Vitamin D supplementation to prevent acute respiratory infection: Updated systematic review and meta-analysis of aggregate data

Study Protocol

Version 1

Dated: 13\textsuperscript{th} April 2020
**Full Title**

Vitamin D supplementation to prevent acute respiratory infections: Updated systematic review and meta-analysis of aggregate data

**Investigators**

Prof Adrian R Martineau (PI)
Centre for Primary Care and Public Health
Barts and The London School of Medicine and Dentistry
Queen Mary University of London
58 Turner St, London E1 2AB, UK

Email: a.martineau@qmul.ac.uk

Dr David Jolliffe
Blizard Institute
Barts and the London School of Medicine and Dentistry
Queen Mary University of London
4 Newark Street, London E1 2AT, UK

Prof John F Aloia
Director, Bone Mineral Research Center
Winthrop University Hospital
222 Station Plaza North, Suite 510
Mineola, NY 11501, USA

Dr Peter Bergman
Department of Laboratory Medicine
Karolinska Institute
SE-171 77 Stockholm, Sweden

Prof Carlos A Camargo Jr
Department of Emergency Medicine, Massachusetts General Hospital,
Harvard Medical School,
326 Cambridge St, Suite 410
Boston, MA 02114, USA

Prof Camilla Trab Damsgaard
Department of Nutrition, Exercise and Sports,
University of Copenhagen,
Rolighedsvej 26, 1958 Frederiksberg C, Denmark

Prof Ganmaa Davaasambuu
Harvard T.H. Chan School of Public Health,
677 Huntington Avenue,
Boston MA 02115, USA

Prof Susanna Esposito
Department of Pathophysiology and Transplantation
Università degli Studi di Milano
Via Commenda 9, 20122 Milano, Italy

Dr Clare Gilham
Department of Non-communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, UK
Dr Cameron Grant  
Department of Paediatrics  
Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Wellesley St, Auckland 1142, New Zealand

Prof Christopher J Griffiths  
Centre for Primary Care and Public Health  
Barts and The London School of Medicine and Dentistry  
Queen Mary University of London  
58 Turner St, London E1 2AB, UK

Prof Piyush Gupta  
University College of Medical Sciences,  
Guru Teg Bahadur Hospital,  
Dilshad Garden, Delhi 110 095, India

Prof Anna Maria Hibbs  
Rainbow Babies and Children’s Hospital, Division of Neonatology, 11100 Euclid Ave,  
Ste 3100, Cleveland, OH 44106, USA

Prof Wim Janssens  
Universitair ziekenhuis Leuven  
Herestraat 49  
3000 Leuven, Belgium

Dr Vaman Khadilkar  
Hirabai Cowasji Jehangir Medical Research Institute  
Block 5, Lower Ground Floor, Jehangir Hospital  
32, Sassoon Road, Pune 411001, India

Dr Ilkka Laaksi  
Tampere School of Public Health  
University of Tampere  
Medisiinarinkatu 3, Tampere, Finland 33014

Prof Margaret T. Lee  
Division of Pediatric Hematology/Oncology/Stem Cell Transplantation, Columbia University Medical Centre,  
3959 Broadway, New York, NY 10032, USA

Prof Mark Loeb  
McMaster University,  
Department of Pathology and Molecular Medicine,  
1280 Main Street West, HSC-2N16,  
Hamilton ON, L8S 4K1, Canada

Dr Semira Manaseki-Holland  
School of Health and Population Sciences  
College of Medical and Dental Sciences  
University of Birmingham  
Birmingham B15 2TT, UK

Dr Hidetoshi Mezawa  
Division of Molecular Epidemiology,
Jikei University School of Medicine,  
Nishi-shimbashi 3-25-8, Minato-ku,  
Tokyo 105-8461, Japan

Prof David Murdoch  
Department of Pathology  
University of Otago  
Christchurch 8140, New Zealand

Dr Rachel Neale  
QIMR Berghofer Medical Research Institute  
Queensland  
Australia

Prof Julian Peto  
Department of Non-communicable Disease Epidemiology,  
London School of Hygiene & Tropical Medicine,  
Keppel St, London WC1E 7HT, UK

