participants experiencing at least one ARI (adjusted OR (aOR) 1.21, 95% CI 0.68 to 2.15, p=0.52), the rate of ARI per participant year (adjusted incidence rate ratio 1.15, 95% CI 0.84 to 1.58, p=0.37) or median ARI duration (adjusted ratio of geometric means 1.17, 95% CI 0.92 to 1.49, p=0.21, table 3). However, when URI and LRI were analysed separately—as prespecified in the protocol and the analysis plan—allocation to the active arm of the trial was associated with increased risk of URI (aHR 1.48, 95% CI 1.02 to 2.16, p=0.039, figure 3), and increased median duration of URI symptoms (7 vs 5 days for active vs control, adjusted ratio of geometric means 1.34, 95% CI 1.09 to 1.65, p=0.005). Allocation did not have a differential effect on time to first URI fulfilling Jackson criteria for cold (aHR 1.40, 95% CI 0.95 to 2.07, p=0.09) versus time to first URI fulfilling criteria for influenza-like illness (aHR 1.38, 95% CI 0.73 to 2.61, p=0.32). No effect of allocation was seen on LRI outcomes (table 3). Vitamin D status improved in both the active arm (40.4 nmol/L mean increase in serum 25(OH)D at 12 months vs baseline, 95% CI for difference 36.0 to 44.7 nmol/L, p<0.001) and the control arm (14.9 nmol/L mean increase in serum 25(OH)D at 12 months vs baseline, 95% CI for difference 10.5 to 19.3 nmol/L, p<0.001). At 12 months, mean serum 25(OH)D was higher in the active versus the control arm of the trial (adjusted mean difference 25.7 nmol/L, 95% CI 20.6 to 30.7 nmol/L, p<0.001), but mean serum corrected calcium concentration at 12 months was not significantly different between arms (p=0.21, table 4). A random sample of active medication assayed at the end of the study was found to contain 99.2% of its original vitamin D_3 content.

No effect of allocation was seen on unscheduled healthcare attendance or use of antibiotics or over-the-counter medications for ARI (see online supplementary table S1) or on quality-of-life outcomes as evaluated with the EQ5D questionnaire (see online supplementary table S2). Allocation did not influence risk of work absence due to ARI among carers (see online supplementary table S3) or health economic outcomes (ARI-associated costs, QALY and incremental net benefit; see online supplementary table S4). The cost-effectiveness acceptability curve displayed in online supplementary figure S2 shows that the probability that the active intervention is cost-effective for prevention of ARI is less than 60% at a realistic willingness to pay (£20 000) for a QALY gain.

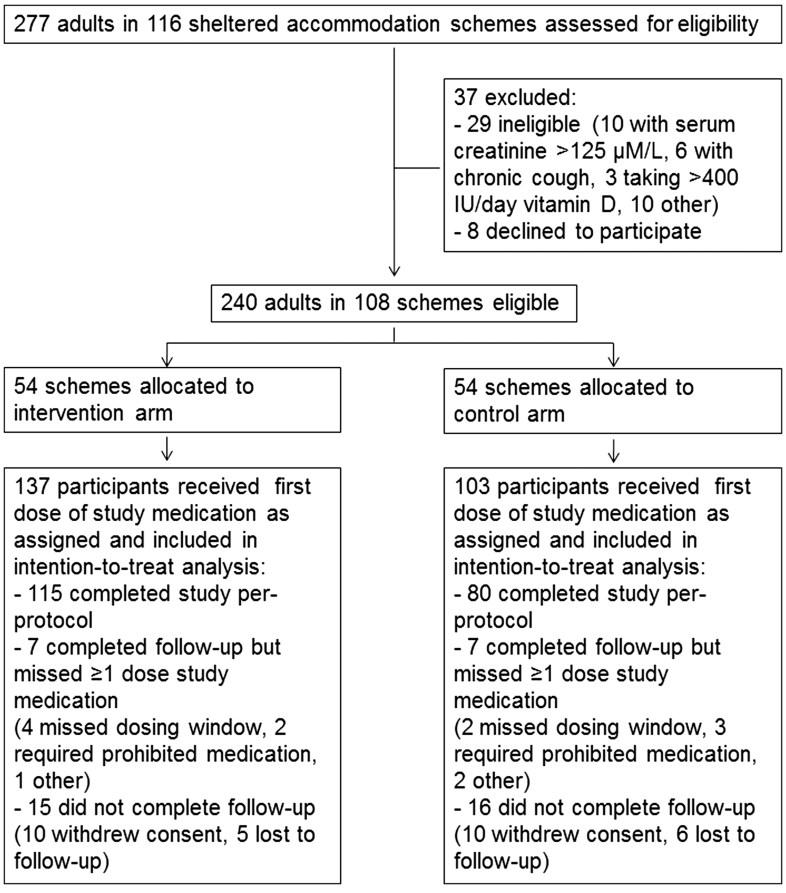


Figure 1 Trial profile.

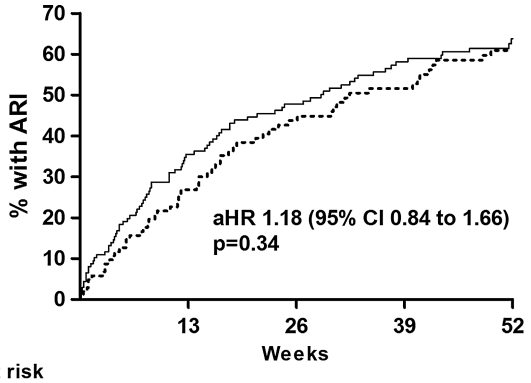
Table 2 Baseline characteristics by allocation

