Individual Patient Data meta-analysis of randomised controlled trials of vitamin D supplementation to prevent acute respiratory infection (AVID-ARI)

Study Protocol

Version 1

Dated: 29 September 2014

Full Title	Vitamin D Supplementation to Prevent Acute Respiratory Infection: Individual Patient Data Meta-Analysis of Randomised Controlled Trials
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1 Glossary of terms and abbreviations

25(OH)D	25-hydroxyvitamin D
ARI	Acute Respiratory Infection
COPD	Chronic Obstructive Pulmonary Disease
DBP	Vitamin D Binding Protein
GP	General Practitioner
IPD	Individual Patient Data
JRMO	Joint Research Management Office
LRI	Lower Respiratory Infection
NICE	National Institute for Health Care and Excellence
Participant	An individual who takes part in a randomised clinical trial
PI	Principal Investigator
QALY	Quality-Adjusted Life Year
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
URI	Upper Respiratory Infection
VDR	Vitamin D Receptor

2 Summary

Short Title	Vitamin D Supplementation to Prevent Acute Respiratory Infection (ARI): Individual Patient Data (IPD) Meta-Analysis of Randomised Controlled Trials
Methodology	IPD meta-analysis of randomised controlled trials
Research Site	Centre for Primary Care and Public Health, Barts and The London School of Medicine and Dentistry
Primary Objectives	To determine whether there are differential effects of vitamin D supplementation on incidence of ARI and acute exacerbations of asthma and Chronic Obstructive Pulmonary Disease (COPD) in participant sub-groups categorised by baseline vitamin D status, age, sex, race/ethnic origin, body mass index, environmental exposure to particulate matter, nutritional supplement use, presence or absence of respiratory comorbidity, severity of asthma / COPD (where applicable), vitamin D-related genotype, vitamin D dose administered, type of vitamin D administered, frequency of vitamin D administration, duration of supplementation and degree of compliance with protocol
Number of Participants/Patients	11,638 individual participants in 21 randomised controlled trials.
Main Inclusion Criteria	Studies will be eligible to contribute primary data to this meta-analysis if they are: • Randomised controlled trials of vitamin D supplementation in which data relating to incidence of ARI or exacerbation of asthma / COPD have been prospectively collected using a directed, closed question routinely directed at all participants • Approved by a research ethics committee
Statistical Methodology and Analysis (if applicable)	Statistical analyses of the effectiveness of vitamin D supplementation vs. placebo will be performed on the combined study population for the following outcomes: • Incidence of ARI • Incidence of asthma exacerbation • Incidence of COPD exacerbation • Health service use • Medication use • Work absence • Safety
Proposed Start Date	1st October 2014
Proposed End Date	31st December 2015
Study Duration	Fifteen months

3 Introduction

Acute respiratory infections (ARI) are major causes of morbidity and health service use that impose significant human and economic costs [1]. People with chronic respiratory illnesses such as asthma and chronic obstructive pulmonary disease (COPD) are particularly susceptible, and they suffer more serious consequences, as ARI can precipitate acute exacerbations of these conditions [2]. Although vaccines are available for some of the pathogens responsible for ARI, their protective efficacy is limited by poor uptake, narrow spectrum of protection and failure to induce protection in some groups, e.g. older adults. New interventions offering a broader spectrum of protection, higher degree of patient-acceptability and lower cost are urgently needed.

A growing body of evidence suggests that vitamin D supplementation might prevent ARI by inducing protective innate immune responses to a wide range of viral and bacterial respiratory pathogens [3-5]. Several randomised controlled trials of vitamin D supplementation with primary outcome of ARI have been published to date. Additionally, at least two other trials of vitamin D supplementation have investigated effects on ARI as a secondary outcome [6] [7]: published meta-analyses of aggregate data from these studies have yielded conflicting results, with one [8] reporting a protective effect of vitamin D supplementation (OR, 0.64; 95% CI, 0.49 to 0.84, p=0.0014) and another [9] reporting no effect (RR 0.98; 95% CI 0.93-1.03, p = 0.45). The former meta-analysis reported a high degree of heterogeneity between studies.