Dr Judy R Rees  
Dartmouth-Hitchcock Medical Centre  
1 Medical Centre Drive  
HB 7927, Lebanon  
NH 03756, USA

Prof Robert Scragg  
School of Population Health,  
Faculty of Medical and Health Sciences,  
University of Auckland,  
Private Bag 92019, Auckland Mail Centre 1142, New Zealand

Dr Yoshiki Shimizu  
FANCL Research Institute, FANCL Corporation,  
12-13 Kamishinano, Totsuka-ku,  
Yokohama, Kanagawa 244-0806, Japan

Dr John Sluyter  
School of Population Health,  
Faculty of Medical and Health Sciences,  
University of Auckland,  
Private Bag 92019, Auckland Mail Centre 1142, New Zealand

Dr Jenni Rosendahl  
Children’s Hospital, Pediatric Research Center,  
University of Helsinki and Helsinki University Hospital,  
Helsinki, Finland

Prof Iwona Stelmach  
Department of Pediatrics and Allergy  
Medical University of Lodz,  
Aleja Tadeusza Kościuszki 4, Lodz, Poland

Dr Geeta Trilok-Kumar  
Institute of Home Economics  
University of Delhi  
F-4 Haus Khas Enclave  
New Delhi - 110016, India
Prof Mitsuyoshi Urashima
Division of Molecular Epidemiology,
Jikei University School of Medicine,
Nishi-shimbashi 3-25-8, Minato-ku,
Tokyo 105-8461, Japan

Dr Madhu Yadav
Department of Pediatrics
Rao Tula Ram Memorial Hospital,
New Delhi, India
# TABLE OF CONTENTS

1. Glossary of terms and abbreviations ................................................................. 7
2. Summary .................................................................................................................. 8
3. Introduction ............................................................................................................ 10
4. Aims and objectives .............................................................................................. 10
5. Methodology .......................................................................................................... 11
   5.1 Eligibility Criteria ............................................................................................ 11
   5.2 Data collection, entry and checking and study quality .................................... 15
   5.3 Study procedures ............................................................................................. 15
   5.4 Statistical analysis ........................................................................................... 15
       5.4.1 Summarising overall effect of vitamin D supplementation ...................... 15
       5.4.2 Examining heterogeneity and potential sub-group effects ....................... 16
       5.4.3 Exploration of sources of bias, unavailable data and publication bias .... 16
6. Ethics ..................................................................................................................... 17
7. Dissemination of findings and manuscript authorship ......................................... 17
8. REFERENCES ......................................................................................................... 18
# Glossary of terms and abbreviations

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>ARI</td>
<td>Acute Respiratory Infection</td>
</tr>
<tr>
<td>URI</td>
<td>Upper Respiratory Infection</td>
</tr>
<tr>
<td>LRI</td>
<td>Lower Respiratory Infection</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health Care and Excellence</td>
</tr>
<tr>
<td>Participant</td>
<td>An individual who takes part in a randomised clinical trial</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>URI</td>
<td>Upper Respiratory Infection</td>
</tr>
<tr>
<td>ADMA</td>
<td>Aggregate Data Meta-analysis</td>
</tr>
<tr>
<td>IPDMA</td>
<td>Individual Participant Data Meta-analysis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>COVID-19</td>
<td>Coronavirus disease 2019</td>
</tr>
<tr>
<td>PICO</td>
<td>Population Intervention Comparator Outcome</td>
</tr>
<tr>
<td>RIDT</td>
<td>Rapid Influenza Diagnostic Test</td>
</tr>
</tbody>
</table>
## Summary

