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The effect of dosing regimen on outcomes of vitamin D supplementation trials
A study of current literature

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Abstract

Results from observational studies have indicated associations between vitamin D and extra-skeletal outcomes, including respiratory tract infections (RTI) and all-cause mortality. However, available trial-data have shown inconsistent results. The main objective of this thesis was to investigate whether a beneficial effect of daily supplementation of vitamin D on RTI and all-cause mortality could have been masked by the use of less frequent supplementation intervals.

This thesis included data from double-blinded, randomized controlled trials (RCTs) published in the last 10 years. Eligible trials were identified through screening of the reference lists of systematic reviews of meta-analyses (MAs), and of reference lists of MAs on the selected outcomes included in these reviews. Also, additional searches were performed to ensure that also recently published RCTs, not identified in a previous step of the search strategy, were considered for inclusion. The search strategy was designed to promote selection of trials of adequate methodological quality.

To be included the record had to be written in English and report results of a double-blinded placebo-controlled RCT with vitamin D supplementation in a human population. Studies including pregnant women or assessing the effect of prenatal supplementation were not included, nor were studies including populations with chronic kidney disease and/or other diseases known to affect the conversion of active metabolites of vitamin D. Titles and abstracts of identified records were screened for eligibility. Eligible full-text articles were retrieved, and key information extracted and summarized in modified PICO-tables.

This thesis included a total of 21 RCTs reporting effects of vitamin D supplementation on RTI, and 15 RCTs reporting effects of vitamin D supplementation on all-cause mortality. Comparing the effect of dosing regimen on the pooled relative effect estimates showed a significantly lower odds of RTI with daily supplementation compared to less frequent dosing regimens in children. The same trend was observed in adults, but the difference was non-significant. No significant effects of dosing regimen were observed regarding the all-cause mortality outcome.

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Preface – the work process

I caught interest for this fifth-year thesis during my year at the medical student research program, where I collaborated with researchers from the Endocrinology Research Group in Tromsø. During one of their meetings, I was presented with the concern that beneficial effects of vitamin D supplementation might have been masked in clinical trials using less than daily dosing regimens. This thesis has been shaped during my fifth-year clinical rotation and in the periods designated to work with the fifth-year thesis.

Outline of the work process:

March – April 2018 – Work on project description and disposition of the thesis

August 2018 – Work on the introduction and methods

November – December 2018 – Identification and selection of systematic reviews of MAs.

January – February 2019 – Screening of MA reference lists for RCTs, full-text downloaded.

March – April 2019 – Final searches in PubMed for recently published RCTs, articles read and key information for tables extracted.

May 2019 – Completing the results, discussion, layout, and adjustments of the manuscript.

Given the extensive literature on clinical trials on vitamin D supplementation; Benefit was drawn from previously well-performed systematic reviews and meta-analyses. The project deviates from its protocol in the number of selected outcomes, key information extracted for statistics, data analysis and bias assessment.

Anette Uhlving Larsen,

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1 Introduction

1.1 Discovery

Vitamin D was first discovered in the early 1920s, although rickets, a disease caused by vitamin D deficiency, had been known since antiquity (1). Several important contributors are worth mentioning in the discovery of vitamin D, but perhaps some of the most notable were the works of Professor Elmer McCollum (2) and Sir Edward Mellanby (3) demonstrating that heated, oxidized cod liver oil could cure rickets in rats. At the same time, reports on how sunlight and UV exposure could prevent, and cure, rickets emerged. This ultimately led to the hypothesis by Hess et al. (4) that a cholesterol in the skin (namely 7-dehydrocholesterol) could be converted by UV exposure into a substance possessing anti-rachitic properties. Also noteworthy was the demonstration of the structure of vitamin D_2 by Askew et al. in 1931 (5), and of 7-dehydrocholesterol and vitamin D_3 by Windaus et al. only a few years later (6). Although vitamin D is still classified as a vitamin, it became clear during the second half of the 20th century that vitamin D possesses a function more in terms of a prohormone than merely a vitamin (7).

1.2 Vitamin D physiology

Today, it is well known that the production of vitamin D_3 (cholecalciferol) occurs through cutaneous synthesis from 7-dehydrocolesterol when the skin is exposed to UV radiation in a spectrum of 280-320 nm (7, 8). Additional dietary sources of vitamin D, which also includes vitamin D_2 (ergocalciferol), include fatty fish, certain dairy products fortified with vitamin D and vitamin D supplements (7). Whatever the source, vitamin D undergoes hydroxylation in the liver by actions of the 25-hydroxylase converting enzymes (CYP2R1, CYP27A1, and others) to form 25-hydroxyvitamin D (25OHD). 25OHD is the main circulating vitamin D metabolite, and it is traditionally considered as the best biochemical measure of an individual's vitamin D status (7). However, serum levels of 25OHD vary according to vitamin D intake, body composition (9) and genetic factors (10). In the circulation, less than 1% of vitamin D circulates in the free form, as vitamin D metabolites are mainly bound to plasma proteins. The main binding protein, namely vitamin D binding protein (DBP), accounts for 90% of the transportation, whereas minor fractions may be bound to albumin (11).

Activation to the hormonal form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) occurs in the kidneys by actions of the activating enzyme 1-alpha-hydroxylase (CYP27B1). When activated, $1,25(OH)_2D$ binds to the nuclear vitamin D receptor (VDR) in the target tissue. The $1,25(OH)_2D$/VDR complex acts as a transcription factor in combination with the retinoid X receptor (RXR). Its main function is the induction of genes that enable intestinal calcium and phosphate absorption, renal reabsorption of calcium and flow of calcium and phosphate in and out of the skeleton (7, 8). With no or little calcium available for intestinal absorption, vitamin D stimulates osteoblasts to produce receptor activator nuclear factor-κB ligand (RANKL) (12). RANKL then stimulates osteoclastogenesis and activates resting osteoclasts for bone resorption (12). In other words, the main role of vitamin D is to ensure calcium homeostasis, and if necessary, at the expense of bone.

Activation is regulated by parathyroid hormone (PTH) and FGF-23 (7, 11). PTH increases in response to low s-calcium levels and high serum phosphate levels to increase the production of $1,25(OH)_2D$, thus increasing the absorption of intestinal calcium and increasing the urinary output of phosphate (13). Moreover, vitamin D interacts with PTH to stimulate the reabsorption of filtered calcium in the distal renal tubule (7). In contrast, FGF-23 simultaneously inhibits vitamin D activation, while stimulating the 24-hydroxylase enzyme responsible for the conversion of 25OHD to the inactive metabolite 24,25(OH)D, which is excreted in the urine (7).

1.3 Vitamin D deficiency

It is well known that vitamin D deficiency may lead to rickets in children, and osteomalacia and osteoporosis in adults (7, 14). The secondary increase in serum PTH concentration is most likely to cause the skeletal effects of moderate vitamin D deficiency in adults or elderly subjects, as this secondary hyperparathyroidism leads to high bone turnover and associated cortical bone loss (15). However, it has been ferociously debated at what levels vitamin D deficiency causes disease, and if there is a difference between vitamin D deficiency and insufficiency (15, 16). Today, most research communities agree that serum concentrations below 30 nmol/L should be corrected and that levels lower than 50 nmol/L should be avoided (15). However, the optimal vitamin D intake or threshold values of 25OHD to achieve clinically detectable non-skeletal effects remains unsettled (17).

1.4 Extra-skeletal effects

Over the past couple of decades, the presence of VDRs, as well as that of the vitamin D converting enzymes (25-hydroxylase and 1-alpha-hydroxylase), has been demonstrated not only in enterocytes, osteoblasts and distal renal tubule cells, but in a wide variety of tissues and target cells (8). This has led to the consideration that vitamin D might exert extra-skeletal effects (8). Arguments include that $1,25(OH)_2D$ acts on all cells (at least at some stage during their differentiation) and that many of these may produce $1,25(OH)_2D$ independently of renal activation. Also, studies indicate that VDR might act through other ligands than those traditionally being associated with its functions. It has been suggested that RXR is not the only receptor of which the $1,25(OH)_2D$/VDR complex might act as a transcription factor. Furthermore, it has been argued that vitamin D signaling might also be involved in non-genomic mechanisms of actions (8).

This new "paradigm" has found support in several studies, both cross-sectional and longitudinal, showing strong associations between vitamin D deficiency and multiple extra-skeletal outcomes (14). These include both outcomes and risk factors related to infectious and immunologic diseases, cancer, mortality and more (14). Despite unanimous reports from epidemiologic studies, prevention or treatment of these diseases with vitamin D, as tested in randomized controlled trials (RCTs), have not carried the same conviction (18). Many explanations have been suggested, of which the most important include that the studies were underpowered, that recruited populations, in general, have been vitamin D sufficient, that the duration of the intervention periods has been too short or that the design of the study or the dosing regimen applied were wrong (19). In the following, the rationale regarding this latter explanation will be discussed in further detail.

1.5 Intermittent dosing regimens and clinical trials

In recent years, the potentially modifying effects of different dosing regimens have been debated (20). Many studies have used weekly or monthly or even less frequent doses of vitamin D as part of their study design, as these dosing regimens leads to sufficient and stable levels of the biochemical marker of vitamin D status, serum 25OHD, at the same time as they reduce concerns regarding compliance (21-23). However, there are strong indications that serum levels of 25OHD might not reflect the true vitamin D status of the body (24), and that daily supplementation is to be preferred to less frequent doses. Moreover, several reports

indicate that the effects of vitamin D supplementation have been better in trials using daily doses compared to trials using intermittent dosing regimens (20, 25). The rationale for this argument is that the parent compound for tissue activation, serum vitamin D (i.e. cholecalciferol and ergocalciferol), traditionally perceived mainly as a substrate for hepatic 25-hydroxylation, might possess a more direct physiological role through the local tissue autocrine system (24). With a half-life of approximately one day, vitamin D given on a weekly or monthly basis would provide stable levels of 25OHD, but only short, intermittent periods with sufficient levels of circulating vitamin D (24).

Moreover, vitamin D, 25OHD and the active metabolite $1,25(OH)_2D$ are found mainly bound to DBP in the general circulation, although some also circulate in a free unbound form. From this it can be understood that 6 forms (or complexes) may be found in the general circulation:

- free vitamin D and a DBP-vitamin D complex
- free 25OHD and a DBP-25OHD complex
- free $1,25(OH)_2D$ and a DBP-$1,25(OH)_2D$ complex.

To exert any physiologic effects, the active form, $1,25(OH)_2D$, must first bind to the intracellular VDR. Some cells, including the renal tubular cells, mammary gland cells, myocytes, and the PTH cells, possess the ability to internalize the entire DBP-vitamin D(metabolite) complex through the megalin-cubilin system (26). However, most other cell types depend on the availability of vitamin D (metabolites) circulating in the free form in their paracrine/autocrine environment. As previously mentioned, most of the vitamin D metabolites circulate bound to DBPs. As 25OHD binds more strongly to DBP than both vitamin D and $1,25(OH)_2D$, with binding coefficients being 10^{-9} M, 10^{-8} M and 10^{-7} M respectively (27), vitamin D is likely more accessible for internalization, than 25OHD. Moreover, as the circulating concentration of vitamin D is 100 to 1000-fold higher compared to that of $1,25(OH)_2D$, it is also highly plausible that most of the intracellular $1,25(OH)_2D$ is derived from passive diffusion (and subsequent hydroxylation) of the free form of vitamin D into the cells. This theory is also supported by the fact that the enzymes necessary for hydroxylation of both vitamin D and 25OHD to $1,25(OH)_2D$ have been demonstrated in most cells (7). Accordingly, some of the discrepancies between observational and interventional studies might be resolved by also including serum vitamin D in the evaluation of vitamin D status (24).

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This hypothesis is controversial, although highly relevant, as studies assessing the effect of daily versus less frequent dosing regimens are lacking. This thesis focused on the effects of daily versus less frequent vitamin D supplementation with regards to respiratory tract infections (RTIs) and all-cause mortality. These outcomes were selected based on previous findings in systematic reviews, in which some support was lent to an effect on respiratory tract infections (RTIs) and all-cause mortality, among only a few other non-skeletal outcomes (17, 28).