We hypothesise that much of this heterogeneity can be attributed to inter-trial variation in vitamin D dosing regimens and / or specific characteristics of study populations that have potential to modify the effects of vitamin D supplementation on immune responses to respiratory pathogens: trial populations differed widely in terms of dose of vitamin D administered, baseline prevalence of vitamin D deficiency, mean age, race/ethnic origin and prevalence of asthma and COPD, for example. Identification of more effective dosing regimens and / or sub-groups of patients who may particularly benefit from vitamin D supplementation would allow optimisation and targeting of this intervention, with significant implications in terms of improved clinical effectiveness and cost savings. However, aggregate data meta-analysis cannot demonstrate sub-group effects, as event rates within different sub-groups of trial participants are not consistently reported. Conduct of further primary studies to compare different dosing regimens or to identify sub-group effects is impractical due to a) the very large sample sizes that would be required, and b) the ethical problem of prospectively identifying patients with profound vitamin D deficiency (who may particularly stand to benefit) and randomising them to placebo.

We therefore propose to obtain individual participant data (IPD) from 21 trials in order to perform IPD meta-analysis with health economic evaluation to answer the following question:

“Is there a differential effect of vitamin D supplementation on incidence of ARI and acute exacerbations of asthma and COPD in various sub-groups categorised by baseline vitamin D status, age, race/ethnic origin, presence or absence of respiratory comorbidity, vitamin D dose administered, frequency of vitamin D administration, duration of supplementation and degree of compliance with protocol?”

Our consortium comprises principal investigators from 21 primary trials of vitamin D supplementation for prevention of ARI and/or exacerbations of asthma or COPD, giving us access to IPD from over 11,000 patients. This approach gives us adequate power to generate valid, reliable answers to many of the questions above.

4 Aims and objectives

The overarching aims of the proposed project are

i) to determine whether there are differential effects of vitamin D supplementation on incidence of ARI and acute exacerbations of asthma and COPD in particular sub-groups of participants, and

ii) to determine whether any effects of vitamin D supplementation on the outcomes above vary according to dosing regimen.

Our primary objective is to conduct IPD meta-analysis of RCTs to determine the differential effects of vitamin D supplementation on risk of ARI and acute exacerbations of asthma and COPD in sub-groups categorised according to the following potential effect-modifiers:

i) Baseline vitamin D status

ii) Age

iii) Race/Ethnic origin

iv) Presence *vs.* absence of respiratory comorbidity: asthma vs. COPD vs. neither condition reported

v) Vitamin D dose administered

vi) Frequency of vitamin D administration

vii) Duration of supplementation

viii) Degree of compliance with protocol (as evidenced by supervised dosing, self-report of compliance with study medication, self-reported use of vitamin D supplements in addition to study medication, and / or attained 25[OH]D level)

5 Methodology

We will conduct an IPD meta-analysis of raw, individual-level data from each trial, and then summarise the evidence by synthesising the data whilst preserving the randomisation and clustering of patients within studies. The 'PICO' structured question addressed in our project is summarised in Table 1 below.

Table 1. 'PICO' structured question for IPD meta-analysis of trials of vitamin D supplementation for prevention of acute respiratory infection

Population	Males and females of any age and any race/ethnic origin with and without vitamin D deficiency at baseline
Intervention	Supplementation with vitamin D (either vitamin D_3 [cholecalciferol] or vitamin D_2 [ergocalciferol]) administered at any dose with any frequency via any route
Comparator	Placebo or alternate dose of vitamin D
Outcomes	Incidence of ARI and acute exacerbations of asthma and COPD

5.1 Eligibility Criteria

Studies will be eligible to contribute primary data to the proposed IPD meta-analysis if they are:

- Randomised controlled trials of vitamin D supplementation in which data relating to incidence of ARI or exacerbation of asthma / COPD have been prospectively collected using a directed, closed question routinely directed at all participants
- Approved by a research ethics committee

Table 2: Trials to be included in the proposed IPD meta-analysis, by date of publication

Study first author and year of publication	Country	Participants	Mean / median 25(OH)D, baseline	Dose of vitamin D administered	Outcomes	Sample size
Li-Ng 2009	USA	Healthy adults, mean age 59 yr	25.8 ng/ml	2,000 IU/day	URI	162
Urashima 2010	Japan	Healthy schoolchildren aged 6-15 yr	Not reported	1,200 IU/day	URI	334
Manaseki-Holland 2010	Afghanistan	Children with previous pneumonia aged 1-36 mo	Not reported	Single bolus, 100,000 IU	Recurrent LRI	453
Laaksi 2010	Finland	Healthy males aged 18-28 yr	31.5 ng/ml	400 IU/day	ARI	164
Trilok-Kumar 2011	India	Low birthweight infants	36.0 nmol/L	200 IU/day	Hospital admission / death (primary); 'severe morbidity', including ARI (secondary)	2,079
Majak 2011	Poland	Children with asthma aged 5-18 yr	36.1 ng/ml	500 IU/day	Asthma exacerbation	48
Camargo 2012	Mongolia	Healthy schoolchildren, mean age 10 yr	7 ng/ml	300 IU/day	ARI	247
Murdoch 2012	New Zealand	Healthy adults, mean age 47 yr	29 ng/ml	2 x 200,000 IU bolus, 100,000 IU monthly thereafter	URI	322
Manaseki-Holland 2012	Afghanistan	Infants aged 1-11 months	17.2 ng/ml	100,000 IU boluses 3-monthly	LRI	3,046
Lehouck 2012	Belgium	Patients with COPD, mean age 68 yr	20.1 ng/ml	100,000 IU bolus monthly	COPD exacerbation	182
Bergman 2012	Sweden	Adults with antibody deficiency or recurrent ARI	20.6 ng/ml	4,000 IU/day	ARI	140
Rees 2013	USA	Adults aged 45-75 with baseline 25(OH)D >12 ng/ml	25 ng/ml	1,000 IU/day +/- 1200 mg calcium/day	URI	2,259
Marchisio 2013	Italy	Children aged 1-5 yr with recurrent acute otitis media	26 ng/ml	1,000 IU/day	Acute otitis media	116
Yadav 2013	India	Children aged 3-14 yr with asthma	Not reported	60,000 IU/month	Asthma exacerbation	100
Martineau, unpublished	UK	Adults with asthma	20.2 ng/ml	6 x 120,000 IU boluses over one year	URI and asthma exacerbation	250
Martineau, unpublished	UK	Adults with COPD	18.3 ng/ml	6 x 120,000 IU boluses over one year	URI and COPD exacerbation	250
Martineau, unpublished	UK	Older adults and their carers	17.0 ng/ml	6 x 120,000 IU boluses over one year	ARI	240
Urashima, unpublished	Japan	High school students aged 15-18 years	Not reported	2,000 IU/day	Influenza A	247
Mezawa, unpublished	Japan	Children with asthma aged 6-15 years	28.4-32.6 ng/ml	800 IU/day	Asthma exacerbation	89
Grant, unpublished	New Zealand	Pregnant women and their offspring	22 ng/ml (maternal)	1000/2000 IU/day (mothers), 400/800 IU/day (infants)	Acute respiratory infection	266
Neale, Unpublished ARI data	Australia	Healthy adults aged 60-84 at recruitment, mean age 72 years	42 nmol/L	30,000IU per month or 60,000 IU/month	Acute upper respiratory tract infection	644

5.2 Data collection, entry and checking and study quality

Table 3 details the availability of data for each trial included in our consortium. Where necessary, datasets will be re-analysed to identify the proportion of participants experiencing ARI / exacerbations re-defined using diagnostic criteria that are harmonised between trials.