<table>
<thead>
<tr>
<th><strong>Short Title</strong></th>
<th>Vitamin D Supplementation to Prevent Acute Respiratory Infection (ARI): Aggregate Data Meta-Analysis of Randomised Controlled Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methodology</strong></td>
<td>Aggregate data meta-analysis of randomised controlled trials</td>
</tr>
<tr>
<td><strong>Research Site</strong></td>
<td>Centre for Primary Care and Public Health, Barts and The London School of Medicine and Dentistry</td>
</tr>
<tr>
<td><strong>Primary Objective</strong></td>
<td>To determine whether vitamin D supplementation influences the proportion of participants experiencing at least one ARI</td>
</tr>
</tbody>
</table>
| **Secondary Objectives** | 1. To determine whether vitamin D supplementation influences the proportion of participants experiencing at least one ARI, in sub-groups categorised by baseline vitamin D status, size of vitamin D dose administered, frequency of vitamin D administration and duration of supplementation  
2. To determine whether there is an effect of allocation to vitamin D vs. placebo on the following secondary outcomes:  
   - Proportion with at least one Upper Respiratory Infection (URI)  
   - Proportion with at least one Lower Respiratory Infection (LRI)  
   - Proportion with at least one school/work absence due to ARI  
   - Proportion taking at least one course of antibiotics for ARI  
   - Proportion with at least one hospital admission or emergency department attendance due to ARI  
   - Proportion with at least one serious adverse event of any cause  
   - Proportion dying of ARI  
   - Proportion dying of any cause  
   - Proportion experiencing at least one episode of hypercalcaemia  
   - Proportion experiencing at least one episode of renal stones |
| **Number of Trials and Participants** | 38 trials with 29,476 randomised participants                                                                               |
| **Main Inclusion Criteria** | Studies will be eligible to contribute primary data to this meta-analysis if they are:  
   - Randomised controlled trials of vitamin D or calcidiol in which data relating to incidence of ARI have been prospectively collected as an efficacy outcome  
   - Approved by a research ethics committee |
| **Statistical Methodology and Analysis (if applicable)** | The effectiveness of vitamin D supplementation vs. control will be assessed by calculation of odds ratios and standard errors from aggregate data for each outcome, within each trial, and subsequent meta-analysis using a random effects model to produce a pooled estimate of effect size and a measure of trial heterogeneity. |
Heterogeneity will be interrogated by sub-group analysis of trial-level characteristics and by multivariate meta-regression analysis, to produce an adjusted estimate of effect size and a p value for interaction.
3 Introduction

Acute respiratory infections (ARI) are major causes of morbidity and health service use that impose significant human and economic costs (1). 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 (2-4). These findings have led to a significant number of randomised controlled trials (RCTs) of vitamin D supplementation for protection against ARI, which our group meta-analysed in 2017 (5). Twenty-five RCTs provided individual participant data (IPD) from n=10,933 subjects for this analysis, which showed that vitamin D supplementation reduced the odds of having ≥1 ARI in all participants (adjusted odds ratio [aOR] 0.88; 95% confidence interval [CI] 0.81 to 0.96) and that this protective effect was greater in participants with profound vitamin D deficiency at baseline (serum 25[OH]D concentration <25 nmol/L; aOR 0.58, 95% CI 0.40 to 0.82). Our analysis also found that within profoundly deficient participants, the protective effects of vitamin D were seen in those administered a daily or weekly dosing regimen (aOR 0.30, 95% CI 0.17 to 0.53), but not in those who received a bolus monthly or two-monthly regimen of vitamin D supplementation (aOR 0.82, 95% CI 0.51 to 1.33). Taken together, these subgroup findings point towards a dosing strategy for improved ARI protection, however our analysis did not offer insights into an optimal vitamin D dose size or duration of supplementation.

In the past 3 years a significant number of new RCTs have come into the fold, including two vitamin D “mega trials” (n>5,000 participants). In light of the amount of new data and their potential to address any attendant power issues we may have encountered with previous sub-group analyses, together with the potential relevance of this research theme to the emergent COVID-19 pandemic, we endeavoured to update our meta-analysis by synthesis of aggregate level data in order to provide a rapid answer to the following question:

“Does vitamin D supplementation influence the proportion of participants who experience acute respiratory infections?”.

Our consortium comprises principal investigators from 38 primary trials of vitamin D supplementation for prevention of ARI, giving us access to aggregate data from over 29,000 participants. This approach gives us adequate power to generate valid, reliable answers to the questions above.