	Active (n=137)	Control (n=103)
Participant characteristics		
Type of participant		
Resident, n (%)	115 (84%)	79 (77%)
Carer, n (%)	22 (16%)	24 (23%)
Age, years		
Mean (SD)	67.5 (12.8)	66.6 (13.4)
Sex		
Male, n (%)	51 (37%)	31 (30%)
Female, n (%)	86 (63%)	72 (70%)
Ethnicity		
Asian/Asian British, n (%)	5 (4%)	6 (6%)
African/Afro–Caribbean, n (%)	17 (12%)	24 (24%)
White, n (%)	110 (80%)	71 (69%)
Mixed, n (%)	2 (1%)*	1 (1%)†
Chinese, n (%)	2 (1%)	1 (1%)
Other, n (%)	1 (1%)‡	0 (0%)
Occupational class		
1 (Managerial, administrative and professional occupations), n (%)	53 (39%)	55 (53%)
2 (Intermediate occupations), n (%)	18 (13%)	10 (10%)
3 (Small employers and own-account workers), n (%)	9 (7%)	3 (3%)
4 (Lower supervisory and technical occupations), n (%)	18 (13%)	12 (12%)
5 (Semiroutine and routine occupations), n (%)	39 (28%)	20 (19%)
Never/long-term (>5 years) unemployed, n (%)	0 (0%)	3 (3%)
Body mass index, kg/m^2		
Mean (SD)	29.5 (6.3)	29.0 (5.9)
Smoking status		
Current smoker, n (%)	26 (19%)	17 (17%)
Non-smoker, n (%)	111 (81%)	86 (83%)
Vaccination history		
Seasonal flu vaccine in the year preceding enrolment, n (%)	88 (64%)	72 (70%)
Pneumococcal vaccine in 5 years preceding enrolment, n (%)	56 (41%)	35 (34%)
Biochemical parameters		
Mean serum 25(OH)D, nmol/L (SD)	42.4 (23.4)	43.6 (22.6)
Serum 25(OH)D<25 nmol/L, n (%)	36 (26%)	24 (23%)
Serum 25(OH)D 25–49 nmol/L, n (%)	55 (40%)	38 (37%)
Serum 25(OH)D 50–74 nmol/L, n (%)	32 (23%)	35 (34%)
Serum 25(OH)D≥75 nmol/L, n (%)	14 (10%)	6 (6%)
Mean serum corrected calcium, mmol/L (SD)	2.24 (0.10)	2.24 (0.09)
Unit characteristics		
Level of care		
<24 h care, n (%)	133 (97%)	96 (93%)
24 h care, n (%)	4 (3%)	7 (7%)
Number of residents		
<30, n (%)	42 (31%)	31 (30%)
≥30, n (%)	95 (69%)	72 (70%)
Month of randomisation		
November–April, n (%)	92 (67%)	66 (64%)
May–October, n (%)	45 (33%)	37 (36%)

*One mixed white/Asian origin, one mixed Filipino/Spanish origin.
†Mixed black African/white origin.
‡Venezuelan.
25(OH)D, 25-hydroxyvitamin D.

In order to dissect out effects of the distinct study regimens administered to residents versus carers, we conducted a prespecified subgroup analysis to determine the influence of allocation on time to ARI, URI and LRI within each of these groups (see online supplementary table S5). Allocation to the intervention arm of the trial was associated with increased risk of URI among residents (aHR 1.58, 95% CI 1.02 to 2.43, p=0.039), but not among carers (aHR 1.24, 95% CI 0.47 to 3.26, p=0.67). No other statistically significant effect of allocation was seen for other outcomes investigated in this subgroup analysis.

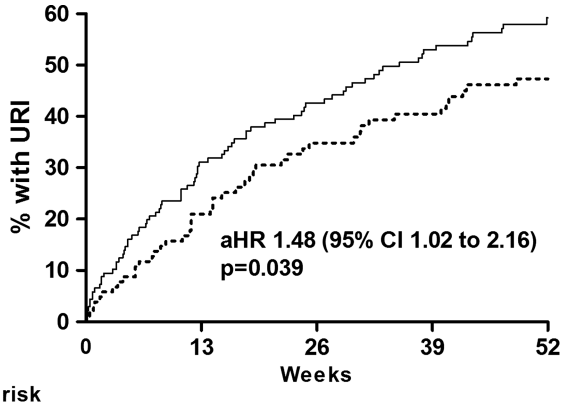
Forty-seven serious adverse events were reported in 39/240 participants receiving at least one dose of study medication; none of these were attributed to study medication, and no



Number at risk

Intervention	137	85	66	51	28
Control	103	70	52	43	25

Figure 2 Time to first ARI by allocation. Numbers of participants yet to experience ARI (number at risk) at 0, 13, 26, 39 and 52 weeks are shown. aHR, adjusted HR; ARI, acute respiratory infection. Solid line, active arm; dotted line, control arm.



No. at risk

Intervention	137	91	72	57	30
Control	103	75	60	53	35

Figure 3 Time to first URI by allocation. Numbers of participants yet to experience URI (number at risk) at 0, 13, 26, 39 and 52 weeks are shown. aHR, adjusted HR; URI, upper respiratory infection. Solid line, active arm; dotted line, control arm.

participant died during the study (see online supplementary table S6). A total of 1132 non-serious adverse events were reported in 221/240 participants: other than a trend towards more self-reported ARI in active versus control arms (267 vs 238, respectively), these were equally distributed between study arms (see online supplementary table S7).

To validate the diary definition for ARI, paired nasopharyngeal and throat swabs were taken in study participants during 21 symptomatic events meeting ARI criteria, and on 145 occasions during which participants were asymptomatic, and tested for the presence of ten respiratory pathogens as described in Methods. Symptomatic events were strongly associated with detection of at least one of the pathogens above (detected in 11/21 symptomatic episodes vs 7/145 patients who were asymptomatic, p<0.0001). Pathogens detected during symptomatic episodes were rhinovirus alone (n=5), influenza A (n=2), enterovirus alone, influenza B, parainfluenza 3 (n=1 each) and mixed rhinovirus/enterovirus infection (n=1).