A database will be set up and authors will be allowed to supply data in whatever way convenient to them. All data supplied will be subjected to range and consistency checks. This will ensure that all randomised patients are included; that all non-randomised patients are excluded; that data are as accurate as possible; and that intention-to-treat analysis is performed for all analyses except for that exploring the effect of protocol compliance on trial outcomes (a per-protocol analysis will be employed in this one instance). Any missing data, obvious errors, inconsistencies between variables or outlying values will be queried and rectified as necessary through input from the original authors.

The quality of each study will also be assessed at this stage, in order to evaluate the integrity of the randomisation and follow-up procedure for each trial. The Risk of Bias tool developed by the Cochrane Collaboration will be used to score the quality of each study [10]. In subsequent meta-analysis, sensitivity analyses will be used to examine the robustness of statistical and clinical conclusions to the inclusion / exclusion of trials deemed at high risk of bias, if applicable.

5.3 Study procedures

Procedures for individual studies are documented in original trial reports [11-21] and individual study protocols.

Table 3: Data availability for individual trials included in the proposed meta-analysis

		Li-Ng 2009	Urashima 2010	Manaseki-Holland 2010	Laaksi 2010	Majak 2011	Kumar 2011	Camargo 2012	Murdoch 2012	Manaseki-Holland 2012	Lehouck 2012	Bergman 2012	Rees 2013	Marchisio 2013	Yadav 2013	Martineau ViDiAs	Martineau ViDiCO	Martineau ViDiFlu	Urashima unpublished	Mezawa unpublished	Grant unpublished	Neale
Study characteristics	Dose of vitamin D administered	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Type of vitamin D administered (D_2 vs. D_3)	✓	✓	D2	✓	D3	✓	✓	✓	D2	✓	D3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Frequency of administration	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Duration of administration	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EXT	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Type of randomisation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Participant characteristics	Baseline 25(OH)D	✓	×	×	✓	✓	×	✓	✓	S	✓	✓	✓	✓	×	✓	✓	✓	×	✓	✓	✓
	Age	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Sex	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Race/Ethnicity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Body mass index		✓	✓	✓	✓	EXT	✓	✓	✓	✓	X	✓	✓	×	✓	✓	✓		✓	✓	✓
	Smoking history	✓	NA	NA	✓	✓	NA	NA	✓	NA	✓	✓	✓	NA	NA	✓	✓	✓	NA	NA	✓	✓
	Exposure to air pollution	X	NA	✓	NA	×	×	×	×	✓	×	X	X	✓	✓	EXT	EXT	EXT	NA	NA	x	X
	Exposure to second-hand cigarette smoke	X	NA	✓	NA	×	×	×	×	✓	×	X	X	✓	✓	×	×	×	NA	NA	✓	X
	Use of calcium supplements	✓	NA	✓	✓	×	×	✓	EXT	✓	✓	✓	EXT	✓	×	✓	✓	✓	NA	NA	X	X
	Use of vitamin A supplements	X	NA	S	✓	×	×	✓	EXT	S	×	✓	EXT	✓	×	✓	✓	✓	NA	NA	x	X
	Use of vitamin D supplements in addition to study medication	✓	NA	✓	✓	×	×	✓	✓	✓	✓	✓	EXT	✓	✓	✓	✓	✓	NA	NA	✓	✓
	Vaccination history	✓	✓	S	✓	✓	✓	✓	✓	S	✓	✓	✓	✓	×	✓	✓	✓	×	×	✓	X
	DNA available	×	×	×	X	×	×	×	×	×	✓	✓	(✓)	✓	×	✓	✓	✓	×	×	×	✓
	Asthma diagnosis recorded?	✓	✓	E	✓	✓	×	✓	✓	✓	E	✓	EXT	✓	✓	✓	E	E	×	✓	✓	X
	History of wheeze at baseline? (children)	NA	✓	✓	NA	NA		✓	NA	✓	NA	NA	-	✓	✓	NA	NA	NA	✓	✓	✓	X
	COPD diagnosis recorded?	✓	NA	NA	NA	NA	NA	NA	✓	NA	✓	✓	EXT	✓	NA	E	✓	E	NA	NA	NA	X
	Compliance with protocol recorded?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EXT	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Follow-up 25(OH)D recorded?	✓	NA	✓	✓	✓	S	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	NA	✓	✓	✓
Event characteristics	Date of onset of ARI / exacerbation recorded?	✓	✓	✓	✓	×	S	×	✓	✓	✓	✓	EXT	✓	×	✓	✓	✓	✓	✓	✓	X
	Asthma exacerbation recorded?	✓	✓	NA	✓	✓	X	×	✓	NA	NA	✓	EXT	✓	✓	✓	NA	NA	×	✓	✓	X
	COPD exacerbation recorded?	✓	NA	NA	NA	NA	NA	NA	✓	NA	✓	✓	EXT	✓	NA	NA	✓	NA	NA	NA	NA	X
	N (%) with ≥1 event recorded?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Health service use for event recorded?	✓	×	EXT	✓	✓	✓	×	✓	EXT	✓	×	✓	✓	✓	✓	✓	✓	×	✓	✓	X
	Hospitalisations recorded?	✓	✓	✓	✓	✓	✓	×	EXT	✓	✓	✓	EXT	✓	✓	✓	✓	✓	✓	✓	✓	X
	Death recorded?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EXT	NA	✓	✓	✓	✓	✓	✓	NA	✓
	Rates of drop-out from the study recorded?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EXT	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Medication use for event recorded (antibiotics / corticosteroids) ?	✓	×	EXT	✓	✓	✓	×	✓	EXT	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	X
	Absence from work due to event recorded?	✓	✓	NA	✓	✓	NA	×	✓	NA	×	✓	×	X	✓	✓	✓	✓	✓	✓	x	X
	Hypercalcaemia recorded?	✓	NA	x	NA	X	×	×	✓	x	✓	✓	EXT	NA	×	✓	✓	✓	NA	✓	✓	✓