4 Aims and objectives

The primary aim of the proposed project is to determine whether vitamin D supplementation influences the proportion of participants who experience at least one ARI.
Our secondary aims are 1. To determine whether vitamin D supplementation influences the proportion of participants who experience at least one ARI, in sub-groups categorised according to the following potential effect-modifiers:

i) Baseline vitamin D status
ii) Size of vitamin D dose administered
iii) Frequency of vitamin D administration
iv) Duration of supplementation

and 2. To determine whether vitamin D supplementation influences the following efficacy or safety outcomes:

i) Proportion with at least one Upper Respiratory Infection (URI)
ii) Proportion with at least one Lower Respiratory Infection (LRI)
iii) Proportion with at least one school/work absence due to ARI
iv) Proportion taking at least one course of antibiotics for ARI
v) Proportion with at least one hospital admission or emergency department attendance due to ARI
vi) Proportion with at least one serious adverse event of any cause
vii) Proportion dying of ARI
viii) Proportion dying of any cause
ix) Proportion experiencing at least one episode of hypercalcaemia
x) Proportion experiencing at least one episode of renal stones

5 Methodology

We will conduct an aggregate data meta-analysis of proportional 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 aggregate data meta-analysis of trials of vitamin D supplementation for prevention of acute respiratory infection

<table>
<thead>
<tr>
<th>Population</th>
<th>Males and females of any age and any race/ethnic origin with and without vitamin D deficiency at baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>Supplementation with vitamin D (either vitamin D₃ [cholecalciferol], vitamin D₂ [ergocalciferol] or 25-hydroxyvitamin D [calcidiol]) administered at any dose with any frequency via any route</td>
</tr>
<tr>
<td>Comparator</td>
<td>Placebo or alternate dose of vitamin D</td>
</tr>
<tr>
<td>Primary Outcome</td>
<td>Proportion of participants experiencing one or more ARI</td>
</tr>
</tbody>
</table>

5.1 Eligibility Criteria

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

- Randomised controlled trials of vitamin D supplementation in which data relating to incidence of ARI 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 aggregate data meta-analysis, by date of publication