DISCUSSION

We present results of the first randomised controlled trial to compare the efficacy of intermittent bolus-dose versus low-dose

Table 3 Respiratory outcomes by allocation

	Active (n=137)	Control (n=103)	Adjusted HR/OR/incidence rate ratio/ratio of geometric means (95% CI)*	p Value
ARI (upper or lower)				
Median time to first ARI, days (IQR)	203 (55 to --)	227 (83 to --)	1.18 (0.84 to 1.66)	0.34
Proportion of participants with ≥1 ARI (%)†	83/125 (66%)	58/92 (63%)	1.21 (0.68 to 2.15)	0.52
Rate of ARI per participant-year	279/130.0=2.15	185/93.6=1.98	1.15 (0.84 to 1.58)	0.37
Median duration of symptoms per ARI, days (IQR)‡	6 (3 to 13)	5 (3 to 11)	1.17 (0.92 to 1.49)	0.21
URI				
Median time to first URI, days (IQR)	247 (75 to --)	-- (107 to --)	1.48 (1.02 to 2.16)	0.039
Proportion of participants with ≥1 URI (%)†	77/124 (62%)	45/91 (49%)	1.83 (0.99 to 3.41)	0.055
Rate of URI per participant-year	149/130.0=1.15	94/93.6=1.00	1.34 (0.94 to 1.90)	0.10
Median duration of symptoms per URI, days (IQR)‡	7 (4 to 12)	5 (3 to 10)	1.34 (1.09 to 1.65)	0.005
Median peak Jackson symptom score per URI (IQR)§	9 (6 to 14)	8 (5 to12)	1.11 (0.91 to 1.35)	0.30
LRI				
Median time to first LRI, days (IQR)¶	-- (109 to --)	-- (137 to --)	1.12 (0.75 to 1.66)	0.57
Proportion of participants with ≥1 LRI (%)†	63/124 (51%)	44/90 (49%)	1.11 (0.64 to 1.92)	0.72
Rate of LRI per participant-year	130/130.0=1.00	91/93.6=0.97	1.06 (0.71 to 1.58)	0.77
Median duration of symptoms per LRI, days (IQR)‡	6 (2 to 13)	5 (3 to 11)	1.07 (0.76 to 1.51)	0.70
Median peak Macfarlane symptom score per LRI (IQR)¶	4 (3 to 7)	4 (3 to 6)	1.11 (0.92 to 1.33)	0.27

*Adjusted for study group (resident vs carer) and minimisation variables (level of care, size of scheme and season of randomisation).
†These analyses exclude participants who withdrew from the trial without experiencing ARI/URI/LRI prior to date of withdrawal.
‡Duration of symptoms defined as the number of consecutive days associated with a URI/LRI in which the total Jackson/Macfarlane symptom score is greater than the median total Jackson/Macfarlane symptom score recorded during the run-in period.
§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 to calculate symptom score; peak score is the highest total symptom score recorded during the course of the URI.
¶Macfarlane symptoms (cough, sputum production, dyspnoea, wheeze, chest pain/discomfort) each scored from 0 (no symptoms) to 3 (severe symptoms) and summed to calculate symptom score; peak score is the highest total symptom score recorded during the course of the URI.
--, undefined; ARI, acute respiratory infection; LRI, lower respiratory infection; URI, upper respiratory infection.

Table 4 Biochemical outcomes by allocation

	Active (2 months: n=131; 12 months: n=118)	Control (2 months: n=97; 12 months: n=86)	Adjusted mean difference (95% CI)*	p for allocation-time interaction	p for individual time point†
Mean trough serum 25(OH)D, nmol/L (SD)					
2 months	65.5 (19.8)	52.9 (21.7)	13.2 (8.3 to 18.0)	<0.001	<0.001
12 months	85.3 (24.3)	59.1 (26.0)	25.7 (20.6 to 30.7)		<0.001
Mean serum corrected calcium, mmol/L (SD)					
2 months	2.40 (0.09)	2.40 (0.09)	0.00 (–0.02 to 0.02)	0.21	–
12 months	2.43 (0.13)	2.41 (0.07)	0.02 (0.00 to 0.04)		–

*Adjusted for study group (resident vs carer) and minimisation variables (level of care, size of scheme and season of randomisation).
†p for individual time point only presented where p for allocation-time interaction <0.05.
25(OH)D, 25-hydroxyvitamin D.

daily vitamin D_3 supplementation for the prevention of ARI. In a population with high baseline prevalence of vitamin D deficiency, addition of intermittent bolus-dose vitamin D_3 supplementation to standard of care (400 IU/day for older adults, nil for younger adults) elevated vitamin D status, but did not influence risk of ARI. However, when URI and LRI were analysed separately, allocation to the active intervention was found to increase risk of URI and duration of URI symptoms.

One potential explanation of this finding is that higher serum 25(OH)D levels achieved in the intervention arm might have impaired immunity to pathogens causing URI. A U-shaped relationship between serum 25(OH)D and risk of TB has previously been reported, with very low and very high levels both associated with increased risk of disease.[21] However, observational studies reporting that serum 25(OH)D levels >75 nmol/L[22] or >96 nmol/L[23] associate with protection against ARI do not support this interpretation. Alternatively, it may be that the intermittent bolus dosing regimen we used in the active intervention is less effective than daily dosing at inducing protective immunity to URI. Administration of vitamin D using large intermittent oral boluses has been hypothesised to dysregulate vitamin D metabolism in extrarenal tissues by causing inappropriately low 1-alpha-hydroxylase activity coupled with inappropriately high 24-hydroxylase activity, which may limit local concentrations of the active vitamin D metabolite 1,25-dihydroxyvitamin D.[24] It has also been suggested that 'parent vitamin D_3' (cholecalciferol) may itself play an important physiological role:[25] since the half-life of this compound is only ~24 h, daily dosing with 400 IU would have caused sustained elevation of cholecalciferol levels, whereas dosing at 2-month intervals would not have. In this context, it is interesting to note that relatively low daily doses of vitamin D have been shown to offer protection against URI despite causing relatively modest increments in 25(OH)D,[10 26] and that trials of intermittent bolus dosing regimens for URI prevention have tended to be null.[27–29]

Our study has several strengths. Inadequate vitamin D status was very common among the study population at baseline, and adherence to bolus doses of study medication was high as administration of all such doses was directly supervised by study staff. We collected detailed prospective data on outcomes by using a PCR-validated case definition. This allowed us to detect potential effects of the intervention on episodes that did not come to medical attention, and to determine the influence of allocation on symptom severity and duration as well as incidence of ARI. We also collected data on quality of life and ARI-associated costs, allowing us to conduct a health economic evaluation of the intervention.