'Event' = acute respiratory infection or exacerbation of asthma / COPD; ✓, present in all; ×, absent in all; E, patients with this diagnosis were excluded from the trial; S, available for a sub-set of participants; EXT can be extracted; NA, not applicable (e.g. diagnosis of COPD or absence from work are not applicable in infants).

5.4 Statistical analysis

5.4.1 Summarising overall effect of vitamin D supplementation

Our IPD meta-analytical approach will follow existing guidelines [22]. We will adopt a three-tiered approach to inclusion of different studies, categorised as follows:

Tier 1 studies: Vitamin D RCTs that have primary outcome of ARI or asthma/COPD exacerbation listed in the study protocol.

Tier 2 studies: Vitamin D RCTs that have ARI or asthma /COPD exacerbation as a secondary efficacy outcome, with data on this outcome collected prospectively using a directed, closed question routinely directed at all participants.

Tier 3 studies: Vitamin D RCTs that have ARI or asthma /COPD exacerbation as a safety outcome, with data on this outcome collected prospectively using a directed, closed question routinely directed at all participants.

Separate analyses will be conducted as follows:

1. Analysis incorporating data from tier 1 studies only

2. Analysis incorporating data from tier 1/2 studies

3. Analysis incorporating data from tier 1/2/3 studies.

Analyses 1 and 2 above will be the major analyses, and resource will be focused on these analyses in the first instance. The volume of studies in analysis 3 is uncertain.