<table>
<thead>
<tr>
<th>Study first author, year</th>
<th>Setting</th>
<th>Participants</th>
<th>Mean baseline 25(OH)D, nmol/L (s.d.)</th>
<th>Baseline 25(OH)D &lt;25 nmol/L (%)</th>
<th>Intervention/control</th>
<th>Oral dose of vitamin D₃, intervention arm</th>
<th>Study duration</th>
<th>ARI definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li-Ng 2009 (6)</td>
<td>USA</td>
<td>Healthy adults</td>
<td>63.7 (25.5)</td>
<td>3/150 (2.0)</td>
<td>84.78</td>
<td>50 µg daily</td>
<td>3 mo</td>
<td>URI: ≥2 URI symptoms in absence of allergy symptoms</td>
</tr>
<tr>
<td>Urashima 2010 (7)</td>
<td>Japan</td>
<td>Schoolchildren</td>
<td>Not determined</td>
<td>--</td>
<td>217:213</td>
<td>30 µg daily</td>
<td>4 mo</td>
<td>URI: influenza A/B diagnosed by RIDT or RIDT-negative ILI</td>
</tr>
<tr>
<td>Manaseki-Holland 2010 (8)</td>
<td>Afghanistan</td>
<td>Pre-school children with pneumonia</td>
<td>Not determined</td>
<td>--</td>
<td>224:229</td>
<td>2.5 mg bolus once</td>
<td>3 mo</td>
<td>LRI: repeat episode of pneumonia – age-specific tachypnoea without wheeze</td>
</tr>
<tr>
<td>Laaksi 2010 (9)</td>
<td>Finland</td>
<td>Military conscripts</td>
<td>75.9 (18.7)</td>
<td>0/73 (0.0)</td>
<td>80:84</td>
<td>10 µg daily</td>
<td>6 mo</td>
<td>ARI: medical record diagnosis</td>
</tr>
<tr>
<td>Majak 2011 (10)</td>
<td>Poland</td>
<td>Children with asthma</td>
<td>88.9 (38.2)</td>
<td>0/48 (0.0)</td>
<td>24:24</td>
<td>12.5 µg daily</td>
<td>6 mo</td>
<td>ARI: self-report</td>
</tr>
<tr>
<td>Trilok-Kumar 2011 (11)</td>
<td>India</td>
<td>Low birthweight infants</td>
<td>Not determined</td>
<td>Not determined</td>
<td>1,039:1,040</td>
<td>35 µg weekly</td>
<td>6 mo</td>
<td>ARI: medical record diagnosis of events causing hospitalisation</td>
</tr>
<tr>
<td>Lehouck 2012(12)</td>
<td>Belgium</td>
<td>Adults with COPD</td>
<td>49.8 (29.2)</td>
<td>31/182 (17.0)</td>
<td>91.91</td>
<td>2.5 mg bolus monthly</td>
<td>1 yr</td>
<td>URI: self-report</td>
</tr>
<tr>
<td>Manaseki-Holland 2012 (13)</td>
<td>Afghanistan</td>
<td>Infants</td>
<td>Not determined</td>
<td>Not determined</td>
<td>1524:1522</td>
<td>2.5 mg bolus 3-monthly</td>
<td>1.5 yr</td>
<td>LRI: pneumonia confirmed by chest radiograph</td>
</tr>
<tr>
<td>Camargo 2012 (14)</td>
<td>Mongolia</td>
<td>3rd/4th grade schoolchildren</td>
<td>18.9 (9.7)</td>
<td>192/245 (78.4)</td>
<td>143:104</td>
<td>7.5 µg daily</td>
<td>7 wk</td>
<td>ARI: parent-reported 'chest infections or colds'</td>
</tr>
<tr>
<td>Murdoch 2012 (15)</td>
<td>New Zealand</td>
<td>Healthy adults</td>
<td>72.1 (22.1)</td>
<td>5/322 (1.6)</td>
<td>161:161</td>
<td>2 x 5 mg bolus monthly then 2.5 mg bolus monthly</td>
<td>1.5 yr</td>
<td>URI: assessed with symptom score</td>
</tr>
<tr>
<td>Bergman 2012 (16)</td>
<td>Sweden</td>
<td>Adults with increased susceptibility to ARI</td>
<td>49.3 (23.2)</td>
<td>15/131 (11.45)</td>
<td>70:70</td>
<td>100 µg daily</td>
<td>1 yr</td>
<td>URI: assessed with symptom score</td>
</tr>
<tr>
<td>Marchisio 2013 (17)</td>
<td>Italy</td>
<td>Children with recurrent acute otitis media</td>
<td>65.3 (17.3)</td>
<td>2/116 (1.7)</td>
<td>58:58</td>
<td>25 µg daily</td>
<td>6 mo</td>
<td>URI: doctor-diagnosed acute otitis media</td>
</tr>
<tr>
<td>Rees 2013 (18)</td>
<td>USA</td>
<td>Adults with previous colorectal adenoma</td>
<td>62.5 (21.3)</td>
<td>0/759 (0.0)</td>
<td>399:360</td>