In the following, the main outcomes of this thesis, RTIs and all-cause mortality, will be presented.

1.5.1 Respiratory tract infections

The immune system has been connected to vitamin D through the presence of VDR and vitamin D metabolic enzymes in both innate and adaptive immune signaling (15). Both epidemiological and in vitro data have reported consistent and independent associations between low serum concentrations of 25OHD and risk of RTI (29, 30). A potential mechanism with regards to how vitamin D might mediate a protective effect has been suggested, in that 25OHD supports the introduction of antimicrobial peptides in response to both viral and bacterial stimuli (31, 32). Moreover, it has been reported that vitamin D metabolites may also induce other innate antimicrobial effector mechanisms, such as induction of autophagy and synthesis of reactive intermediates of nitrogen and oxygen (33). In a meta-analysis (MA) by Martineau and colleagues from 2016 on individual participant data from RCTs (25), it was concluded that vitamin D supplementation significantly decreased the risk of RTI and that the protective effect was greater in subjects with lower baseline 25OHD concentrations. Similar findings were also reported in a review by Bouillon from 2018, concluding that *"vitamin D possesses a role with regards to sensitivity to infections and autoimmune diseases, and that vitamin D deficiency enhances the risk of upper respiratory tract infections"* (15). Interestingly, beneficial effects appeared to be limited to patients receiving vitamin D on shorter intervals compared to those receiving bolus doses. A subgroup analysis of the MA by Martineau and colleagues showed a similar trend, with protective effects of supplementation seen in those receiving daily or weekly vitamin D without additional bolus-doses, but not in those who received one or more bolus doses (25). We included RTI as an outcome because the effect of daily versus less frequent

supplementation with vitamin D on RTIs has not previously been assessed.

1.5.2 All-cause mortality

Vitamin D_3 has been connected to mortality in a vast number and variety of studies, yet findings have been inconsistent (17). Most observational studies suggest that a suboptimal vitamin D status is associated with an increased risk of death (34, 35). In a MA by Bjelakovic from 2014 (36), the overall effect of vitamin D supplementation was associated with a small, but significant reduction in all-cause mortality in middle-aged and older adults. Similar reductions in all-cause mortality were reported in a MA by Bolland and colleagues (37) from the same year, although with a slightly different trial selection. Moreover, both of these MAs also included trials that assessed the effect of vitamin D in combination with calcium supplementation. Little is known regarding the mechanisms by which vitamin D might increase life expectancy, although increased resistance to acute infectious episodes has been suggested (28). As the effect of daily versus less frequent supplementation with vitamin D on all-cause mortality has not previously been assessed, and as it is a highly relevant, hard endpoint of interest for the general public, all-cause mortality was included.

1.6 The aim of the thesis

The main objective of this thesis was to investigate whether beneficial effects of daily vitamin D supplementation on the prevention of RTI and all-cause mortality are dependent on whether the supplementation was given daily or by less frequent dosing. In the following, a comparison of RCTs published within the last 10 years (i.e. published after 01.01.2009) will be presented.

2 Methods

2.1 Criteria for considering studies to be included in this thesis

2.1.1 Delimitations

The interest in vitamin D research has exploded over the past two decades, and at the time of writing this thesis, PubMed lists more than 80,000 publications on vitamin D. Restricting the search to clinical trials done within the past 10 years results in almost 3000 publications (15/04: 2418). Therefore, the search for eligible RCTs was designed to limit the sample of

studies and to promote a selection of trials that were of adequate methodological quality. This was ensured by including results from RCTs published within the last 10 years, identified through screening of the reference lists of the most recent systematic reviews of MAs, and reference lists of MAs included in these reviews (See Section 2.3). Also, an additional search was executed to identify RCTs published after the inclusion period in the most recent MA on the outcome of interest. This intention of this additional search was to ensure that recently published RCTs, not identified in a previous step of the search strategy, were considered for inclusion. All electronic searches were performed through the PubMed database by the use of both MeSH terms and free-text words.

2.1.2 Inclusion criteria
The following inclusion criteria were applied when searching for eligible records:

- **Timeframe**: To be considered for inclusion, studies had to be published within a set timeframe from 01.01.2009 until 01.04.19. Unpublished or ongoing trials were not considered for inclusion.
- **Study design:** Double-blinded RCTs.
- **Population**: Human studies including subjects of either sex, and any age
- **Intervention**: Vitamin D, including vitamin D3 (cholecalciferol) or D2 (ergocalciferol), administered at any dose, at any frequency, and via any route, as a supplement (including fortified food or drinks) alone, or as a co-intervention when this regime was compared to placebo with an identical co-intervention. Studies in which supplementation was given as an initial bolus, followed by a daily supplementation regimen of vitamin D > 400 IU was registered according to a daily dosing regimen.
- **Control**: Placebo or placebo with co-intervention when the co-intervention was applied in both the treatment and control arm of the study.
- **Outcomes**: Publications in which the effect of vitamin D supplementation (except prenatal supplementation) on prevention of RTIs (as defined in the individual studies) and/or on life expectancy/all-cause mortality were reported.

2.1.3 Exclusion criteria
Studies that met the following criteria were excluded from the study:

- **Full-text**: Records in which full-text was not available.
- **Language**: Records in which full-text was not available in English.
- **Populations**: Trials including pregnant women and/or subjects with diseases affecting vitamin D metabolism such as current liver or kidney disorders; a history of hypercalcemia; nephrolithiasis or sarcoidosis. Studies assessing the risk of RTI, studies including subjects with HIV infection were not included.
- **Sample size**: As smaller trials tend to show greater treatment effects when included in MAs than larger trials, trials in which the sample size included > 50 subjects were not included.
- **Intervention/Control**: Studies in which the control group actively received any vitamin D supplementation alone or together with placebo.
- **Intervention length**: Trials with an intervention length of less than one month.
- **Outcomes**: Studies in which relative risk estimates, or numbers for calculation of such estimates, were not available, were excluded from the study.

2.2 Outcomes

2.2.1 Respiratory tract infections
In records reporting effects of vitamin D supplementation on respiratory tract infections (RTIs), the outcome of interest was incidence of RTI, as defined in the individual trials. To be included in the statistical analyses it was required that outcomes were expressed as counts in two-by-two tables, as numbers of successes and failure in the treatment and control group. Thus, the proportion of patients experiencing one or more RTIs was identified and then used to calculate relative risk estimates (odds ratios (ORs)) for comparison between studies. If unavailable, and no ORs were reported, the study was not included in the statistical analyses. Additional RTI-related outcomes were not considered to be part of this thesis.

2.2.2 All-cause mortality
Records reporting effects of vitamin D supplementation were included in the analyses if numbers of deaths (of any cause) and survival in the treatment and control group were reported. In cases where numbers for two-by-two tables were extracted from flow-charts or similar and/or the study did not primarily assess the effect of vitamin D supplementation on

all-cause mortality, this information was used for subsequent sensitivity/subgroup analyses. Cause-specific death was not assessed.

2.3 Literature search strategy

2.3.1 Summary
The literature search for RCTs started with the identification of the most recent systematic review summarizing MAs on nonskeletal outcomes, including respiratory tract infections and all-cause mortality. Next, all MAs described in these reviews were assembled, and the reference lists of these MAs were screened for eligible RCTs. Finally, the results of trials reported since the last MA on RTI was submitted for publication, were added, as were trials reported since the last MA on all-cause mortality was submitted for publication.

2.3.2 Search for systematic reviews of meta-analyses
A search was set up using PubMed advanced search builder, to identify the most recent systematic review of MAs summarizing trial data on extra-skeletal outcomes (the full search strings used may be found in the appendix, figure S1). The search was based on three modules; the 1st module concerning vitamin D, the 2nd module concerning the outcome of interest and the 3rd module concerning publication type (i.e. systematic reviews of MAs). Finally, filters were added with restrictions regarding language, date of publication and populations studied (i.e. humans).

2.3.3 Screening of identified meta-analyses' reference lists
Identified systematic reviews were screened for MAs matching the pre-specified outcomes (i.e. RTI and all-cause mortality). MAs matching the pre-specified outcomes were downloaded in full-text and reference-lists were screened for eligible RCTs.

2.3.4 Supplemental search for and screening of recently published randomized controlled trials
A supplemental search was made in PubMed for each outcome to identify RCTs published after the inclusion period of the most recent systematic review conducted on that outcome. The search was built in a similar fashion as the search for systematic reviews of MAs,

including three modules: 1st module concerning vitamin D, the 2nd module concerning publication type and the 3rd module concerning the outcome of interest (the full search strings used may be found in the appendix, figure S2 and S3). Resulting RCTs were filed through the reference manager, and titles/abstracts screened for eligibility. Eligible RCTs were downloaded in full-text, read and key information extracted.

2.4 Data collection

2.4.1 Selection of studies
Titles and abstracts of identified RCTs were screened for eligibility by Anette Uhlving Larsen (AUL). Eligible RCTs were downloaded to the reference manager (EndNote Version X8.2) and downloaded in full-text as a PDF-file. Studies in which certain aspects were unclear as to whether or not they conflicted with the eligibility criteria, were discussed on a consensus meeting between AUL and the project supervisor Rolf Jorde (RJ) on April 23rd, 2019. Figure 1 and Figure 2 summarizes the flow through the selection processes.

2.4.2 Data extraction and synthesis
Finally, eligible RCTs were read and key information was extracted. Data extracted included publication details (authors, year, country, trial duration), patients characteristics (age, gender distribution, number of subjects, baseline vitamin D levels and associated standard deviations (SD)), intervention (vitamin D type, dose and dosing interval), control group design, and finally outcome measures including as numbers of successes and failures in the treatment versus in the control group for RTI studies, and numbers of deaths in the treatment versus control group in all-cause mortality studies. Inclusion and exclusion criteria, as well as the route of administration, were not registered. Extracted data were registered in a modified PICO-table (38) and transferred to excel/STATA for further analyses.

2.4.3 Assessment of risk of bias
Methodological quality and risk of bias in included studies were assessed by the use of GRADE criteria (39), and the results of these assessments may be found in the Appendix.

2.5 Data analysis – Measures of treatment effect

Study results were quantitatively combined for each outcome; Numbers expressed as counts of successes and failures with regards to prevention of RTI and death were extracted into two-by-two tables, and the OR with the corresponding 95% confidence intervals (CIs) were calculated.

To investigate the effect of vitamin D supplementation on the occurrence of RTIs and death from any cause, a MA was conducted for each outcome and applied a random-effects model (DerSimonian and Laird) (40) to obtain the pooled intervention effects. Next, subgroup analyses, stratified by supplementation regimen, were done to assess whether the pooled effect estimates were influenced by the supplementation regimen used (i.e. daily versus less frequent supplementation, including weekly, monthly or less frequent bolus administration). Heterogeneity was quantified with the I^2 statistic (41). This was reported on a scale from 0-100%, in which values of >50% were interpreted as substantial statistical heterogeneity being present.

Meta-regression analyses of the log OR were used to evaluate dose interval (daily versus less frequent) as a predictor of the effect of vitamin D supplementation on the pooled effect estimates by adding the [effect estimate × interval] interaction term. This analysis was redone to assess also the effect of daily <u>or</u> weekly supplementation versus less frequent bolus regimens, to allow for comparisons with previous MAs (25, 42).

Also, meta-regression was used in exploratory analyses to identify factors modifying the influence of dosing regimen on the pooled estimates for each outcome, by adding dose interval-covariate interaction terms (dosing interval × *modifying variable) to the above-mentioned meta-regression analysis. Covariates were tested independently and included length of the intervention (< 1 year versus ≥ 1 year), age (RTI: whether the study included children < 12 years of age or not; All-cause mortality: whether participants' mean age was above or below 60 years), mean baseline 25OHD-level (≤ 50 nmol/L versus > 50 nmol/L), risk of bias (low versus moderate/high) and ethic-assessment (described in section 2.6).

The risk of publication bias was explored by visual inspection of funnel plot asymmetries.

All analyses were performed using Stata version 15.1 for Mac (StataCorp 4905 Lakeway Dr. College Station, TX 77845 USA).