Our trial also has some limitations. By using a cluster-randomised design, we hoped to demonstrate benefits of achieving 'herd immunity' to pathogens causing ARI by enrolling the majority of residents and carers in a given scheme and allocating them all to the same intervention. The proportion of residents/carers enrolling in the trial at each scheme was lower than expected, however, and this precluded detection of such effects. The total number of carers enrolled was small; null effects of the intervention observed in this subgroup could, therefore, be due to lack of power. Sampling of vitamin D status was limited to 2-month and 12-month time points, so the 25(OH)D concentrations measured represent 'trough' values only; the 25(OH)D response to bolus-dose supplementation would have been better characterised if vitamin D status had additionally been measured at 3–7 days post dose when it would have been expected to peak.

In summary, we have shown that addition of intermittent bolus-dose vitamin D supplementation to standard of care elevated vitamin D status among residents and their carers in sheltered accommodation units, but increased risk of URI. Our findings suggest that intermittent bolus dose vitamin D supplementation is less effective than daily supplementation for prevention of URI. The influence of dosing interval on immune function and susceptibility to ARI should be evaluated in clinical trials comparing regimens that deliver the same total dose of vitamin D administered in daily versus intermittent doses.

Author affiliations
[1]Centre for Primary Care and Public Health, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
[2]Asthma UK Centre for Applied Research, Blizard Institute, Queen Mary University of London, London, UK
[3]London School of Hygiene and Tropical Medicine, London, UK
[4]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang, Malaysia
[5]Homerton University Hospital, London, UK
[6]Royal London Hospital, London, UK

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THORAX

Double-blind randomised controlled trial of vitamin D_3 supplementation for the prevention of acute respiratory infection in older adults and their carers (ViDiFlu)

Adrian R Martineau, Yasmeen Hanifa, Karolina D Witt, Neil C Barnes, Richard L Hooper, Mital Patel, Natasha Stevens, Zinat Enayat, Zuhur Balayah, Asmat Syed, Aishah Knight, David A Jolliffe, Claire L Greiller, David McLaughlin, Timothy R Venton, Marion Rowe, Peter M Timms, Duncan Clark, Zia Sadique, Sandra M Eldridge and Christopher J Griffiths

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Supplementary Information

Methods

Participants

Sheltered accommodation schemes in London, UK, were identified by searching the online directory at http://www.housingcare.org/ and assessed for their eligibility to host the trial: schemes offering care exclusively for clients with dementia, learning disability, mental health needs and alcohol or drug dependency were excluded. Housing associations responsible for potentially eligible sheltered accommodation schemes were then approached for permission to conduct the trial on their premises. Individual residents and their carers at sheltered accommodation schemes managed by participating housing associations were sent a letter inviting them to attend a screening visit. Respondents were excluded from participation in the trial if they had cognitive impairment or a communication problem precluding informed consent; if they had a medical record diagnosis of asthma, chronic obstructive pulmonary disease, active tuberculosis, sarcoidosis or any other condition causing chronic cough, hyperparathyroidism, nephrolithiasis, renal or hepatic failure, terminal illness or malignancy other than non-melanoma skin cancer not in remission at the time of recruitment; if they were taking a dietary supplement or prescribed therapy containing >10 μg (400 IU) vitamin D per day up to 2 months before first dose of study medication; if they were taking a cardiac glycoside, carbamazepine, phenobarbital, phenytoin, primidone or long-term immunosuppressant therapy, or

applying a medication containing a topical vitamin D analogue; if they were taking a benzothiadiazine derivative at a dose higher than that recommended in the British National Formulary (1), or in combination with a calcium supplement; if they were aged <16 years; if they had been treated with any investigational medical product or device up to 4 months before the first dose of study medication; if serum corrected calcium was >2.65 mmol/L; if serum creatinine was >125 μmol/L; or if they failed to complete the symptom diary during the run-in period. Female carers who were breastfeeding, pregnant or planning a pregnancy at screening were excluded; other female carers of child-bearing potential were also excluded from the study unless a) they were sexually abstinent, or b) they had a negative pregnancy test within 7 days of recruitment and agreed to use a reliable form of contraception until they had completed the study. The trial was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/112) and written informed consent was obtained from all participants before enrolment.

Procedures

Screening visit

Participants attending the screening visit completed the EuroQoL EQ-5D questionnaire (2). They also underwent a baseline clinical assessment including measurement of height and weight and collection of a blood sample for determination of serum concentrations of calcium, albumin and total 25-hydroxyvitamin D (25[OH]D). A urine sample was collected from women of childbearing potential for a pregnancy test (SA Scientific, San Antonio, TX USA).

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 S1) recorded the presence or absence of cough, cold or 'flu symptoms for each day of participation in the trial. When symptoms were present, participants were also asked to record the severity of the following symptoms, scored from 0 (no symptoms) to 3 (symptoms severe enough to interfere with activity or sleep): headache, sneezing, rhinorrhoea, nasal congestion, sore throat, dyspnoea, wheeze, chest pain, cough, sputum production, sensation of fever or chilliness, myalgia and general malaise. The diary also recorded details of time off work (for carers only), health care use, medication use and out-of-pocket expenses incurred as a result of acute respiratory infections.

Randomisation

As soon as compliance with diary completion was demonstrated and serum concentrations of corrected calcium and creatinine were available for at least one participant at a given sheltered accommodation scheme, this scheme was randomly assigned to active or control arms of the trial with a 1:1 ratio. Individual participants at randomised schemes then received one of the regimens detailed in Table 1, according to a) the allocation of the scheme at which they were enrolled, and b) whether they were a resident or a carer at that scheme. All participants in the intervention arm received a total dose of 3 mg vitamin D_3 over a two-month period: for carers this was given as a single bolus of 3 mg once every two months, while for residents this was given as a daily dose of 10 µg plus a bolus dose of 2.4 mg once

every two months. This regimen was designed to accommodate recommendations from the Department of Health that adults aged 65 years or more should receive a daily dose of 10 µg vitamin D in order to meet their Reference Nutrient Intake (9).