For each analysis, we will include all patients ever randomised (except in the one instance where a sub-group analysis of participants completing studies per-protocol is proposed) and will base analysis on the intention-to-treat principle. Results will be displayed graphically using odds ratio plots. Analyses of the effectiveness of vitamin D supplementation *vs.* placebo will be performed on the combined study population for the following outcomes:

a) Incidence of ARI, incorporating URI (including colds, influenza-like illness, ear infections, acute rhinosinusitis) and LRI (including pneumonia); URI and LRI may be analysed separately or together.

b) Incidence of asthma exacerbation

c) Incidence of COPD exacerbation

d) Health service use

e) Medication use (including antimicrobials for ARI treatment and oral corticosteroids for exacerbation of asthma / COPD)

f) Work / school absence

g) Adverse events (including hypercalcaemia, drop-out rates, hospitalisations / serious adverse events, mortality)

Incidence may be expressed in several different ways, including the proportion of participants experiencing at least one event, time to first event, time to second event or overall event rate. Events will be categorised according to season of onset (e.g. ‘Winter

URI'). Season will be defined by quarter of the year. The incidence of clinical outcomes will be compared for different time windows post-randomisation (e.g. <1 month, 1-2 months).

Case definitions of clinical events will be established by the consortium (e.g. 'Pneumonia 1: consolidation on chest radiograph + treated with antibiotics')

Initially, all studies will be reanalysed separately; the original authors will then be asked to confirm accuracy of this reanalysis, and any discrepancies will be resolved. Then, for each outcome separately, we will perform both a one-step and a two-step IPD meta-analysis to obtain the pooled intervention effect. The one-step approach analyses the IPD from all studies simultaneously, while accounting for the clustering of patients within studies. In contrast, the two-step approach first estimates the intervention effect from the IPD in each study separately, and then pools them using a conventional meta-analysis of the intervention effect estimates obtained. Given the heterogeneity identified in meta-analyses of aggregate data [8,9] we also expect to observe significant heterogeneity in the IPD meta-analyses. Thus, we will use a random effects meta-analysis approach, which allows for between-study heterogeneity in intervention effect. If no between-study heterogeneity is found to exist, this model will revert to a fixed effect model. Heterogeneity will be summarised using the I^2 statistic (which provides the proportion of total variability that is due to between-study heterogeneity) and the estimated between-study variance ('tau-squared').

For binary outcomes we will synthesise odds ratios with the binomial nature suitably modelled (e.g. using a one-step logistic regression adjusting for clustering), as recommended by The Cochrane Collaboration. At the study level, the random effects to account for heterogeneity will be assumed to be normally distributed, allowing us to estimate the average intervention effect and its confidence interval as well as the between-study variance ('tau-squared'). To reveal the impact of heterogeneity more clearly, we will also calculate a 95% confidence interval for the effect of vitamin D supplementation when applied in an individual clinical setting [23].

5.4.2 Examining heterogeneity and potential sub-group effects

To consider the causes of heterogeneity and factors that may modify the effects of vitamin D supplementation, we will perform pre-specified sub-group analyses according to:

i) Baseline vitamin D status

ii) Age

iii) Race/Ethnic origin

iv) Presence *vs.* absence of respiratory comorbidity: asthma vs. COPD vs. neither condition reported

v) Vitamin D dose administered

vi) Frequency of vitamin D administration

vii) Duration of supplementation

viii) Degree of compliance with protocol (as evidenced by supervised dosing, self-report of compliance with study medication, self-reported use of vitamin D supplements in addition to study medication, and / or attained 25[OH]D level)

Sub-group analyses, if not carefully planned, can lead to misleading results e.g. due to the play of chance with multiple testing [24]. Thus caution will be used in interpretation of the

collective set of sub-group results, and adjustment for multiple testing considered as necessary. However, we reiterate here that our IPD meta-analysis will increase the power to detect genuine sub-group effects (treatment-covariate interactions) and will also allow us to examine if there is consistency in the sub-group effect from study to study, rather than being a chance finding in a single study for example.