2.6 Ethical aspects

Although a study of previously performed trials to a lesser extent require a written consent and ethics approvals, concerns regarding ethical standard still ought to be considered. Reviews and MAs, like all other biomedical research, may be prone to conflict of interests. Moreover, there is always a risk of including studies with ethical insufficiencies or to include studies in which the informed consent given for the original study is no longer valid at the review level (43). In this thesis, we assessed ethical standard by screening the included studies for a set of relevant key terms according to the following criteria:

- The study had been approved by a research ethics committee, institutional review board or similar.
- The record included a paragraph regarding conflict of interests.
- The record included a declaration of financial support/funding sources.
- The record included a paragraph on adverse events reported in the study.

Key terms used in this screening included: ethic; approved; interest; conflict; fund; grant; support; declare; adverse; side; safe. Each study then received a comment on whether or not the above-mentioned criteria for an appropriate ethical standard was met. As a sensitivity analysis, these data were used to assess for potential modifying effects of ethical standard on dosing regimen (Section 2.5).

3 Results

3.1 Results of the literature search strategy

The search for systematic reviews of MAs resulted in 35 independent records in PubMed. On screening of these results, in addition to the most recent systematic review done by Autier and colleagues in 2017 (28), it was decided to include also a systematic review by Rejnmark et al. (17) as this review was done at approximately the same time, however including a slightly different selection of MAs.

Overall, a total of 238 records within the set timeframe were identified through screening of MA reference lists, 112 records on screening for RTI and 126 for all-cause mortality. In both PubMed searches for trials published after the inclusion period in the most recent MA on RTI and all-cause mortality, an additional 72 records were identified for each outcome. The search strategy results for RTI and all-cause mortality is summarized in Figure 1 and Figure 2, respectively.

3.1.1 Respiratory tract infection
In total, 54 duplicates were removed manually from the identified records, which left a total of 130 unique records for screening of titles and abstracts. Five of these records were excluded because the full-text was unavailable. Of the remaining records, 40 records were eligible after the screening of titles and abstracts and were retrieved in full-text. After reading the full-texts, 21 articles fulfilled the inclusion criteria (Table 1), and 19 articles were excluded. A summary of the excluded trials is found in the appendix (Supplemental Table S1).

3.1.2 All-cause mortality
Regarding all-cause mortality, a total of 64 duplicate studies were removed manually, which left a total of 140 records for screening of titles and abstracts. Of these, 2 records were excluded because the full-text was unavailable excluded because the full-text was unavailable. The remaining articles that were available and eligible (n= 32) were retrieved. After reading the full-texts, 15 articles fulfilled the inclusion criteria (Table 2), whereas 17 articles were excluded. A summary of the excluded trials is found in the appendix (Supplemental Table S1).

3.2 Study and participant characteristics of eligible studies
The characteristics of the eligible studies on RTIs and all-cause mortality are summarized in Table 1 and Table 2, respectively.

3.2.1 Respiratory tract infection
Trials were conducted in 15 different countries, enrolled a total of 10,663 participants, with 45.7% being women. 6 studies included infants or school-children only, and 15 included

adults or elderly people. Mean baseline 25OHD concentrations were measured in 15 of 21 included trials, with BL-values ranging from 18.9 nmol/L to 75.9 nmol/L. All but one study assessed the effect of vitamin D3 supplementation, with the one study being that of Bergmann et al. (44) assessing the effect of vitamin D2 supplementation. Vitamin D was given daily in 10 studies (44-53); weekly in two trials (54, 55); and monthly or less frequent in six trials (22, 56-61); and as a single bolus dose in two trials (62, 63). Trial duration varied from 7 weeks to 5 years. In trials using daily dosing regimens, the length of intervention was $\geq$ 1 year in three of 11 trials using daily dosing regimens, as compared to seven of 10 trials administering vitamin D on less frequent intervals. Of the trials that administered vitamin D on a daily basis, doses varied from 300 IU to 4000 IU. RTI was assessed as the primary or co-primary outcome in 13 studies and as a secondary outcome in the remaining eight studies.

3.2.2 All-cause mortality

Trials were conducted in 10 (+) countries, enrolled a total of 25,871 participants, with 48.5% being women. Mean age was 65.7 ± 8.4 years (not including two trials done in children, in which mean age were 0.8 ± 0.6 years). In 2 studies, age-related data was not available (64, 65). Mean age was below 60 years in 4 studies, and 60 or above in 10 studies. 1 study (66) included subjects in a critical care setting. BL 25OHD concentrations were measured in 9/15 trials, ranging from 22.1 to 78.3. All but one study assessed the effect of vitamin D3 supplementation, with the one study being that of Witham et al. (67) assessing the effect of vitamin D2 supplementation. Vitamin D was given daily in only four trials (65, 68-70); weekly in two trials (71, 72); monthly or less frequent in eight trials (22, 23, 57, 64, 66, 67, 73, 74); and as a single bolus in one trial (62). Trial duration varied from 12 weeks to more than 6 years. It was noted that three of four trials using daily dosing regimens had intervention length $\geq$ 1 year, as compared to five of 11 trials administering vitamin D on less frequent intervals. Of the trials that administered vitamin D on a daily basis, doses varied from 800 IU to 4000 IU. All-cause mortality was assessed as a primary or co-primary outcome in four trials, and as a secondary outcome in three trials. Eight of the included trials did not assess all-cause mortality as an independent outcome but reported numbers of deaths in the intervention group and control groups, thus being included in the all-cause mortality analyses.

3.3 Excluded studies

A total of 234 studies were excluded (109 on RTIs and 125 on all-cause mortality) after trial duplicates were removed and titles and abstracts were screened. A summary of the most common reasons for exclusion is shown in Figure 1 and Figure 2. Four studies were excluded because full-text was not available. Of the 72 records that were downloaded and screened in full-text, a total of 36 were excluded (19 studies on RTI and 17 studies on mortality). Appendix table S1 and S2 present brief summaries on study characteristics and reason for exclusion of studies screened in full-text. Of 16 records were excluded because the outcome of interest (RTI or all-cause mortality) were missing, six records were excluded because they were studies on treatment effect (and not **prevention** efficacy), five records were excluded because the sample size was too small (< 50 subjects randomized), and seven records were excluded because the study design, including intervention/control group design, were inappropriate. The distribution of trials using daily versus less frequent dosing regimens was comparable across the different categories for exclusion.

3.4 Risk of bias in included studies

3.4.1 GRADE assessments

A detailed description of the individual studies' risk of bias is found in the appendix for each of the included studies.

In trials assessing the effect of vitamin D supplementation on RTIs, all but two received a moderate to high GRADE (Supplemental Table S2). Two trials received a low or low to moderate GRADE, in which one was downgraded due to inclusion of only 41% of the invited (46), and the other was downgraded due to a small sample size combined with a low response rate on the questionnaire used (51). Both trials receiving a low or low to moderate GRADE used daily dosing regimens.

In trials assessing the effect of vitamin D supplementation on all-cause mortality, nine studies received a low or low to moderate GRADE, and six received a moderate or moderate to high GRADE (Supplemental Table S3). Eight trials were downgraded because all-cause mortality was not assessed as an outcome in the original paper (i.e. trials presenting data on deaths in each study group in flow-charts; as an adverse event; or similar). The study by Punthakee et

al. (69) received a low GRADE because the study was ended prematurely. Of trials that received a low GRADE, all but one trial used less frequent than daily dosing regimens. Four trials received a high GRADE, and the distribution of high-quality trials was similar between trials using a daily dosing regimen and studies in which a less frequent regimen was applied.

3.4.2   Assessment of publication bias

Funnel plots of studies assessing the effect of vitamin D supplementation on incidence of RTI were found symmetrical, representing a low risk of publication bias among trials included in the analyses (Figure 3). There was no evident difference in the risk of publication bias in trials using daily versus less frequent dosing regimens (data not shown).

In studies assessing the effect of vitamin D supplementation on the incidence of all-cause mortality, funnel plots were found asymmetrical, indicating presence of publication bias (Figure 4). Comparison of publication bias in studies using daily versus less frequent dosing regimens, were not appropriate, as only three studies assessed the effect of daily supplementation (data not shown).

3.5   Effect of interventions

In the following, a short narrative of the main results of this thesis will be presented. The individual trials included are summarized in Table 1 and Table 2.

3.5.1   Incidence of respiratory tract infection

As shown in Figure 5, the pooled ORof experiencing at least one RTI was 0.83 in the vitamin D group compared to the placebo group, and the result was statistically significant (OR 0.83; 95% CI 0.70 to 0.97). Between-study variability was large ($I^2 = 50.6\%$). When stratified by supplementation regimen, the odds of experiencing at least one RTI was lower in trials using daily compared to less frequent dosing regimens, but the results were not statistically significant (OR_{daily} 0.77, 95% CI 0.54 to 1.01, *versus* $OR_{less\ frequent}$ 0.89, 95% CI 0.73 to 1.04). Also, between study variability was greater in trials using daily supplementation compared to less frequent dosing regimens ($I^2_{daily} = 57.3\%\ v\ I^2_{less\ frequent} = 35.1\%$).

In the meta-regression analysis, supplementation regimen (daily versus less frequent) turned out as a non-significant predictor of the effect of vitamin D supplementation on the pooled

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effect estimates (*p*=0.10). However, when redoing the analysis for daily <u>or</u> weekly supplementation versus less frequent bolus regimens, the dose regimen was a significant predictor of the effect of vitamin D supplementation on the pooled effect estimates (*p*=0.01).

 In the exploratory meta-regression analysis age turned out to significantly modify the influence of dosing regimen on the effect of vitamin D on RTI prevention, both in daily versus less frequent dosing (*p*=0.03) and in daily or weekly supplementation versus less frequent dosing regimens (*p* < 0.01). In the age-stratified analysis (i.e. trials including adults versus trials including subjects < 15 years of age), for trials done in children it was observed a significant effect in trials using daily dosing regimens (OR_{Daily} 0.59 [0.32 to 0.86]), but not in trials using less frequent dose intervals ($OR_{Less\_frequent}$ 0.86 [0.54 to 1.19]) (Figure 6). In trials done in adult populations, the effect of vitamin D on RTI prevention was non-significant for both dosing regimens (OR_{Daily} 0.88 [0.55 to 1.22] versus $OR_{Less\_frequent}$ 0.90 [0.71 to 1.08]) (Figure 7).

None of the other covariates tested appeared to modify the influence of a daily versus less frequent dosing regimen on the pooled effect estimates (length of intervention: *p*=0.59; mean baseline 25OHD-level: *p*=0.186; risk of bias: *p*=0.45; ethic-assessment: *p*=0.33), however, length or intervention and baseline mean 25OHD level significantly modified influence of a daily or weekly dosing regimen versus less frequent bolus regimens (*p*=0.06 and *p*=0.01, respectively).

3.5.2   Incidence of all-cause mortality

In studies assessing the effect of vitamin D supplementation on all-cause mortality, vitamin D was associated with an overall risk reduction (pooled OR: $OR_{overall}$ =0.97, 95% CI 0.89 to 1.061, I^2= 0%), although according to the 95% CI limits, this difference was non-significant (Figure 8). When stratified by supplementation regimen, there was no difference between trials using daily compared to less frequent than daily dosing regimens (OR_{daily} = 0.97, 95% CI 0.88 to 1.07, I^2= 0% *v.* $OR_{less\_frequent}$ = 0.97, 95% CI 0.80 to 1.18, I^2= 0 %).

From Figure 6 it was noted that trials in which mortality was not assessed as an outcome in the original record, or in which a low GRADE was obtained on screening, were associated with low precision, giving wide CIs. However, excluding these trials (n=9/15), did not change the pooled ORs of neither overall effect of vitamin D supplementation ($OR_{overall}$ =0.97 [0.89,

1.06]), nor stratified by dose interval (OR_{daily} =0.97 [0.88, 1.08] $v.$ $OR_{less\_frequent}$ =0.97 [0.69, 1.35]). Excluding trials in which one or fewer deaths occurred in one treatment group (n=5/15) also did not change the pooled ORs (data not shown).

In the meta-regression analysis, the supplementation regimen (daily versus less frequent) was a non-significant predictor of the effect of vitamin D supplementation on the pooled effect estimates (p=0.88). Redoing the analysis for daily or weekly supplementation versus less frequent bolus regimens did not affect this status (p=0.79).