<td>25 µg daily</td>
<td>13 mo (average)</td>
<td>URI: assessed from daily symptom diary</td>
</tr>
<tr>
<td>Tran 2014 (19)</td>
<td>Australia</td>
<td>Healthy older adults</td>
<td>41.7 (13.5)</td>
<td>66/643 (10.3)</td>
<td>430:214</td>
<td>0.75 mg bolus vs. 1.5 mg bolus monthly</td>
<td>1 yr</td>
<td>URI: self-reported cold</td>
</tr>
<tr>
<td>Goodall 2014 (20)</td>
<td>Canada</td>
<td>Healthy university students</td>
<td>Not determined</td>
<td>--</td>
<td>300:300</td>
<td>0.25 mg weekly (factorial with gargling)</td>
<td>8 wk</td>
<td>URI: self-reported cold</td>
</tr>
<tr>
<td>Urashima 2014 (21)</td>
<td>Japan</td>
<td>High school students</td>
<td>Not determined</td>
<td>--</td>
<td>148:99</td>
<td>50 µg daily</td>
<td>2 mo</td>
<td>URI: influenza A diagnosed by RIDT or RIDT-negative ILI</td>
</tr>
<tr>
<td>Grant 2014 (22)</td>
<td>New Zealand</td>
<td>Pregnant women and offspring</td>
<td>54.8 (25.8)</td>
<td>30/200 (15.0)</td>
<td>173:87 (mothers 164:85 (offspring)</td>
<td>Mothers: 25 µg vs. 50 µg daily Infants: 10 µg vs. 20 µg daily</td>
<td>9 mo (3 mo in pregnancy + 6 mo in infancy)</td>
<td>ARI: doctor-diagnosed ARI precipitating primary care consult</td>
</tr>
<tr>
<td>Martineau 2015a (23)</td>
<td>UK</td>
<td>Adults with COPD</td>
<td>46.1 (25.7)</td>
<td>50/240 (20.8)</td>
<td>122:118</td>
<td>3 mg bolus 2-monthly</td>
<td>1 yr</td>
<td>URI: assessed from daily symptom diary</td>
</tr>
<tr>
<td>Martineau 2015b (24)</td>
<td>UK</td>
<td>Adults with asthma</td>
<td>49.6 (24.7)</td>
<td>36/250 (14.4)</td>
<td>125:125</td>
<td>3 mg bolus 2-monthly</td>
<td>1 yr</td>
<td>URI: assessed from daily symptom diary</td>
</tr>
<tr>
<td>Martineau 2015c (25)</td>
<td>UK</td>
<td>Older adults and their carers</td>
<td>42.9 (23.0)</td>
<td>60/240 (25.0)</td>
<td>137:103</td>
<td>Older adults: 2.4 mg bolus 2-monthly + 10 µg daily Carers: 3 mg 2-monthly</td>
<td>1 yr</td>
<td>URI &amp; LRI, both assessed from daily symptom diary</td>
</tr>
<tr>
<td>Study first author, year</td>
<td>Setting</td>
<td>Participants</td>
<td>Mean baseline 25(OH)D, nmol/L (s.d.)</td>
<td>Baseline 25(OH)D (&lt;25 nmol/L (%))</td>
<td>Intervention: control</td>
<td>Oral dose of vitamin D₃ intervention arm</td>
<td>Study duration</td>
<td>ARI definition</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>--------------</td>
<td>------------------------------------</td>
<td>---------------------------------</td>
<td>----------------------</td>
<td>----------------------------------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Simpson 2015 (26)</td>
<td>Australia</td>
<td>Healthy adults</td>
<td>67.9 (23.0)</td>
<td>0/23 (0.0)</td>
<td>18:16</td>
<td>0.5 mg weekly</td>
<td>17 wk</td>
<td>ARI assessed with symptom score</td>
</tr>
<tr>
<td>Dubnov-Raz 2015 (27)</td>
<td>Israel</td>
<td>Adolescent swimmers with vitamin D deficiency</td>
<td>60.4 (11.9)</td>
<td>0/54 (0.0)</td>
<td>27:27</td>
<td>50 µg daily</td>
<td>12 wk</td>
<td>URI assessed with symptom score</td>
</tr>
<tr>
<td>Denlinger 2016 (28)</td>
<td>USA</td>
<td>Adults with asthma</td>
<td>47.0 (16.9)</td>
<td>0/55 (13.5)</td>
<td>201:207</td>
<td>2.5 mg bolus then 100 µg daily</td>
<td>28 wk</td>
<td>URI assessed with symptom score</td>
</tr>
<tr>
<td>Tachimoto 2015 (29)</td>
<td>Japan</td>
<td>Children with asthma</td>
<td>74.9 (24.6)</td>
<td>1/89 (1.1)</td>
<td>54:35</td>