The randomisation process was performed as follows. Before the start of recruitment, Nova Laboratories Ltd. prepared kits of study medication for the trial, according to Good Manufacturing Practice. The contents of each kit varied according to a) the allocation of the sheltered accommodation scheme where the participant was recruited, and b) whether the participant was a resident or a carer at that scheme (Table 1). Kits prepared for residents of schemes allocated to the active arm of the study comprised 6 bottles each containing 4.8 ml Vigantol® Oil (2.4 mg [96,000 IU] vitamin D_3) plus 6 dropper bottles each containing sufficient Vigantol oil to dispense a daily drop of 20 µl Vigantol® Oil (10 µg [400 IU] vitamin D_3) for two months. Kits prepared for residents of schemes allocated to the control arm of the study comprised 6 bottles each containing 4.8 ml Miglyol Oil (placebo) plus 6 dropper bottles each containing sufficient Vigantol oil to dispense a daily drop of 20 µl Vigantol® Oil (10 µg [400 IU] vitamin D_3) for two months. Kits prepared for carers at schemes allocated to the intervention arm of the study comprised 6 bottles each containing 6 ml Vigantol® Oil (3 mg [120,000 IU] vitamin D_3). Kits prepared for carers at schemes allocated to the control arm of the study comprised 6 bottles each containing 6 ml Miglyol Oil (placebo). Kits were packed into 108 batches: 54 batches contained sufficient kits for residents and carers of a single scheme allocated to the active arm of the study, and 54 batches contained sufficient kits for residents and carers of a single scheme allocated to the control arm of the study. Each batch of kits was allocated a batch number from 001 to 108 using a computer-generated

random sequence. Individual kits within a given batch were then labelled with a unique randomisation number, composed of the batch number and a kit number separated by a decimal point. Nova Laboratories Ltd were responsible for generation of the random batch number sequence and for packing and labelling kits and batches as above. Nova Laboratories Ltd also provided a copy of the batch randomisation code to the participating pharmacy, members of the Data Monitoring Committee, and to a statistician not involved in analysis of trial results. This statistician randomised units using minimisation software and maintaining allocation concealment and blinding from the Chief Investigator and other researchers. Minimisation criteria were a) number of eligible residents per unit (<30 *vs.* ≥ 30); b) season of randomisation (November to April *vs.* May to October); and c) type of scheme, defined according to the level of care provided (no care or scheme manager only *vs.* housing with care). Once this statistician had assigned a batch number to the unit, study staff were informed of the batch number, and consecutive kit numbers were assigned to participants according to whether they were residents or carers. This process continued until a total of 108 schemes had been randomised. Treatment allocation was concealed from participants and study staff. Randomised participants were invited to attend a subsequent 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 were asked to complete 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 bolus doses of study medication were administered at 2-monthly intervals following the first dose under direct supervision. Repeat blood samples were taken at 2 and 12 months, and serum was separated by centrifugation and frozen for subsequent assay of concentrations of 25(OH)D, albumin and calcium. Completion of the EQ5D questionnaire was repeated at 2, 6 and 12 months of follow-up. 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 and use of concomitant medications 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. Diary data were then imported into Stata and episodes of ARI (categorised as either URI or LRI) were identified using algorithms based on the following definitions. URI was defined as a) influenza-like illness, as indicated by the presence of cough, feeling of fever/chilliness and muscle pain (3) or b) a cold, defined as follows using the Jackson criteria (4). Scores (from 0-3) for each of 8 Jackson symptoms (sneezing, sore throat, headache, subjective sensation of fever or chilliness, malaise, nasal discharge, nasal obstruction, cough) were summed for each day to generate a total Jackson score. A cold was defined as i) total Jackson symptom score of ≥14 + subjective impression of having a cold, or ii) total Jackson symptom score of ≥14 + increased nasal discharge for at least 3 days, or iii) total Jackson symptom score <14 + subjective impression of having a cold + increase in nasal discharge score above median run-in nasal discharge score for ≥ 3 days (4).

LRI was defined according to the Macfarlane criteria as follows. Each of 5 Macfarlane symptoms (cough, sputum production, dyspnoea, wheeze, chest discomfort/pain) was scored from 0-3 as above, and a LRI was defined as presence of cough with symptom score at least one point over that recorded during the run-in period, plus at least one other Macfarlane symptom scoring at least one point over that recorded during the run-in period (5).

Validation of ARI definition

In order to validate the diary definition for ARI, we performed paired nasopharyngeal and throat swabs on study participants during 21 symptomatic events meeting ARI criteria, and on 145 occasions during which participants were asymptomatic. Patients were sampled using flocked nasopharyngeal swabs (Copan Diagnostics, Murietta, CA, USA). Swabs were transferred to the laboratory in Universal Transport Medium (Copan Diagnostics) and tested for the presence of nucleic acids for ten respiratory pathogens (adenovirus, enterovirus, influenza A, influenza B, metapneumovirus, parainfluenza 1, 2 and 3, rhinovirus and respiratory syncytial virus) using real-time polymerase chain reaction (6).

Sample size and statistical analysis

This trial was powered to detect a clinically significant difference in time to first ARI among participants enrolled in sheltered accommodation schemes allocated to active *vs.* control arms of the trial. The proportion of the population experiencing at least one ARI per year is variously reported to be between 68% and 92% (5, 7, 8). Employing the Xie and Waksman formula for sample size estimation in clinical trials

with clustered survival times as the primary endpoint (9) and assuming an average of 3 participants per unit, with intra-cluster coefficient of 0.05, equal numbers of units allocated to active and control arms of the study and 25% loss to follow-up of units, we calculated that a total of 108 units would need to be randomised to demonstrate a 20% reduction in proportion of participants experiencing at least one ARI in one year from 80% to 64%, with 80% power at the two-sided 5% significance level. This calculation was revised from the original power calculation, which indicated that we would need to randomise a total of 36 sheltered accommodation schemes, based on the assumption that 15 participants would be recruited in each scheme.