In the meta-regression analysis, none of the assessed covariates turned out to be significant modifying factors on the influence of dosing regimen on the pooled effect estimates (length of intervention: p=0.91; age: p=0.77; mean baseline 25OHD-level: p=0.64; risk of bias: p=0.33; ethic-assessment: p=0.33).

4 Discussion

4.1 Summary and discussion of main results

This thesis demonstrates that a beneficial effect of vitamin D supplementation may have been masked in previously performed trials on RTI prevention due to the application of a less frequent than daily dosing regimen, but that this was not the case in trials on all-cause mortality.

A beneficial effect of vitamin D supplementation on the prevention of RTI events is supported by findings in previous MAs (25, 75, 76). Whether this effect is modified by choice of a daily dosing regimen compared to a less frequent dose interval has not previously been assessed. In this thesis, it was not possible to demonstrate a significant difference between dosing regimens, although findings indicated a more pronounced effect of daily as compared less frequent supplementation. However, in age-stratified analyses of trials done in children, daily vitamin D supplementation significantly reduced the odds of an RTI event, whereas this effect was less pronounced, and also non-significant, with less frequent dosing regimens. In adults, results indicated a more pronounced effect from daily compared to less frequent supplementation in reducing odds of an RTI event, but the risk reduction was not statistically significant neither with daily nor with less frequent dosing regimens. These findings are in line with those in previous MAs on RTI (25, 42), in which results indicated a more

pronounced effect from vitamin D supplementation in children compared to adults, and from daily <u>or</u> weekly vitamin D supplementation compared to less frequent bolus administration.

Regarding RTI prevention, this thesis differs from previous MAs in a slightly different study selection, and in the number of outcomes assessed. All but one study on RTI reported numbers for calculation of ORs; In the study by Bergman et al. (75), only the OR and the associated 95% CI were reported. In the MA by Martineau and colleagues (25), these numbers were reported, however, as they did not correspond to the OR reported by Bergmann et al, and as redoing the analyses by crude numbers including these values, did not change the overall results of the analyses, it was decided to present MA results regarding RTI based on the ORs and corresponding 95% CIs upper and lower limits (i.e. not crude numbers). Individual participant data (IDP) was not collected (as has been done in a previous MA (25).

In contrast with previous findings of a beneficial effect of vitamin D supplementation on reduced mortality risk (36, 77), this thesis was not able to demonstrate a significant effect of vitamin D supplementation regarding this outcome. This finding was not affected by the dosing regimen applied, and age-trends similar to those seen in RTI trials were not found when evaluating the all-cause mortality outcome. The results of this thesis correlate with that of Bolland et al. (37), in which no significant effect of vitamin D supplementation alone was found on reduction of all-cause mortality.

This thesis differs from previous reviews of vitamin D supplementation for increasing life expectancy in a slightly different study selection and inclusion of two recently published large scale RCTs. In this thesis, MAs reporting data on all-cause mortality were included regardless of whether all-cause mortality was assessed as a primary or secondary outcome, thus including other trials compared to previous MAs, in which more strict inclusion criteria were applied (36, 37).

Regarding the main hypothesis, this thesis was not able to confirm that daily supplementation is better than less frequent dosing regimens, although an effect was found in trials done in infants and children for the RTI outcome. Weak or missing effects from vitamin D supplementation overall might be due to that several of the included studies were of short trial duration, were underpowered or that the subjects included were vitamin D sufficient at baseline. Therefore, the hypothesis could still be that a long-lasting, well-powered study with vitamin

D deficient patients could show a beneficial effect of daily dosing of vitamin D.

4.2 Limitations of the thesis

This thesis has several limitations worth considering when interpreting the results. First, the literature search strategy was performed in a single database. However, the MAs of which reference lists were screened for eligible RCTs did comprehensive searches in multiple databases, including Embase and the Cochrane central register. Thus, the number of RCTs missed by the search strategy is most likely to be small. Nevertheless, this must be regarded as a major limitation of the study. Moreover, it was not searched systematically in databases such as clinicaltrials.gov for ongoing or unpublished trials, and so selective reporting was not assessed.

Second, only one person reviewed studies for eligibility. This is known to increase the risk of authors "cherry picking" studies inducing a potential selection bias in the review process. To compensate for this limitation, strict and detailed eligibility criteria were applied, as well as a transparent method for the study selection process. A consensus meeting was arranged between AUL and her supervisors, to discuss inclusion of studies in which certain aspects were unclear regarding whether or not they conflicted with the eligibility criteria. In addition, a table presenting characteristics and reason for exclusion of excluded trials are enclosed in the appendix.

Third, only trials in which the article manuscript was available in English and full-text were included, thus introducing potential selection bias related to language and access. However, the total number of trials excluded due to missing full-text was small, including only four trials.

Fourth, the study deviates from its protocol in the number of selected outcomes, which was delimited from the suggested five outcomes (cardiovascular disease, RTI, cancer, multiple sclerosis, and all-cause mortality) to include only two outcomes (namely RTI and all-cause mortality). This delimitation was necessary as it would have been too extensive to include all endpoints. Both statistical analyses and bias assessments were adjusted as they were insufficiently described in the original protocol. Retrospectively, the protocol ought to have been registered in a database for planned systematic reviews, such as Prospero, prior to the execution of the study.

Fifth, there was substantial clinical heterogeneity due to major differences in studies included concerning population, setting, trial duration, the number of subjects randomized and outcome measurements. Moreover, this thesis did not assess whether the proportion of vitamin D deficiency subjects at baseline of the study influenced the effect of dose interval on the overall effect estimates, as these data turned out insufficient for statistical analyses. This thesis did also not assess the effect of factors such as BMI, attained vitamin D status at trial end, vaccination status, or underlying chronic disease (i.e. asthma, COPD, heart disease and so forth). Neither was the effect of prenatal supplementation or supplementation in pregnant women and thus, extrapolation of results with regards to these populations is inappropriate.

Sixth, regarding outcomes related to RTI, this thesis did not assess other outcomes than the risk of one or more RTIs, such as infection rate or time to first infection. However, it is unlikely that results regarding these outcomes would differ substantially from incidence of RTI events or that such a difference would have clinical implications.

Seventh, it was observed that the inclusion of trials that did not assess all-cause mortality as an independent outcome of the original RCT, were associated with much wider CIs compared to trials assessing all-cause mortality as the primary or secondary outcome. However, this is not surprising given that such trials would be more likely not to have applied an equally robust design with regards to this outcome, thus leading to lower precision in the effect estimate. Nevertheless, excluding these trials from the analyses did not change the result of the overall effect of vitamin D supplementation of the influence of dosing regimen on this effect.

Eight, ORs were chosen as the main effect estimate, to include the study by Bergmann et al. (44). The OR is a different way of describing the relation than RR: At low prevalence, RR and OR are almost identical, whereas at high prevalence, OR and RR are different numbers (78). Nevertheless, both numbers are correct, and results based on RRs did not affect the thesis conclusions, despite excluding the one trial reporting only the OR (44).

Finally, this thesis included RCTs published within the last 10 years. The potential effect of RCTs published outside this timeframe is not known.

4.3 Strengths of the thesis

This thesis also has some strengths, as it is the first MA to compare the effect of using a daily compared to a less frequent dosing regimen of vitamin D supplementation in the prevention of RTI events and to reduce all-cause mortality. Clear eligibility criteria and well-defined outcomes (success versus failure) were applied.

5 Conclusion and venues for future research

This thesis aimed to review whether the dosing regimen applied could have masked a beneficial effect of vitamin D supplementation in previously performed RCTs. However, among most of the RCTs included in this thesis, neither supplementation regimen showed significant effects, thus, no difference between dose regimens was to be expected.

In conclusion, the current thesis lends some support to the hypothesis that the application of a less frequent than daily dosing regimen could have masked a beneficial effect of vitamin D supplementation in previously performed trials assessing an effect of vitamin D supplementation in prevention of RTI, but lends little support to that this was true regarding all-cause mortality. One reason to why an effect of daily vitamin D supplementation compared to weekly supplementation is difficult to show, could be that MAs including trials with less than weekly dosing regimens dilutes the results with poor quality studies. However, our finding is associated with substantial uncertainty and cautious interpretation of these results is warranted.

Future MAs ought to perform a full systematic search, and preferably collect individual participant data, including information on the proportion of subjects with vitamin D deficiency as well as 25OHD levels attained at the end of the trial. Also, further assessment of age-differential effects is needed to confirm the findings of the exploratory analyses of this thesis. Inclusion of other outcomes, including multiple sclerosis and other immune modulatory diseases, is warranted.

Figures and Tables

Table 1 – PICO table of studies on respiratory tract infections

PICO table of included studies studying the effect of vitamin D supplementation on prevention of respiratory tract infections.

Number	RTI as outcome (RTI definition)	Ref Publication year Country (stat) *Population*	N (VD/placebo) %F (n) Mean age (SD) (Age range)	Intervention vs control Dose Interval Length of trial Season	BL 25OHD nmol/l, mean (SD) BL 25OHD nmol/l, range n < 25nmol/L (%)	Outcome extracted Incidence of 1 or more RTI event, (n/total n) in intervention group vs control group)	Study conclusion Positive (1) Indifferent (0) Negative (-1) NA	Ethical standards met on screening, GRADE
1	**Primary** (URTI: ≥2 URTI symptoms in absence of allergy symptoms)	**Li-Ng 2009** USA (NY) *Healthy adults*	**148 (78/70)** 59% (128) 57.9 (13.6) (21.4-80.6)	**D3 vs placebo** 50ug/2000 IU Daily 3 months Dec-June (winter)	63.7 (25.5) 16.0-156.0 3/150 (2,0%)	1 or more URTI: VD 28/78 vs P 29/70	0	√ Moderate-High
2	**Primary** (ARTI: Medical record diagnosis)	**Laaksi 2010** Finland (Säkylä) *Military conscripts*	**164 (80/84)** 0% (0) 19.1 (0.6) (18 -21)	**D3 vs. placebo** 10 ug/400 IU Daily 6 months Oct-Mar	75.9 (18.7) 41.9-129.0 0 (0%)	1 or more days absent from work due to ARTI: VD 80-41=39/80 vs. P 84-30=50/80	0	Missing: Adverse events Moderate-Low
3	**Primary** (URTI: influenza A/B diagnosed by RIDT or RIDT neg ILI)	**Urashima 2010** Japan (ND) *Schoolchildren*	**334 (167/167)** 43.7% (188) 10.2 (2.3) (6 -15)	**D3 vs placebo** 30ug/1200IU Daily 4 months Dec-Mar	ND	1 or more URTI (Influenza A): VD 18/167 vs P 31/167	1	Missing: Funding Moderate

4	**Secondary** (URTI: Assessed with symptom score)	**Bergman 2012** Sweden (Huddinge) *Adults with increased suscept. to ARTI*	**140 (70/70)** 72.9% (102) 53.1 (13.1) (20-77)	**D2 vs. placebo** 100 ug/4000IU Daily 1 year (52w) All seasons	49.3 (23.2) 8.0-135.0 15/131 (11.45%)	<u>1 or more antibiotic-required event</u> *OR 0.35* **Comment:** - Missing crude numbers for 1 or more airway infection	1	√ Moderate
5	**Secondary** (ARTI: Parent reported "chest infection or cold")	**Camargo 2012** Mongolia (Ulaanbaatar) *3rd/4th grade schoolchildren*	**245(141/104)** 47.8% (118) 10.0 (0.9) (7-12.7)	**D3 vs. placebo** 7.5 ug/300 IU Daily 7 weeks Jan-Mar	18.9 (9.7) 3.3-61.2 192/245 (78.4%)	<u>1 or more parent-reported ARI</u> VD: 141-69= 72/141 vs. P: 104-49= 55/104 **Comment:** D3-fortified milk	1	√ (approval provided in the original publication) Moderate
6	**Primary** (URTI: Assessed with symptom score)	**Murdoch 2012** NZ (Christchurch) *Healthy adults*	**322 (161/161)** 74.8% (241) 48.1 (9.7) (18-67.6)	**D3 vs. placebo** First 2x5000ug/200k monthly, then 2500ug/100,000 IU monthly 1.5 years (72w) All seasons	72.1 (22.1) 13-142 5/322(1.6%)	<u>Risk of URTI:</u> VD 63/70 vs. P 64/70 **Comment:** - Missing crude numbers for incidence of 1 or more URTI	0	√ Moderate-High
7	**Primary** (URTI: Doctor diagnosed acute media otitis)	**Marchisio 2013** Italy (Milano) *Children with recurrent AOM*	**116 (58/58)** 44.8% (52) 2.8 (1.0) (1.3-4.8)	**D3 vs. placebo** 25 ug/1000IU Daily 6 months (24w) Nov-Mar	65.3 (17.3) 24.7-120.6 2/116 (1.7%)	<u>1 or more AOM:</u> VD 26/58 vs P 38/58	1	√ Moderate