<td>20 µg daily, first 2 mo.</td>
<td>6 mo</td>
<td>URI: assessed with symptom score</td>
</tr>
<tr>
<td>Ginde 2016 (30)</td>
<td>USA</td>
<td>Institutionalised older adults</td>
<td>67.3 (22.7)</td>
<td>0/1207 (11.2)</td>
<td>55:52</td>
<td>2.5 mg bolus monthly + ≤25 µg per day equivalent</td>
<td>1 yr</td>
<td>ARI: medical record diagnosis</td>
</tr>
<tr>
<td>Gupta 2016 (31)</td>
<td>India</td>
<td>Children with pneumonia</td>
<td>--</td>
<td>--</td>
<td>162:162</td>
<td>2.5 mg bolus, single dose</td>
<td>6 mo</td>
<td>Physician confirmed recurrent pneumonia</td>
</tr>
<tr>
<td>Arihiro 2018 (32)</td>
<td>Japan</td>
<td>Adults with diagnosis of ulcerative colitis or Crohn's disease</td>
<td>58.6 (22.0)</td>
<td>--</td>
<td>108:115</td>
<td>12.5 µg daily</td>
<td>6 mo</td>
<td>Lab confirmed influenza; physician confirmed URI</td>
</tr>
<tr>
<td>Hibbs 2018 (33)</td>
<td>USA</td>
<td>Healthy infants</td>
<td>Median (IQR): 47.7 (38.4-69.9) intervention; 52.4 (42.4-62.4) control</td>
<td>--</td>
<td>153:147</td>
<td>10 µg daily</td>
<td>1 yr</td>
<td>ARI: self-reported URI/LRI</td>
</tr>
<tr>
<td>Lee 2018 (34)</td>
<td>USA</td>
<td>Sickle cell disease</td>
<td>35.7 (--</td>
<td>--</td>
<td>31:31</td>
<td>2.5 mg bolus monthly</td>
<td>1 yr</td>
<td>Self-reported respiratory events, including ARI</td>
</tr>
<tr>
<td>Loeb 2018 (35)</td>
<td>Vietnam</td>
<td>Healthy children and adolescents</td>
<td>65.5 (16.8)</td>
<td>6/1300 (0.4)</td>
<td>650:650</td>
<td>50 µg weekly</td>
<td>8 mo</td>
<td>Lab confirmed influenza; lab confirmed non-influenza respiratory virus</td>
</tr>
<tr>
<td>Rosendahl 2018 (36)</td>
<td>Finland</td>
<td>Healthy infants</td>
<td>81.5 (25.9)</td>
<td>--</td>
<td>495:492</td>
<td>30 µg daily</td>
<td>2 yrs</td>
<td>Parent reported infections, including ARI</td>
</tr>
<tr>
<td>Shimizu 2018 (37)</td>
<td>Japan</td>
<td>Healthy adults</td>
<td>48.9 (13.5)</td>
<td>--</td>
<td>126:126</td>
<td>10 µg daily</td>
<td>4 mo</td>
<td>URI: self-reported</td>
</tr>
<tr>
<td>Aloia 2019 (38)</td>
<td>USA</td>
<td>Healthy older adults</td>
<td>54.4 (16.7)</td>
<td>--</td>
<td>130:130</td>
<td>50 µg daily</td>
<td>3 mo</td>
<td>ARI: self-reported cold/flu</td>
</tr>
<tr>
<td>Camargo 2019 (39)</td>
<td>New Zealand</td>
<td>Healthy older adults</td>
<td>63.0 (24.0)</td>
<td>--</td>
<td>2558:2552</td>
<td>5 mg bolus loading dose; then 2.5 mg bolus monthly</td>
<td>3 yrs</td>
<td>ARI: self-reported cold/flu</td>
</tr>
<tr>
<td>Hauger 2019 (40)</td>
<td>Denmark</td>
<td>Healthy children</td>
<td>56.7 (12.3)</td>
<td>--</td>
<td>40:38:41</td>
<td>20 µg /10 µg daily</td>
<td>5 mo</td>
<td>ARI: self-reported cold/flu</td>
</tr>
<tr>
<td>Mandlik 2020 (41)</td>
<td>India</td>
<td>Healthy children</td>
<td>58.4 (10.3)</td>
<td>--</td>
<td>120:124</td>
<td>25 µg daily</td>
<td>8 mo</td>
<td>URI: self-reported</td>
</tr>
<tr>
<td>Rake 2020 (42)</td>
<td>England</td>
<td>Healthy older adults</td>
<td>50.2 (--</td>
<td>127:787 (16.1)</td>
<td>395:392</td>
<td>2.5 mg bolus monthly</td>
<td>2 yrs</td>
<td>URI/LRI: GP recorded</td>
</tr>
<tr>
<td>Ganmaa, unpublished</td>
<td>Mongolia</td>
<td>Healthy school children</td>
<td>29.7 (10.5)</td>
<td>2813/8851 (31.8)</td>
<td>4418:4433</td>
<td>0.35 mg weekly</td>
<td>3 yrs</td>
<td>ARI: self-reported cold/flu</td>
</tr>
</tbody>
</table>
5.2 Data collection, entry and checking and study quality