Examination of sub-group effects will be undertaken by extending the one-stage meta-analysis framework to include treatment-covariate interaction terms, which provide the change in intervention effect for a 1-unit change in the covariate. In conducting this analysis, we will ensure that we estimate the pooled within-trial interaction of interest separately from the across-trial (meta-regression) interaction. Between-study heterogeneity in the within-trial treatment-covariate interaction will also be measured, summarised and, if necessary, accounted for in the analysis.

Continuous covariates, such as baseline serum 25(OH)D concentration and age, will be analysed on their continuous scale, and not categorised. However, to translate the results clinically, after the analysis we will report the effect of the covariate-treatment interaction on the intervention effect at clinically relevant covariate values, e.g. for vitamin D status, reporting effects of supplementation in sub-groups defined by widely used clinical cut-offs of serum 25(OH)D concentration defining profound deficiency (<25 nmol/L), moderate deficiency (25-49.99 nmol/L), mild deficiency (50-74.99 nmol/L) and sufficiency (≥ 75 nmol/L).

5.4.3 Exploration of sources of bias, unavailable data and publication bias

For the analyses detailed above, we will explore the potential for, and possible impact of, both publication bias and unavailable data, according to recent guidelines [25]. For each analysis containing 10 or more studies the likelihood of publication bias will be investigated through the construction of contour-enhanced funnel plots and appropriate statistical tests for ‘small-study effects’ [26]; that is, the tendency for smaller studies to provide more positive findings. We recognise that, especially where heterogeneity exists, publication bias may be one of a number of reasons for any small study effects identified. The restriction of 10 studies is due to the low power of identifying small study effects with few studies [27].

In addition, if at some point in the future any eligible studies are identified for which IPD are not provided to us, we will seek to extract suitable aggregate data from their study publications. Where possible we will then, using the two-step meta-analysis framework, combine the IPD trials with the aggregate data from other trials using suitable statistical methods, to examine if conclusions change by the inclusion of additional trials. If the inclusion of studies lacking IPD seems to have an important statistical or clinical impact on the findings of our analysis, we will compare the characteristics of the studies with IPD and of those without to see if there are any key differences (such as in their quality, follow-up length, statistical methods, etc). We recognise, however, that this approach is likely to only be achievable when examining the overall treatment effect, and our main IPD analyses of the sub-group effects are unlikely to be able to include any suitable aggregate data for sub-group effects from non-IPD studies (indeed this a key reason for undertaking IPD meta-analysis in the first place).

5.4.4 Sample size and power

We are well powered to detect clinically important sub-group effects in individuals with different baseline characteristics and in those receiving different dosing regimens of vitamin D : using simulation we have confirmed that 10 studies each of 200 participants would be sufficient to detect an interaction odds ratio of 0.5 with 80% power at the 5% significance level, assuming an odds ratio of 0.85 for the effect of vitamin D in the absence of the effect modifier, an odds ratio for the main effect of the treatment modifier of 1.5, and a one-year risk of ARI of 0.5 in the absence of vitamin D treatment or the effect modifier, and further assuming that the population proportion of people with the effect modifier varied between studies with a beta distribution with mean 0.5, 95% CI 0.1-0.9. Data on baseline vitamin D status are available for participants in 15/21 trials: we are therefore well powered to detect any clinically significant differential effects of vitamin D supplementation in patients who are vitamin D deficient vs. replete at baseline.

6 Ethics

Individual trials contributing primary data to this IPD meta-analysis will all be approved by Research Ethics Committees in the countries where they took place. All data from primary trials will be anonymised on importing into our database.

7 Indemnity

This study will be sponsored by Queen Mary, University of London, who will indemnify the study.

8 Dissemination of findings and manuscript authorship

Findings of this study will be presented at scientific conferences and submitted for publication in peer-reviewed journals. Any publication of results of this IPD meta-analysis will include one PI for each trial included in that meta-analysis as a named co-author. Other investigators named on this protocol who have made a substantive contribution to the IPD meta-analysis, but who are not PIs for individual studies, may also be named co-authors on manuscripts arising from this study.

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