8	**Secondary** (URTI: Assessed from daily symptom diary)	**Rees 2013** USA (ND) *Adults w/previous colorectal adenoma*	**2228 (1113/1115)** 42.3% (321) 61.2 (6.6) (47.1-77.9)	**D3 vs. placebo** 25 ug/1000IU Daily 13 months (on average) All seasons	62.5(21.3) 30.2-171.6 0 (0%)	1 or more influenza or ILI episode since last semiannual phone call: VD 106/1113 vs. P 96/1115	0	Missing Adverse events Moderate
9	**Primary** (URTI: influenza A diagnosed by RIDT or RIDT negative ILI)	**Urashima 2014** Japan (Tokyo) *High school students*	**247 (148/99)** 34.4% (85) 16.5 (1.0) (15-18)	**D3 vs. placebo** 50 ug /2000IU Daily 2 months Sep-Oct	ND	1 or more influenza A: VD 20/148 vs. P 12/99	0	√ Moderate
10	**Primary** (URTI assessed with symptom score)	**Dubnov-Raz 2015** Israel (ND) *Adolecent swimmers with vitamin D insufficiency*	**55 (28/27)** 37% (20) 15.2 (1.6) (12.9-18.6)	**D3 vs. placebo** 50 ug /2000IU Daily 12 weeks Nov-Jan	60.4 (11.9) 28.0-74.6 0 (0%)	1 or more URTI: VD 11/28 vs. P 11/27 **Comment:** *In total, 33 subjects did not complete the diary (treated as if non URTI occurred)*	0	Missing: Adverse events Low
11	**Secondary** (URTI: assessed with symptom score)	**Denlinger 2016** USA (ND) *Adults with asthma*	**408 (201/207)** 68.1% (278) 39.2 (12.9) (18-85)	**D3 vs. placebo** 2.5 mg bolus then 100 ug daily 28 weeks All seasons	47.0 (16.9) 10.0-74.6 55/408 (13.5%)	1 or more episode with cold: VD 161/201 vs P 139/207	0	Missing: Ethics approval Moderate
12	**Secondary** (LRTI)	**Manaseki-Holland 2010** Afghanistan(Kabul) *Infants 1-36 months w/pneumonia*	**453 (224/229)** 43,2% (196) 1.1 (0.8) (0.1-3.3)	**D3 vs. placebo** 2.5 mg/100,000 IU One-time bolus 3 months Dec-Feb	ND	1 or more repeated episodes of pneumonia: VD 92/204 vs. P 122/211	1	Missing: Conflict of interest Moderate

13	**Secondary** (URTI: Self-reported)	**Lehouck 2012** Belgium (Leuven) *Adults with COPD*	**182 (91/91)** 20.3% (37) 67.9 (8.3) (48-86)	**D3 vs. placebo** 2.5 mg/100,000 IU Monthly 1 year All seasons	49.8 (29.2) 9.0-159.7 31/182 (17.0%)	<u>1 or more exacerbation after 4 moths:</u> VD 91-31=60/91 vs. P 91-30=61/91	0	√ High
14	**Primary** (LRTI: Pneumonia confirmed by chest radiography)	**Manaseki-Holland 2012** Afghanistan (Kabul*) *Infants 1-11 months*	**3046 (1524/1522)** 47.8% (1455) 0.5 (0.3) (0.0-1.0)	**D3 vs. placebo** 2.5 mg/100,000 IU Bolus every 3rd month 1.5 years All seasons	ND	<u>Roughly estimated from fig 2:</u> After 360 days (1 year) ≈13% of children in VD group and in P group had first RTI event: VD: 13%*1524 ≈ 198/1524 vs.P: 13%*1522 ≈ 198/1522	0	√ High
15	**Primary** (URTI: Self-reported cold)	**Goodall 2014** Canada (Ontario) *Healthy university students*	**600 (300/300)** 50.2% (301) 19.6 (2.2) (17-33)	**D3 vs. placebo** 0.25 mg/10,000 IU Weekly 2 months Sep-Oct	ND	<u>1 or more clinical URTI:</u> VD 70/300 vs 80/300	1	Missing: Adverse events Moderate
16	**Secondary** (URTI: Self-reported cold)	**Tran 2014** Australia (Multicenter) *Healthy older adults*	**410 (205/205)** *46.7% (301) 71.7 (6.9) (60.3-85.2)*	**D3 vs. placebo** 1.5 mg/60,000 IU Monthly 1 year All seasons	41.7 (13.7) *12.6-105.0 66/643 (10.3%)*	<u>1 or more AB prescription:</u> AB: VD60 76/205 vs. P 92/205	0	√ Moderate
17	**Coprimary** (URTI: Assessed from daily symptom dairy)	**Martineau 2015a (ViDiCO)** UK (London) *Adults with COPD*	**240 (122/118)** 40% (96) 64.7 (8.5) (40-85)	**D3 vs. placebo** 3 mg/120,000 IU bolus Every 2nd month 1 year All seasons	46.1 (25.7) 0.0-160.0 50/240 (20.8%)	<u>1 or more URTI:</u> 1 or more URTI: VD 76/102 vs. P 75/103	0	√ High

18	**Coprimary** (URTI: Assessed from daily symptom dairy)	**Martineau 2015b (ViDiAs)** UK (London) *Adults with Asthma*	250 (125/125) 56.4% (141) 47.9 (14.4) (16-78)	D3 vs. placebo 3 mg/120,000 IU bolus Every 2nd month 1 year All seasons	49.6 (24.7) 0.0-139.0 36/250 (14.4%)	1 or more URTI: VD 85/115 vs. P 93/117	0	√ High
19	**Coprimary** (URTI and LRTI, both assessed from daily symptom dairy)	**Martineau 2015c (ViDiFlu)** UK (London) *Older adults*	240 (137/103) 65.8% (158) 67.1 (13.0) (21.4-94)	D3 vs. placebo+10ugD3 2.4 mg bolus every 2nd month + 10 ug daily 1 year All seasons	42.9 (23.0) 0.0-128.0 60/240 (25%)	1 or more ARTI: VD 83/125 vs. P 58/92	0	√ Moderate
		Martineau 2015c (ViDiFlu) UK (London) *Carers*		D3 vs. placebo 3 mg/120,000 IU Bolus every 2nd month 1 year All seasons				
20	**Coprimary** *(pneumonia: assessed by clinical diagnose)*	**Gupta 2016** India (New Dehli) *Tertiari care children w/pneumonia*	**324 (162/162)** 30.2% (98) 16.7 (13.2) 6 months – 5 yrs	**D3 vs. placebo** 100,000 IU 1 time bolus 6 month follow-up	ND ND 39% (126) < 30 nmol/L	1 or more recurrent pneumonia within 6 months: VD 39/156 vs. P 36/158	0	√ Moderate
21	**Secondary Infections** (URTI: Self-reported cold bronchitis, influenza and/or ILI last 6 months)	**Jorde 2016** Norway (Tromsø) *Adults with prediabetes*	**511 (256/255)** 38.6% (197) 62.1 (8.7) 38-80	**D3 vs. placebo** 20,000 IU Weekly 5 years All	60.5 (21.6) ND ND	One or more ILI: VD 256-137=119/256 vs. P 255-158=97/255	0	√ Low - Moderate

ND = No data; NR = Not relevant; NA = Not Assessed; DL = Downloaded; VD = Vitamin D; BL = Baseline; URTI = Upper respiratory tract infection; ARTI = Acute respiratory tract infection; ILI = Influenza-like-illness; RIDT = Rapid influenza diagnostic test; LBW = Low birthweight infants; AOM = Acute Otitis Media, PP = Per Protocol;

Ethics screening terms: approved, interest, conflict, fund, grant, support, declar, adverse, side, safety. Ng/mL $\rightarrow$ multiplying by 2.496 $\rightarrow$ nmol/L, 1 ug = 40 IU.

Table 2 – PICO table of studies on all-cause mortality

PICO table of included studies studying the effect of vitamin D supplementation on incidence of all-cause mortality.

Number	All-cause mortality as outcome (Primary outcome)	Ref Publication year Country (stat) Latitude *Population*	N (VD/placebo) %F (n) Mean age (SD) (Age range)	Intervention vs control Dose Interval Length of trial Season	BL 25OHD nmol/l, mean (SD) BL 25OHD nmol/l, range n < 25nmol/L (%)	Source from which numbers are extracted Incidence of 1 or more RTI event, (n/total n) in intervention group vs control group)	Study conclusion Positive (1) Indifferent (0) Negative (-1) NA	Ethical standards met upon screening /GRADE
1	**Pre-specified Secondary** (Secondary fracture prevention)	**Avenell, RECORD 2012** UK (England and Scotland) ND *Low-trauma fracture last 10y*	**2675 (1343/1332)** 85 (2274) 77 (6) 70+	**D3 vs. Placebo** 20 ug / 800IU Daily 322 (median 6.2yrs) All	ND	Extracted from table: VD only 421/1343 vs P 434/1332	0	Missing: Adverse events Moderate
2	**Coprimary** (All-cause death or cancers requiring hospitalization, chemo or surgery)	**Punthakee** TIDE **2012** Multi-center *Diabetes or increased HbA1c*	**1221 (607/614)** 41% (499) 66.4 (6.6) *VD: 66.6 (6.3) P: 66.7 (6.7)* ND	**D3 vs. placebo** 1000 IU Daily 23.1 weeks (mean follow up 162 days) ND	ND	Extracted from Table 2 and flow chart: VD 0/607 vs. P 2/614 **Comment:** - Planned intervention time 5 yrs, stopped prematurely due to regulatory concerns	None	√ Low
3	**Primary**	**Zittermann** EVITA **2017**	**400 (199/201)** 44,5% (178) ND 18-79	**D3 vs. placebo** 4000 IU Daily 156 (3 yrs) All	ND	Extracted from text, section: Primary endpoint by treatment group VD 39/199 (19.6%) vs. P 36/201 (17.9%)	0	√ High

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		Germany (North Rhine-Westphalia) 51N *Adults w/advanced heart failure*						
4	**Co-primary**	**Manson,** VITAL **2019** UK *Men 50+ and females 55+*	25,871 (12,927/12,944) 50.6 (13,085) 67.1 (7.1) ND	D3 vs. placebo 50ug/2000IU Daily 275 weeks (5.3 yrs)	76.9 (25.0) ND ND	Extracted from text, section: Results 485/12927 in VD vs. 493/12944 P	0	√ High
5	**Predefined secondary** (TB score reduction)	**Wejse 2009** Guinea-Bissau (ND) ND *Patients with pulmonary TB*	**365 (187/178)** 39,2% (143) 37.5 (13.5) ND	D3 vs. placebo 100,000 IU po Monthly (at BL, 5 and 8 months) 52 weeks ND	78.3 (22.8) ND ND	Extracted from abstract-results: VD 30/187 (16%) vs. P 24/178 (13%) **Comment:** HIV positive subjects included	0	√ Moderate
6	**Not assessed** (Mediolateral body sway)	**Lips 2010** Multi-center: Europe, north America *VD insufficient adults*	**226 (114/112)** ND 78.1 (6.4) 70+	**D3 vs. placebo** 210 ug / 8400 IU Weekly 16 weeks Winter (Oct-Jun)	34.7 (12.2) ND ND	Extracted from text, section: Safety VD 1/114 vs. 0/112 **Comment**: - Co-admin Ca up to 500 mg (in subj using < 1000 mg/d)	NA	√ Low-Moderate
7	**Not assessed** (risk reduction of falls and fractures)	**Sanders 2010** Australia (Victoria) 38S (-38N) *Community-dwelling women*	**2258 (1131/1127)** 100% ND ND	**D3 vs. placebo** 12,500ug/500,000IU Annual 3-5 years Autumn/winter	ND ND ND	Extracted from text, section Adverse Events: VD 40/1131 vs. P 47/1127	NA	√ Low-Moderate