Pre-specified secondary endpoints were the time to first URI and first LRI; the proportion of participants experiencing at least one such episode; the rate of these episodes; the median duration of symptoms per episode; the peak symptom score per episode; mean serum concentrations of 25(OH)D and corrected calcium at 2 and 12 months; unscheduled health care attendance for ARI; use of antibiotics and over-the-counter medications for treatment of ARI; quality of life, as indicated by EQ5D scores; work absence (carers only); health economic outcomes (costs of ARI, quality-adjusted life years [QALY] and incremental net benefit over one year); and incidence of adverse events. Pre-specified sub-group analyses were conducted to determine whether the effect of vitamin D_3 supplementation on co-primary outcomes was modified by type of participant (resident *vs.* carer).

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 (ITT), and significance was tested at the 5% level. A single pre-specified interim efficacy analysis of time to co-primary

outcomes (requiring P<0.001 to stop) was performed after enrolment of 58 schemes. Interim safety analyses (n=5) were conducted at 6-monthly intervals throughout the course of the trial. Results of interim analyses were reviewed by the Data Monitoring Committee, who recommended continuation of the trial following each review.

Time-to-event outcomes were analysed using Cox regression adjusted for minimisation variables (level of care, size of scheme and season of randomisation) and participant study group (resident *vs.* carer), allowing for a shared frailty within the same unit, with frailty following a gamma distribution. When Cox regression would not converge, these outcomes were analysed using fully-parametric time-to-event regression analysis. Effects of allocation on time-to-event outcomes are presented as hazard ratios, with the numerator being the hazard or chance of the outcome occurring in the intervention arm, and the denominator being the hazard or chance of the outcome occurring in the control arm; thus, a hazard ratio >1 represents an increased risk of the outcome occurring in the intervention arm, and vice versa. The assumption of proportional hazards for all survival analyses was confirmed using the methods proposed by Grambsch and Therneau (10).

Analyses of binary outcomes used logistic regression adjusted for minimisation factors and participant study group (resident *vs.* carer), with a random effect of unit to account for clustering. Analyses of event rates (e.g. rate of infection per participant per year) used negative binomial regression adjusted for minimisation factors and participant study group (resident *vs.* carer), accounting for the appropriate length of follow-up, and with a random effect of scheme. Quantitative outcomes assessed more than once in the same participant, but not at fixed times (e.g. duration of

symptoms per episode of infection) were analysed using linear regression adjusted for minimisation factors and participant study group with random effects of scheme and 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 (e.g. serum 25[OH]D concentrations) were analysed using linear regression adjusted for minimisation factors and participant study group with random effects of scheme and 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. Sub-group analyses were performed by repeating analyses of time to ARI, URI and LRI 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.

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 (11). Unit costs for hospital admissions were obtained from the Reference Costs Database (12). Unit drug costs were calculated from the British National Formulary (1). Participants' costs were obtained from study diaries and included travel expenses and out-of-pocket expenses on prescription drugs and over-the-counter medication incurred as a result of ARI (all participants) and time lost from work due to ARI (carers only). Time lost from work due to ARI was valued using age- and sex-adjusted average daily wage rates from the Office for

National Statistics (13). Total health care costs calculated from diary data were validated against those calculated from GP records for 24 randomly selected participants: good correlation between the two estimates was observed (Spearman's r 0.78, 95% CI 0.54 to 0.90, P<0.0001).

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(14) 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 ARI. 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 active *vs.* control at one year, adjusted for minimisation variables and participant status (resident *vs.* carer).

Missing data for health economic analyses were addressed with multiple imputation. The imputation model included minimisation variables, participant status (resident *vs.* carer) and baseline covariates (sex, ethnicity, alcohol use and body mass index) as predictors. We applied analytical methods in each imputed dataset (n=5) and combined the resultant estimates with Rubin's rules (15). 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 the active intervention was cost-effective at different levels of willingness to pay for a QALY gain (£0 to £50,000 per QALY gained) (16).

Laboratory analyses

Serum concentrations of 25(OH)D_2 and 25(OH)D_3 were determined by isotope-dilution liquid chromatography–tandem mass spectrometry (17) and summed to give values for total 25(OH)D concentration. Sensitivity for this assay was 10 nmol/l. 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]). Vitamin D_3 content of active medication was determined by high performance liquid chromatography.

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Supplementary Table 1: Health service and medication use by allocation

		Active (n=137)	Control (n=103)	Adjusted hazard ratio / odds ratio / incidence rate ratio (95% CI)[1]	P
Unscheduled healthcare attendance for ARI	Median time to first attendance, days (IQR)	107 (41 to 229)	152 (77 to 296)	1.31 (0.72 to 2.41)	0.38
	Proportion of participants with ≥1 attendance (%)[2]	34/123 (28%)	22/88 (25%)	1.10 (0.59 to 2.07)	0.76
	Rate of attendances per participant-year	51/130.0 = 0.39	42/93.6 = 0.45	0.79 (0.43 to 1.44)	0.44
Antibiotic use for ARI	Median time to first course of antibiotics for ARI, days (IQR)	107 (49 to 203)	173 (152 to 296)	1.57 (0.82 to 3.03)	0.18
	Proportion of participants taking ≥1 course of antibiotics for ARI (%)[2]	29/123 (24%)	16/87 (18%)	1.33 (0.66 to 2.69)	0.42
	Rate of antibiotic courses per participant-year	38/130.0 = 0.29	22/93.6 = 0.23	1.23 (0.68 to 2.22)	0.50
Use of OTC medication for ARI	Median time to first course of OTC medication for ARI, days (IQR)	109 (35 to 204)	168 (64 to 234)	1.33 (0.93 to 1.95)	0.12
	Proportion of participants taking ≥1 course of OTC medication for ARI (%)[2]	77/123 (63%)	49/90 (54%)	1.43 (0.82 to 2.50)	0.21
	Rate of courses of OTC medication for ARI per participant-year	206/130.0 = 1.59	139/93.6 = 1.48	1.18 (0.83 to 1.68)	0.37

CI, confidence interval; ARI, acute respiratory infection; IQR, inter-quartile range; OTC, over-the-counter.