Where necessary, datasets will be re-analysed to identify the proportion of participants experiencing ARI re-defined using diagnostic criteria that are harmonised between trials.

A shell table requesting the proportion of participants per arm, for each outcome will be provided to authors for populating. 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. Any missing data, obvious errors, inconsistencies 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 (43).

5.3 Study procedures

Procedures for individual studies are documented in original trial reports (6-9, 11-15, 17, 19-21, 23-25, 27, 31-37, 39-42, 44-52) and individual study protocols.

5.4 Statistical analysis

5.4.1 Summarising overall effect of vitamin D supplementation

Our aggregate data meta-analytical approach will follow existing guidelines (53). For each analysis, we will include all participants ever randomised and will base analysis on the intention-to-treat principle.

From the proportion of events in the intervention vs. control arm for each outcome, within each trial, we will calculate a log odds ratio and its standard error, which will be meta-analysed in a random effects model using the metan package within STATA IC v14.2 (College Station, TX) to obtain a pooled odds ratio with a 95% confidence interval and a measure of heterogeneity summarized by the $I^2$ statistic and its corresponding p value.

Analyses of the effectiveness of vitamin D supplementation vs. placebo will be performed on the combined ARI study population for the following outcomes:

a) Proportion of participants who experience at least one 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) Proportion of participants with at least one hospital attendance, defined as Emergency Department attendance / hospital admission for ARI

c) Proportion of participants taking at least one course of antimicrobials for treatment of ARI
d) Proportion of participants with at least one work/school absence due to ARI

e) Proportion of participants experiencing at least one adverse event including hypercalcaemia, renal stones, drop-out/withdrawal rates, serious adverse events (both ARI-related and total) and mortality (both ARI-related and total).

A separate analysis of the effectiveness of high dose vitamin D vs. low vitamin D control will be performed on relevant trials, for the primary outcome only.

Original authors will be asked to confirm accuracy of this reanalysis, and any discrepancies will be resolved.

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 (serum 25[OH]D <25 vs. 25-49.9 vs. 50-74.5 vs. ≥75 nmol/L);

ii) Size of vitamin D dose administered (daily equivalents ≤400 IU vs. 401-1000 IU vs. 1001-2000 IU vs. >2,000 IU);

iii) Frequency of vitamin D administration (daily vs. weekly vs. monthly or less frequently);

iv) Duration of supplementation (<6 months vs. 6-12 months vs. >12 months)

The 25 nmol/L threshold for baseline 25(OH)D concentration in sub-group analyses was selected on the grounds that it is the threshold for vitamin D deficiency defined by the UK Department of Health (54), and the level below which participants in clinical trials have experienced the most consistent benefits of supplementation (55). Thresholds of 50 nmol/L and 75 nmol/L were selected on the grounds that observational studies have reported that less profound states of vitamin D deficiency may also associate independently with increased risk of ARI (56, 57).

Examination of sub-group effects will be undertaken by multivariate meta-regression analysis on trial-level characteristics, namely, dose frequency, dose size and dose duration, to produce an adjusted odds ratio, a 95% confidence interval and a p value for interaction for each factor.

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, publication bias according to recent guidelines (58), through the construction of contour-enhanced funnel plots and appropriate statistical tests for ‘small-study effects’ (59); 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.
6 Ethics

Individual trials contributing summary data to this aggregate data meta-analysis will all be approved by Research Ethics Committees in the countries where they took place. No participant-level data will be used in this analysis.

7 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 meta-analysis will include one PI for each trial whose data are included in that meta-analysis as a named co-author. Other investigators named on this protocol who have made a substantive contribution to the meta-analysis, but who are not PIs for individual studies, may also be named co-authors on manuscripts arising from this study.
8 REFERENCES