70 yrs or older

#	Outcome	Study	N (groups)	Intervention	25OHD	Mortality/Adverse events		Quality
8	**Not assessed** (insulin sensitivity and secretion and lipids)	**Grimnes 2011** Norway (Tromsø) 69N *Healthy adults*	**94 (49/45)** 47.9 (45) 52.1 (9.2) 30-85	**D3 vs. placebo** 500 ug/20,000 IU Twice weekly 6 months	40.8 (13.0) ND ND	Extracted from adverse events/flow chart: VD 0/51 vs. P 1/53	NA	√ Low-Moderate
9	**Not assessed** (Reduce duration of pneumonia, reduce risk of recurrent pneumonia)	**Manaseki-Holland 2010** Afghanistan(Kabul) 34N *Infants (1-36 month of age) with pneumonia*	**453 (224/229)** 43,2% (196) 1.1 (0.8) (0.1-3.3)	**D3 vs. placebo** 2.5 mg/100,000 IU One-time bolus 12 weeks Dec-Feb	ND	Extracted from flow chart: VD 2/224 vs. P 1/229	NA	Missing: Conflict of interest Low-Moderate
10	**Not assessed** (quality of life)	**Witham 2010** UK, Scotland (Tayside and Fife) ND *Systolic heart failure and 25OHD < 50 nmol/L*	**105 (53/52)** 34.3 % (36) 79.7 (5.6) 70+	D2 vs. placebo 100,000 IU Bolus (BL+ at 10w) 20 weeks All seasons	22.1 (9.4) ND ND	Extracted from Table 5, Adverse Events: VD 4/53 vs. P 2/52	NA	√ Low-Moderate
11	**Not assessed** (COPD ex risk-reduction)	**Lehouck 2012** Belgium (Leuven) 50N *Moderate to severe COPD +history of recent*	**182 (91/91)** 20.3 % (37) 67.9 (8.3) 48-86	**D3 vs. placebo** 2,5 mg/100,000 IU Monthly 52 weeks (1 year) All	49,8 (29,2) 9.0-159.7 17,0 (31/182)	Extracted from flow chart VD 9/91 vs. P 6/91 deaths	NA	√ Low-Moderate

exacerbation

12	**Not assessed** (LRTI: Pneumonia confirmed by chest radiography)	**Manaseki-Holland 2012** Afghanistan (Kabul) 34N *Infants 1-11 months*	**3046 (1524/1522)** 47.8% (1455) 0.5 (0.3) (0.0-1.0)	**D3 vs. placebo** 2.5 mg/100,000 IU Bolus every 3rd month 1.5 years All seasons	ND	Extracted from flow chart: VD 10/1524 vs. P 7/1522	NA	√ Low-Moderate
13	**Not assessed** (difference in BP at 3 months)	**Witham** VitDISH **2013** UK (Glasgow) 55N *Community dwelling olders with ISH*	**159 (80/79)** 48.4% (77) 66.6 (6.5) 70+	**D3 vs. placebo** 100,000 IU Monthly (at BL 3-, 6- and 9 months) 52 weeks (1 y) All	44.9 (15.0) ND ND	Extracted from flow chart: VD 0/80 vs. P 1/79 **Comment:** - Death occurred before 3-mo visit	NA	√ Low-Moderate
14	**Secondary** (length of hospital stay)	**Amrein,** VITdAL-ICU **2014** Austria (Graz) 47N *ICU patients with 25OHD < 50 nmol/l*	**475 (237/238)** 34,9% 64.6 (14.7) 18+	**D3 vs placebo** Bolus 540,000IU + 90,000IU monthly 20 weeks All	32.45nmol/L (10.23) ND 42% (< 30nmol/L)	Extracted from Table 2: VD 83/237 vs. P 102/238	0	√ High
15	**Co-primary**	**Scragg,** ViDA **2017** New Zealand (Auckland) 36S (-36N) *Community resident adults*	**5108 (2558/2550)** 41.9 (2139) 65.9 (8.3) 50-84 yrs	**D3 vs. placebo** Bolus 200,000IU + Monthly 100,000IU Montly 171 weeks (3.3 yrs)	66.1 (22.5) ND ND	Extracted from flow chart: VD 65/2558 vs. 58/2550	0	√ High

ND = No data; NR = Not relevant; NA = Not Assessed; DL = Downloaded, BL = Baseline; VD = Vitamin D; PT = physio therapy, ICU = intensive care unit, CLAD = Chronic lung allograft dysfunction; VAP = Ventilator associated pneumonia. Ng/mL to nmol/L: Multiplying by 2.496. ug to IU: Multiplying by 40.

Figure 1 - Selection of studies on RTI
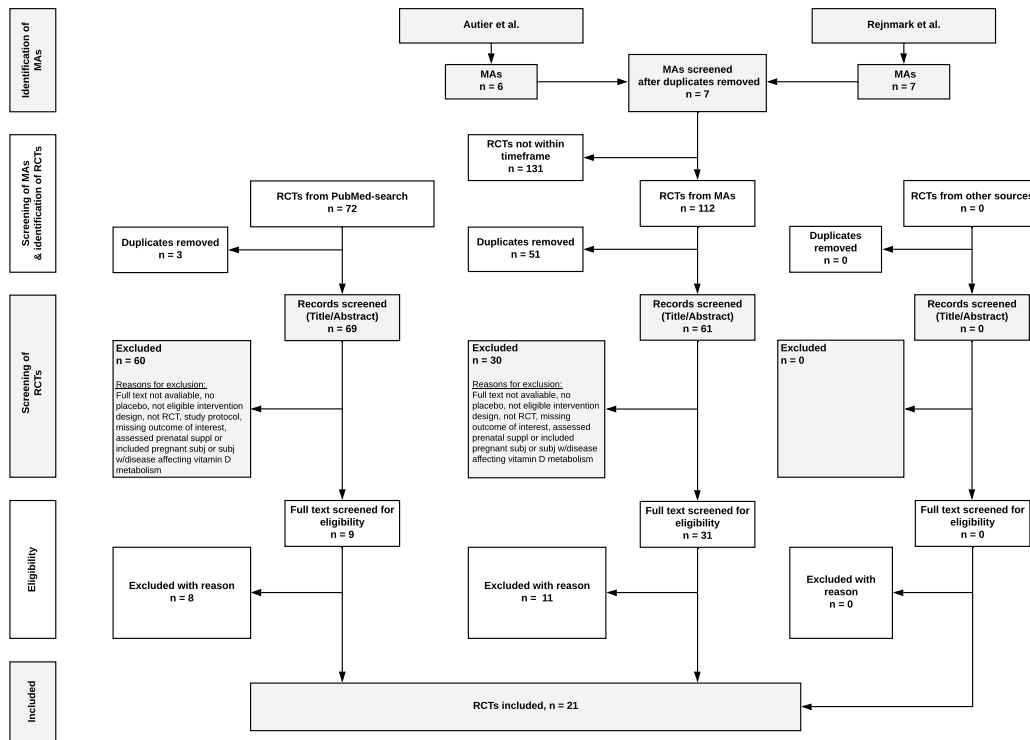
Flow chart on the selection process of studies on respiratory tract infections (RTIs)

Figure 2 - Selection of studies on all-cause mortality

Flow chart on the selection process of studies on all-cause mortality

Figure 3 - Funnel plot RTI

Figure 4 - Funnel plot all-cause mortality

Figure 5 - Forest plot summarizing the results of trials on respiratory tract infections

Author, year	Dose interval	Events vitamin D	Events placebo	Odds ratio (95% CI)	% Weight
Daily					
Li-Ng, 2009	Daily	28/78	29/70	0.79 (0.41, 1.54)	3.89
Laaksi, 2010	Daily	39/80	50/80	0.65 (0.35, 1.20)	5.41
Urashima, 2010	Daily	18/167	31/167	0.53 (0.28, 0.99)	6.44
Bergman, 2012	Daily			0.35 (0.15, 0.78)	7.05
Camargo, 2012	Daily	72/141	55/104	0.95 (0.57, 1.57)	4.50
Murdoch, 2012	Daily	63/70	64/70	0.84 (0.27, 2.65)	1.20
Marchisio, 2013	Daily	26/58	38/58	0.43 (0.20, 0.90)	6.47
Rees, 2013	Daily	106/1113	96/1115	1.12 (0.84, 1.49)	6.84
Urashima, 2014	Daily	20/128	12/99	1.13 (0.53, 2.44)	1.76
Dubnov-Raz, 2015	Daily	11/28	11/27	0.94 (0.32, 2.77)	1.14
Denlinger, 2016	Daily	161/201	139/207	1.97 (1.25, 3.09)	1.87
Subtotal (I-squared = 57.3%, p = 0.009)				0.77 (0.54, 1.01)	46.58
Less frequent					
Manaseki-Holland, 2010	Bolus	92/204	122/211	0.60 (0.41, 0.88)	8.36
Lehouck, 2012	Monthly	60/91	61/91	0.95 (0.51, 1.76)	3.40
Manaseki-Holland, 2012	Monthly	198/1524	198/1522	1.00 (0.81, 1.23)	8.81
Goodall, 2014	Weekly	70/300	80/300	0.84 (0.58, 1.21)	7.02
TranA, 2014	Monthly	76/129	92/205	0.72 (0.49, 1.07)	7.41
MartineauA, 2015	2 monthly	76/102	76/102	1.09 (0.59, 2.03)	2.75
MartineauB, 2015	2 monthly	85/115	93/117	0.73 (0.40, 1.35)	4.79
MartineauC, 2015	2 monthly	83/125	58/92	1.16 (0.66, 2.03)	2.96
Gupta, 2016	Bolus	39/156	36/158	1.13 (0.67, 1.90)	3.49
Jorde, 2016	Weekly	119/256	97/255	1.41 (0.99, 2.01)	4.43
Subtotal (I-squared = 35.1%, p = 0.127)				0.89 (0.73, 1.04)	53.42
Overall (I-squared = 50.6%, p = 0.004)				0.83 (0.70, 0.97)	100.00