1, adjusted for study group (resident *vs.* carer) and minimisation variables (level of care, size of scheme and season of randomisation). **2**, these analyses exclude participants who withdrew from the trial without experiencing the relevant outcome prior to date of withdrawal.

Supplementary Table 2: Quality of life outcomes by allocation

		Active (2 mo: n=132 6 mo: n=128 12 mo: n=122)	Control (2 mo: n=98 6 mo: n=91 12 mo: n=87)	Adjusted odds ratio / mean difference / (95% CI)[1]	P
Mean EQ5D index score (s.d.)	2 mo	0.82 (0.22)	0.87 (0.18)	-0.30 (-1.16 to 0.59)[2]	0.64[2]
	6 mo	0.82 (0.24)	0.84 (0.26)	-0.20 (-1.08 to 0.69)[2]	
	12 mo	0.79 (0.27)	0.80 (0.25)	0.34 (-0.54 to 1.22)[2]	
Proportion reporting any mobility problem (%)	2 mo	45/132 (34%)	29/98 (30%)	0.79 (0.25 to 2.54)	0.27
	6 mo	39/128 (30%	26/91 (29%)	0.58 (0.17 to 1.94)	
	12 mo	42/122 (34%)	34/87 (39%)	0.31 (0.09 to 1.02)	
Proportion reporting any self-care problem (%)	2 mo	7/132 (5%)	2/98 (2%)	2.92 (0.37 to 23.18)	0.79
	6 mo	13/128 (10%)	7/91 (8%)	1.07 (0.25 to 4.68)	
	12 mo	19/122 (16%)	11/87 (13%)	1.04 (0.27 to 3.93)	
Proportion reporting any usual activity problem (%)	2 mo	24/132 (18%)	20/98 (20%)	0.62 (0.21 to 1.81)	0.33
	6 mo	20/128 (16%)	18/91 (20%)	0.43 (0.14 to 1.31)	
	12 mo	22/122 (18%)	20/87 (23%)	0.43 (0.14 to 1.30)	
Proportion reporting any pain / discomfort (%)	2 mo	56/132 (42%)	31/98 (32%)	1.47 (0.65 to 3.34)	0.54
	6 mo	51/128 (40%)	26/91 (29%)	1.50 (0.64 to 3.54)	
	12 mo	49/122 (40%)	33/87 (38%)	0.83 (0.35 to 1.93)	
Proportion reporting any anxiety / depression (%)	2 mo	22/132 (17%)	13/98 (13%)	1.68 (0.55 to 5.15)	0.76
	6 mo	22/128 (17%)	13/91 (14%)	1.25 (0.41 to 3.83)	
	12 mo	22/122 (18%)	16/87 (18%)	0.86 (0.29 to 2.55)	
Mean EQ5D VAS score (s.d.)	2 mo	76.0 (16.9)	77.2 (20.3)	-0.46 (-4.56 to 3.65)	0.73
	6 mo	78.1 (17.1)	78.3 (19.0)	0.68 (-3.53 to 4.89)	
	12 mo	78.3 (19.3)	76.9 (19.0)	2.14 (-2.14 to 6.43)	

s.d., standard deviation; mo, months; VAS, visual analogue scale.

1, adjusted for study group (resident *vs.* carer) and minimisation variables (level of care, size of scheme and season of randomisation). **2**, the distribution of EQ5D scores was bimodal, with the majority of the sample having a value of exactly 1, but with a subgroup with mode around 0.8. Results show adjusted odds ratios and overall P-value from a logistic regression with (EQ5D = 1) as the outcome.

Supplementary Table 3: Work absence by allocation

	Active (n=22)[1]	**Control (n=24)**[1]	**Adjusted hazard ratio / odds ratio / incidence rate ratio (95% CI)**[2]	**P**
Median time to first work absence due to ARI, days	-- (-- to --)	-- (339 to --)	0.82 (0.24 to 2.73)	0.74
Proportion of participants missing ≥ 1 day of work, (%)	5/21 (24%)	7/21 (33%)	0.62 (0.15 to 2.64)	0.52
Rate of days of missed work due to ARI per participant year	16/21.3 = 0.75	34/23.1 = 1.47	0.50 (0.09 to 2.87)[3]	0.44

1, this analysis was conducted for carers only, as the majority of residents were retired. **2**, unless otherwise stated, adjusted for study group (resident *vs.* carer) and minimisation variables (level of care, size of scheme and season of randomisation). **3**, the negative binomial regression in this case was performed ignoring clustering by scheme, as the regression with clustering failed to converge.

Supplementary Table 4: Total one-year costs, quality-adjusted life years and incremental net benefit per participant by allocation

		Active (n=137)[1]	**Control (n=103)**[1]	**Adjusted mean difference (95% CI)**[2]	**P**
Study medication, £		35.00 (0.00)	21.32 (11.81)	12.62 (11.22 to 14.02)	<0.001
ARI-related healthcare use, £	Hospitalisation	9.35 (94.99)	53.30 (388.31)	-49.09 (-116.50 to 18.31)	0.15
	Emergency department attendances	0.66 (7.77)	0.88 (8.97)	-0.11 (-2.39 to 2.17)	0.93
	Primary care consultations	15.47 (33.85)	14.57 (38.54)	0.97 (-8.18 to 10.13)	0.84
ARI-related prescriptions, £	Antimicrobials	0.50 (1.33)	0.35 (1.14)	0.18 (-0.13 to 0.49)	0.25
Out-of-pocket costs paid by participant, £	Travel	0.07 (0.85)	0.42 (2.50)	-0.38 (-0.82 to 0.07)	0.098
	Over-the-counter medication	3.99 (9.46)	3.49 (9.65)	0.30 (-2.14 to 2.73)	0.81
	Prescriptions	0.05 (0.62)	0.25 (1.78)	-0.12 (-0.43 to 0.19)	0.46
Productivity loss, £		11.08 (71.40)	15.25 (80.93)	1.04 (-17.50 to 19.57)	0.91
Total costs associated with ARI over 12 months, £		76.46 (130.47)	109.83 (401.31)	-34.72 (-107.34 to 37.90)	0.35
QALYs over 12 months		0.81 (0.20)	0.82 (0.22)	0.00 (-0.054 to 0.059)	0.93
Incremental Net Benefit, £[3]				82.89 (-1054.76 to 1220.54)	0.89

CI, confidence interval; ARI, acute respiratory infection; QALY, quality-adjusted life-years

1, mean (standard deviation) are presented. **2**, adjusted for study group (resident *vs.* carer) and minimisation variables (level of care, size of scheme and season of randomisation). **3**, incremental net benefit calculated by multiplying the mean QALY gain by £20,000 and subtracting the incremental cost.