NOTE: Weights are from random effects analysis

Favours vitamin D — Favours placebo
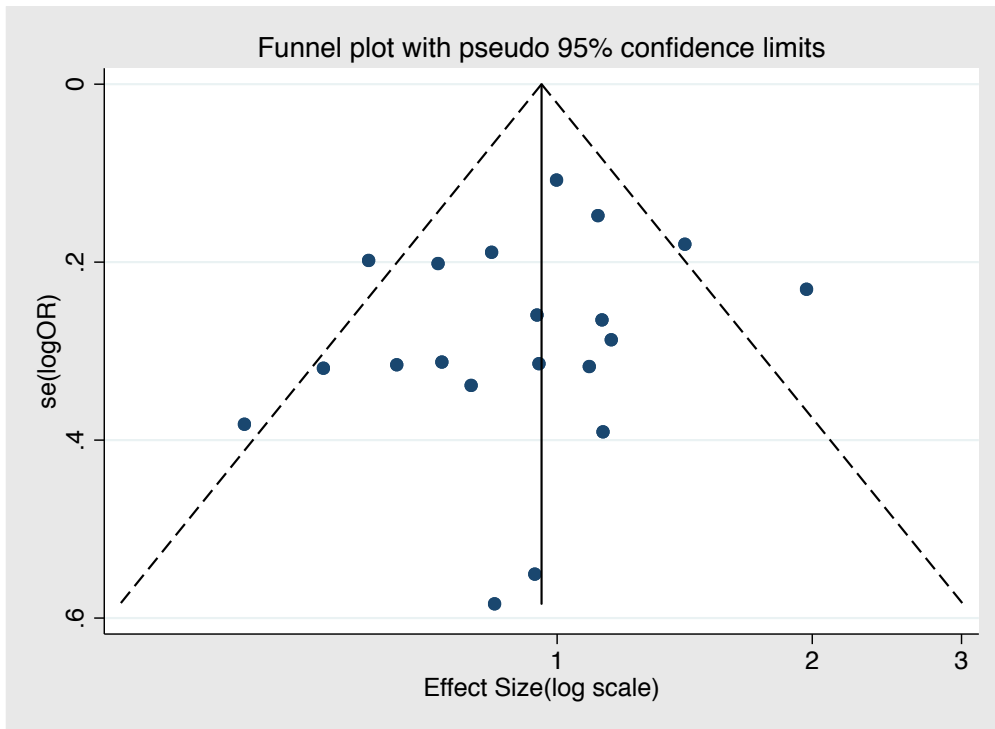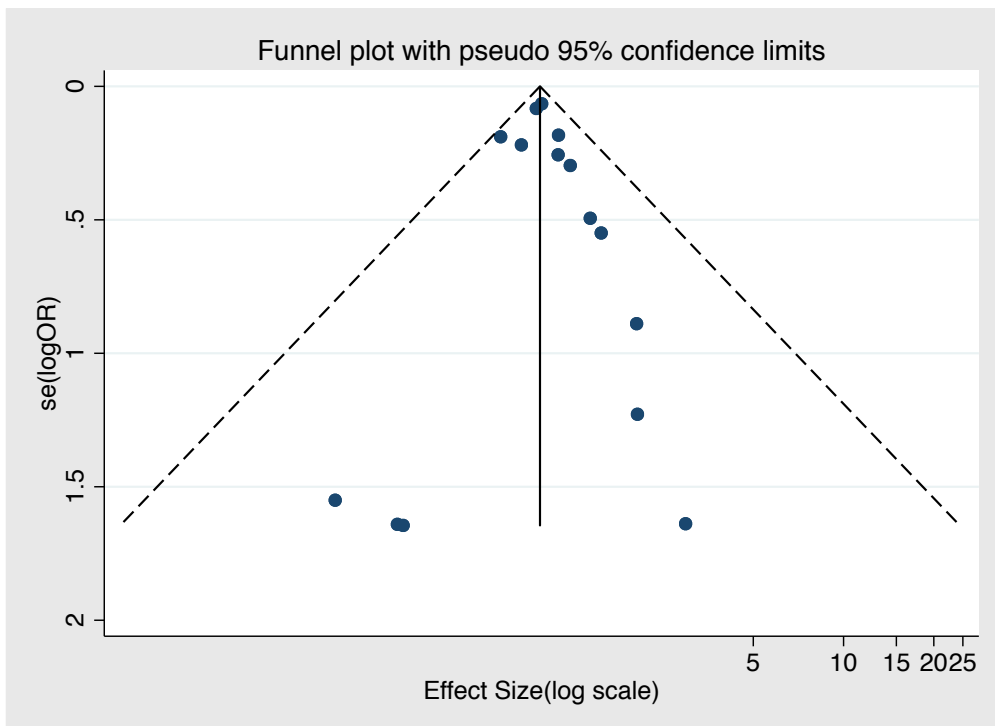
Figure 6 – Forest plot summarizing the results of trials done in children on respiratory tract infections

Author, year	Dose interval	Events vitamin D	Events placebo	Odds ratio (95% CI)	% Weight
Daily					
Urashima, 2010	Daily	18/167	31/167	0.53 (0.28, 0.99)	16.95
Camargo, 2012	Daily	72/141	55/104	0.95 (0.57, 1.57)	11.97
Marchisio, 2013	Daily	26/58	38/58	0.43 (0.20, 0.90)	17.05
Subtotal (I-squared = 29.6%, p = 0.242)				0.59 (0.32, 0.86)	45.97
Less frequent					
Manaseki-Holland, 2010	Bolus	92/204	122/211	0.60 (0.41, 0.88)	21.79
Manaseki-Holland, 2012	Monthly	198/1524	198/1522	1.00 (0.81, 1.23)	22.92
Gupta, 2016	Bolus	39/156	36/158	1.13 (0.67, 1.90)	9.32
Subtotal (I-squared = 71.1%, p = 0.032)				0.86 (0.54, 1.19)	54.03
Overall (I-squared = 63.2%, p = 0.019)				0.74 (0.51, 0.97)	100.00

NOTE: Weights are from random effects analysis

Favours vitamin D — Favours placebo

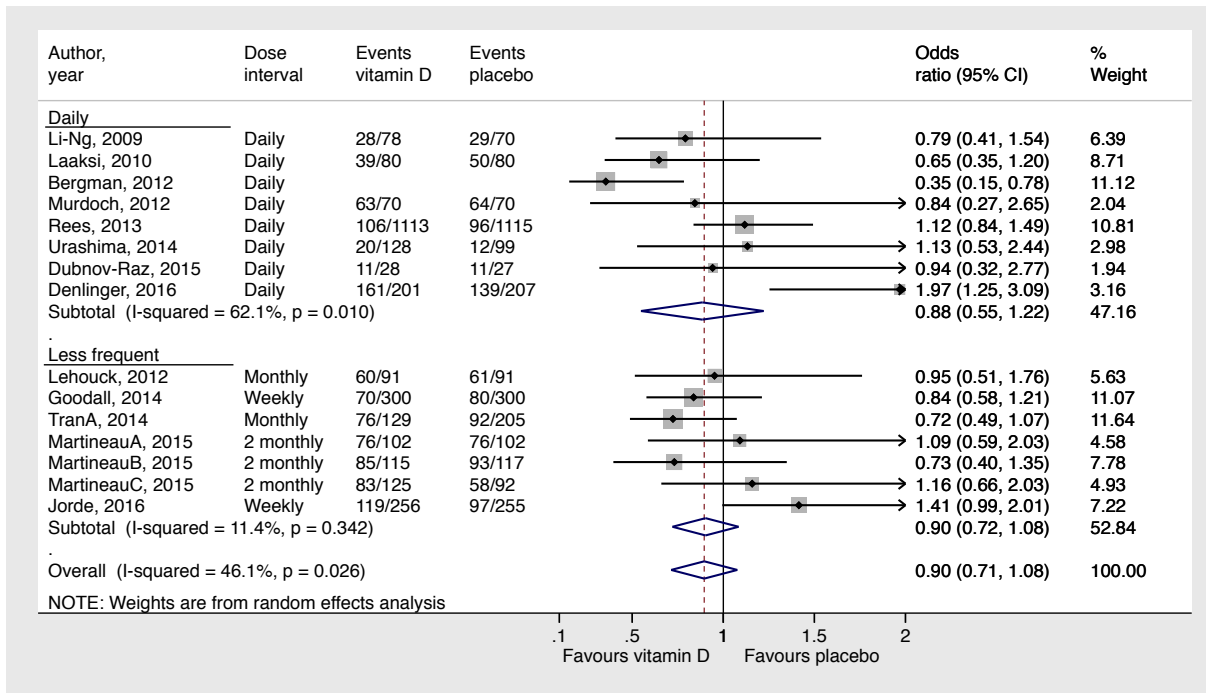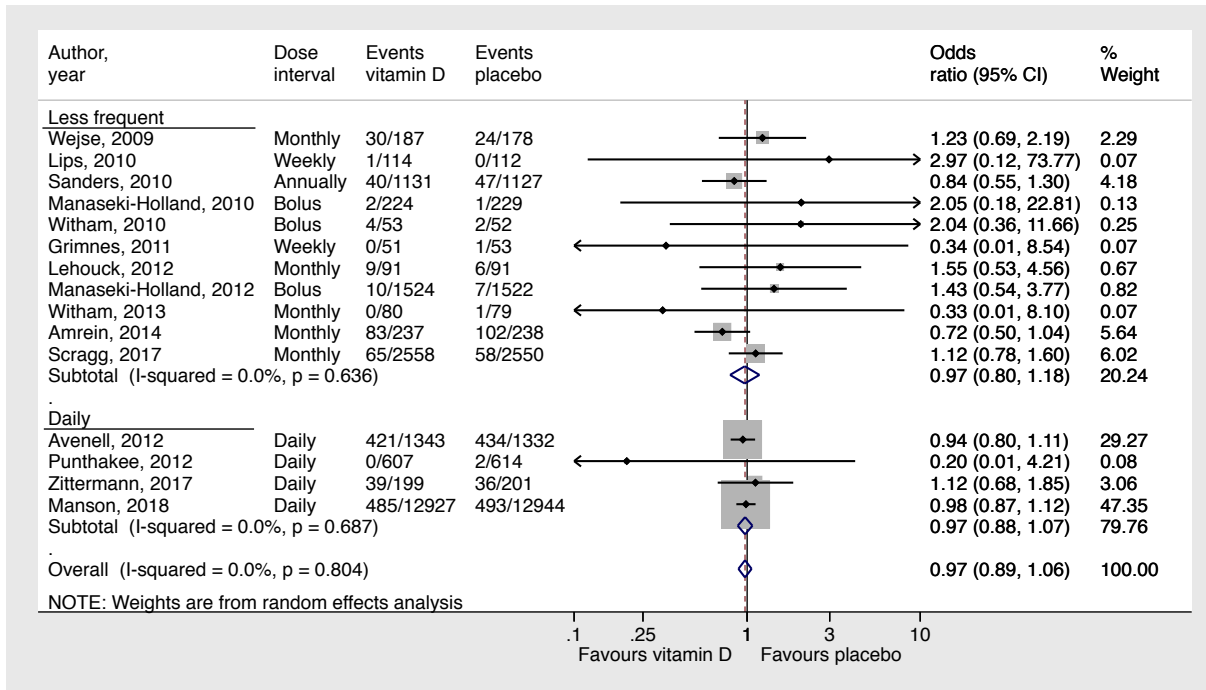*Figure 7 – Forest plot summarizing the results of trials done in adults on respiratory tract infections*

Author, year	Dose interval	Events vitamin D	Events placebo	Odds ratio (95% CI)	% Weight
Daily					
Li-Ng, 2009	Daily	28/78	29/70	0.79 (0.41, 1.54)	6.39
Laaksi, 2010	Daily	39/80	50/80	0.65 (0.35, 1.20)	8.71
Bergman, 2012	Daily			0.35 (0.15, 0.78)	11.12
Murdoch, 2012	Daily	63/70	64/70	0.84 (0.27, 2.65)	2.04
Rees, 2013	Daily	106/1113	96/1115	1.12 (0.84, 1.49)	10.81
Urashima, 2014	Daily	20/128	12/99	1.13 (0.53, 2.44)	2.98
Dubnov-Raz, 2015	Daily	11/28	11/27	0.94 (0.32, 2.77)	1.94
Denlinger, 2016	Daily	161/201	139/207	1.97 (1.25, 3.09)	3.16
Subtotal (I-squared = 62.1%, p = 0.010)				0.88 (0.55, 1.22)	47.16
.					
Less frequent					
Lehouck, 2012	Monthly	60/91	61/91	0.95 (0.51, 1.76)	5.63
Goodall, 2014	Weekly	70/300	80/300	0.84 (0.58, 1.21)	11.07
TranA, 2014	Monthly	76/129	92/205	0.72 (0.49, 1.07)	11.64
MartineauA, 2015	2 monthly	76/102	76/102	1.09 (0.59, 2.03)	4.58
MartineauB, 2015	2 monthly	85/115	93/117	0.73 (0.40, 1.35)	7.78
MartineauC, 2015	2 monthly	83/125	58/92	1.16 (0.66, 2.03)	4.93
Jorde, 2016	Weekly	119/256	97/255	1.41 (0.99, 2.01)	7.22
Subtotal (I-squared = 11.4%, p = 0.342)				0.90 (0.72, 1.08)	52.84
.					
Overall (I-squared = 46.1%, p = 0.026)				0.90 (0.71, 1.08)	100.00