Supplementary Table 5: Respiratory outcomes by allocation: residents *vs.* carers

	Residents (n=194)				Carers (n=46)				
	Active (n=115)	Control (n=79)	Adjusted ratio of hazard ratios (95% CI)[1]	P	Active (n=22)	Control (n=24)	Adjusted ratio of hazard ratios (95% CI)[1]	P	P for interaction
Median time to first ARI, days (IQR)	194 (58 to --)	213 (85 to --)	1.15 (0.79 to 1.68)	0.48	262 (55 to --)	284 (76 to --)	1.59 (0.52 to 4.88)	0.42	0.73
Median time to first URI, days (IQR)	227 (75 to --)	-- (125 to --)	1.58 (1.02 to 2.43)	0.039	266 (55 to --)	284 (76 to --)	1.24 (0.47 to 3.26)	0.67	0.48
Median time to first LRI, days (IQR)	-- (115 to --)	346 (114 to --)	0.96 (0.61 to 1.51)	0.85	313 (109 to --)	-- (284 to --)	2.24 (0.89 to 5.69)	0.09	0.054

CI, confidence interval; ARI, acute respiratory infection; IQR, inter-quartile range; URI, upper respiratory infection; LRI, lower respiratory infection.

1, adjusted for minimisation variables (level of care, size of scheme and season of randomization)

Supplementary Table 6: Serious Adverse Events by allocation[1]

	Active (n=137)	**Control (n=103)**
Cancer diagnosis / treatment		
Malignant melanoma	1[2]	0
Pancreatic carcinoma	0	1[2]
Prostatic carcinoma	1[2]	0
Emergency surgical admission		
Acute cholecystitis	1	0
Acute urinary retention	0	1
Diverticulitis	0	1
Hepatic cyst	0	1
Soft tissue injury following trauma	1	1
Elective surgery		
Knee replacement	4	2
Hip replacement	2	0
Hysterectomy for atypical endometrial hyperplasia	1	0
Interphalangeal joint replacement	1	0
Repair of incisional hernia	1	0
Emergency medical admission		
Atrial fibrillation with rapid ventricular response	0	1
Atypical / musculoskeletal chest pain	1	1
Cellulitis	2	0
Cerebrovascular accident	1	1
Community-acquired pneumonia	2	2
Fall	0	2
Focal seizure	0	2
Headache (cause undetermined)	0	1
Ischaemic optic neuropathy	0	1
Labyrinthitis	1	0
Metabolic acidosis due to metformin / ethanol overdose	0	1
Supra-ventricular tachycardia	0	1
Syncopal episode (cause undetermined)	1	1
Unstable angina pectoris	2	0
Urinary tract infection	2	1
Total number of SAEs	25	22
Number of SAEs leading to discontinuation of study medication	2	1
Death due to any cause during participation in trial	0	0
Number of participants experiencing any serious adverse event (%)	22 (16%)	17 (17%)

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. **2**, these diagnoses led to discontinuation of study medication.

Supplementary Table 7: Non-Serious Adverse Events by allocation

	Active (n=137)	Control (n=103)
Number of non-serious adverse events by system		
Acute upper respiratory infection	218	194
Acute lower respiratory infection	49	44
Other respiratory infection	26	24
Allergic symptoms	8	9
Other ear / nose / throat adverse event	10	1
Hypercalcaemia	0	0
Other biochemical adverse event	15	14
Haematological adverse event	8	8
Cardiovascular adverse event	15	15
Endocrine / metabolic adverse event	9	5
Central nervous system / psychiatric adverse event	35	26
Dermatological adverse event	22	17
Fall	15	12
Fracture	2	2
Other musculoskeletal adverse event	57	59
Gastrointestinal adverse event	29	37
Genitourinary adverse event	18	12
Ophthalmic adverse event	14	19
Oral / dental adverse event	13	8
Other adverse event	33	30
Total number of non-serious adverse events	596	536
Number of non-serious adverse events by relatedness to study medication		
Not related	588	531
Doubtful	5	4
Possible	2[1]	1[2]
Probable	1[3]	0
Number of non-serious adverse events leading to discontinuation of study medication	4[4]	3[5]
Number of participants experiencing any non-serious adverse event (%)	127 (93%)	94 (91%)

1, one diarrhoea, one abdominal cramps; **2**, abdominal pain and vasovagal symptoms leading to discontinuation of study medication; **3**, rash after taking low-dose study medication; **4**, one palpitations, one 'dizzy spell', one diarrhoea, one nausea; **5**, one oral candidiasis, one abdominal pain and vasovagal symptoms, one diagnosis of vitamin D deficiency.

Supplementary Figure 1: Study Diary

	DAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Please complete EVERY DAY	Date (day / month / year)							
	Did you have any cough, cold or flu symptoms today?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	If yes, please complete <u>entire</u> column below, scoring symptoms 0 = none, 1 = mild, 2 = moderate, 3 = severe							
Please complete ENTIRE COLUMN only if you have a cough, a cold or flu symptoms	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
	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
	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
	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
	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
	Shortness of breath	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
	Wheeze	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
	Chest pain	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
	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
	Coughing up phlegm	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
	Fever or chills	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
	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
	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
	Did you take a day off work today because of your symptoms?	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
	Did you see a doctor today for your 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
	Did you take antibiotics or other medicines today for your symptoms?	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
	Did you incur any costs today as a result of cold, flu or chest infection?	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

Supplementary Figure 2: **Probability that vitamin D_3 supplementation is cost effective at alternative levels of willingness to pay for a quality-adjusted life-year (QALY) gain**