NOTE: Weights are from random effects analysis

.1   .5   1   1.5   2
Favours vitamin D   Favours placebo

Figure 8 – Forest plot summarizing the results of trials on all-cause mortality

Author, year	Dose interval	Events vitamin D	Events placebo	Odds ratio (95% CI)	% Weight
Less frequent					
Wejse, 2009	Monthly	30/187	24/178	1.23 (0.69, 2.19)	2.29
Lips, 2010	Weekly	1/114	0/112	2.97 (0.12, 73.77)	0.07
Sanders, 2010	Annually	40/1131	47/1127	0.84 (0.55, 1.30)	4.18
Manaseki-Holland, 2010	Bolus	2/224	1/229	2.05 (0.18, 22.81)	0.13
Witham, 2010	Bolus	4/53	2/52	2.04 (0.36, 11.66)	0.25
Grimnes, 2011	Weekly	0/51	1/53	0.34 (0.01, 8.54)	0.07
Lehouck, 2012	Monthly	9/91	6/91	1.55 (0.53, 4.56)	0.67
Manaseki-Holland, 2012	Bolus	10/1524	7/1522	1.43 (0.54, 3.77)	0.82
Witham, 2013	Monthly	0/80	1/79	0.33 (0.01, 8.10)	0.07
Amrein, 2014	Monthly	83/237	102/238	0.72 (0.50, 1.04)	5.64
Scragg, 2017	Monthly	65/2558	58/2550	1.12 (0.78, 1.60)	6.02
Subtotal (I-squared = 0.0%, p = 0.636)				0.97 (0.80, 1.18)	20.24
.					
Daily					
Avenell, 2012	Daily	421/1343	434/1332	0.94 (0.80, 1.11)	29.27
Punthakee, 2012	Daily	0/607	2/614	0.20 (0.01, 4.21)	0.08
Zittermann, 2017	Daily	39/199	36/201	1.12 (0.68, 1.85)	3.06
Manson, 2018	Daily	485/12927	493/12944	0.98 (0.87, 1.12)	47.35
Subtotal (I-squared = 0.0%, p = 0.687)				0.97 (0.88, 1.07)	79.76
.					
Overall (I-squared = 0.0%, p = 0.804)				0.97 (0.89, 1.06)	100.00

NOTE: Weights are from random effects analysis

.1   .25   1   3   10
Favours vitamin D   Favours placebo

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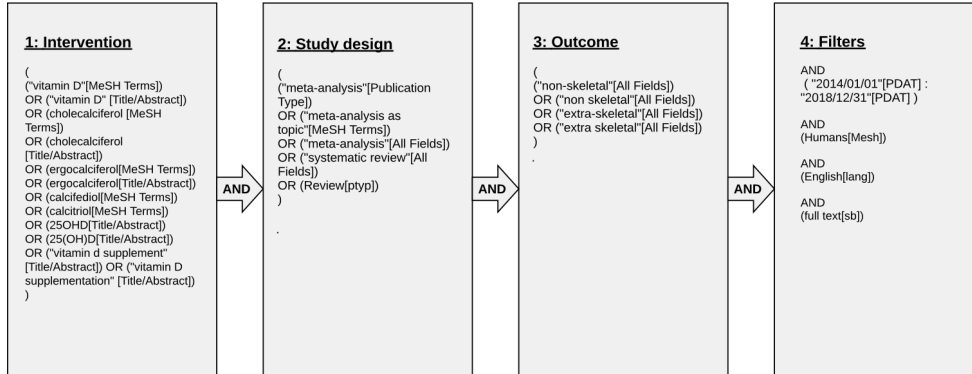
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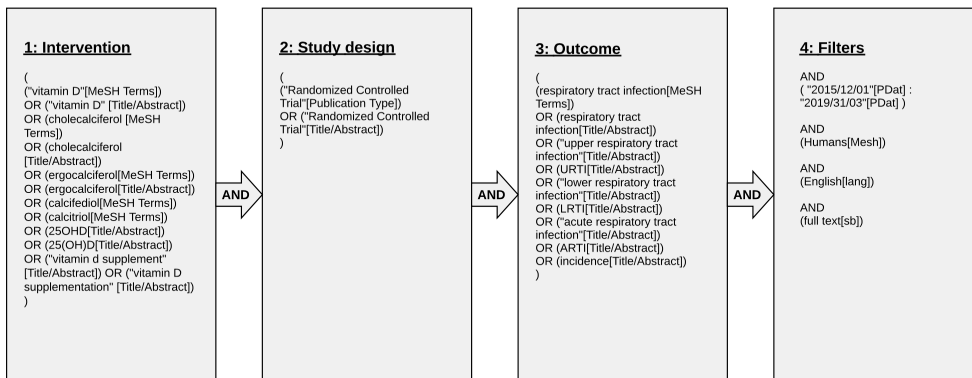
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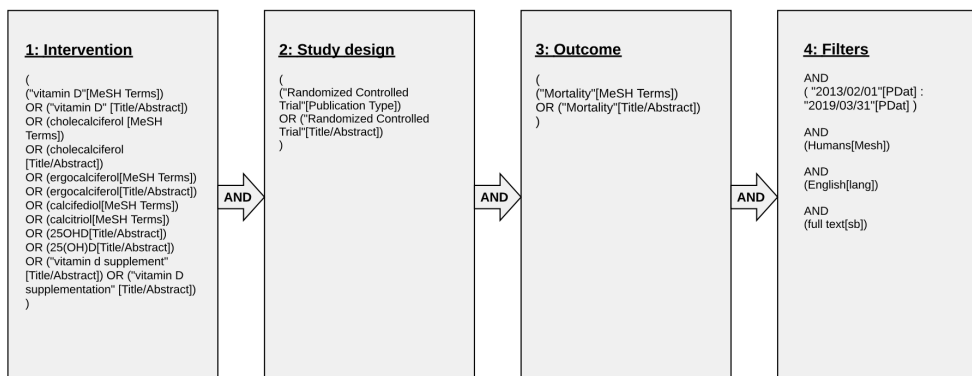
Appendix

Figure S1 – Literature search to identify systematic reviews of meta-analyses summarizing trial data

1: Intervention

(
("vitamin D"[MeSH Terms])
OR ("vitamin D" [Title/Abstract])
OR (cholecalciferol [MeSH Terms])
OR (cholecalciferol [Title/Abstract])
OR (ergocalciferol[MeSH Terms])
OR (ergocalciferol[Title/Abstract])
OR (calcifediol[MeSH Terms])
OR (calcitriol[MeSH Terms])
OR (25OHD[Title/Abstract])
OR (25(OH)D[Title/Abstract])
OR ("vitamin d supplement"
[Title/Abstract]) OR ("vitamin D
supplementation" [Title/Abstract])
)

AND

2: Study design

(
("meta-analysis"[Publication Type])
OR ("meta-analysis as topic"[MeSH Terms])
OR ("meta-analysis"[All Fields])
OR ("systematic review"[All Fields])
OR (Review[ptyp])
)

.

AND

3: Outcome

(
("non-skeletal"[All Fields])
OR ("non skeletal"[All Fields])
OR ("extra-skeletal"[All Fields])
OR ("extra skeletal"[All Fields])
)
.

AND

4: Filters

AND
("2014/01/01"[PDAT] :
"2018/12/31"[PDAT])

AND
(Humans[Mesh])

AND
(English[lang])

AND
(full text[sb])

Figure S2 – Literature search to identify additional randomized controlled trials on respiratory tract infections

1: Intervention

(
("vitamin D"[MeSH Terms])
OR ("vitamin D" [Title/Abstract])
OR (cholecalciferol [MeSH Terms])
OR (cholecalciferol [Title/Abstract])
OR (ergocalciferol[MeSH Terms])
OR (ergocalciferol[Title/Abstract])
OR (calcifediol[MeSH Terms])
OR (calcitriol[MeSH Terms])
OR (25OHD[Title/Abstract])
OR (25(OH)D[Title/Abstract])
OR ("vitamin d supplement"
[Title/Abstract]) OR ("vitamin D
supplementation" [Title/Abstract])
)

AND

2: Study design

(
("Randomized Controlled Trial"[Publication Type])
OR ("Randomized Controlled Trial"[Title/Abstract])
)

AND

3: Outcome

(
(respiratory tract infection[MeSH Terms])
OR (respiratory tract infection[Title/Abstract])
OR ("upper respiratory tract infection"[Title/Abstract])
OR (URTI[Title/Abstract])
OR ("lower respiratory tract infection"[Title/Abstract])
OR (LRTI[Title/Abstract])
OR ("acute respiratory tract infection"[Title/Abstract])
OR (ARTI[Title/Abstract])
OR (incidence[Title/Abstract])
)

AND

4: Filters

AND
("2015/12/01"[PDat] :
"2019/31/03"[PDAT])

AND
(Humans[Mesh])

AND
(English[lang])

AND
(full text[sb])

Figure S3 – Literature search to identify additional randomized controlled trials on all-cause mortality

1: Intervention

(
("vitamin D"[MeSH Terms])
OR ("vitamin D" [Title/Abstract])
OR (cholecalciferol [MeSH Terms])
OR (cholecalciferol [Title/Abstract])
OR (ergocalciferol[MeSH Terms])
OR (ergocalciferol[Title/Abstract])
OR (calcifediol[MeSH Terms])
OR (calcitriol[MeSH Terms])
OR (25OHD[Title/Abstract])
OR (25(OH)D[Title/Abstract])
OR ("vitamin d supplement"
[Title/Abstract]) OR ("vitamin D
supplementation" [Title/Abstract])
)

AND

2: Study design

(
("Randomized Controlled Trial"[Publication Type])
OR ("Randomized Controlled Trial"[Title/Abstract])
)

AND

3: Outcome

(
("Mortality"[MeSH Terms])
OR ("Mortality"[Title/Abstract])
)

AND

4: Filters

AND
("2013/02/01"[PDat] :
"2019/03/31"[PDat])

AND
(Humans[Mesh])

AND
(English[lang])

AND
(full text[sb])

Supplemental Table S1 – Characteristics of studies on respiratory tract infections excluded after full-text screening, and reason for exclusion.

Author, year	Country	Number of participants	Dosing regimen	Length of trial (weeks)	Category of exclusion	Reason for exclusion
Hanson 2011	USA	52	Daily	1	Outcome missing	Missing infectious outcome (assessed effect on 25OHD levels).
Trilok-Kumar 2011	India	2079	Weekly	24	Outcome missing	Missing infectious outcome (assessed effect on mortality, morbidity and growth).
Choudhary, 2012	India	200	Daily	8 (minimum)	Study of treatment effect	Study of treatment effect (assessed effect on time to resolution of severe pneumonia).
Ganmaa, 2012	Mongolia	120	Daily	24	Study of treatment effect	Study of treatment effect (assessed effect on tuberculin skin test conversion).
Jorde, 2012	Multinational	Mixed	Mixed	Mixed	Study design	Mix of several RCTs and dosing-regimens.
Camargo, 2014	Mongolia	107	Daily	4	Outcome missing	Missing infectious outcome (assessed effect on winter-related atopic dermatitis).
Economos, 2014	USA	180	Daily	12	Study of treatment effect	Study of treatment effect (assessed effect on time to resolution of severe pneumonia).
Rajakumar, 2015	USA	157	Daily	24	Outcome missing	Missing infectious outcome (assessed effect on 25OHD and PTH-levels, and markers of bone turnover).
Ginde, 2016	USA	107	Daily and monthly	52	Intervention	Used both daily and monthly dosing in the intervention group.
Pommergaard, 2016	Multinational	1107	Daily	3 years	Co-intervention	Administered co-interventions only in the intervention-group.

Sanjari, 2016	Iran	135	Daily	1	Study of treatment effect	Study of treatment effect (assessed effect on C reactive protein and COPD exacerbation).
Tachimoto, 2016	Japan	89	Daily	2	Study of treatment effect	Study of treatment effect (assessed effect on asthma control).
Chowdhury, 2017	India	960	NR	NR	Intervention	Not VD.
Ganmaa, 2017	Mongolia	390	Biweekly	8	Study of treatment effect	Study of treatment effect (assessed effect on response to antimicrobial therapy in TB-subj).
Lappe, 2017	USA	2303	Daily	4 years	Outcome missing	Missing infectious outcome (assessed effect on cancer-risk reduction).
Rafiq, 2017	Netherland	50	Daily	24	Outcome missing	Missing numbers for RR calculation (assessed effect on respiratory muscle strength).
Somnath, 2017	India	154	Single bolus dose	8 (minimum)	Study design	Open label (no blinding).
Jung, 2018	South-Korea	25	Daily	4	Sample size Outcome missing	N < 50 + Missing numbers for RR calculation.
Lee, 2018	USA	70	Monthly	96	Control	No placebo.

Supplemental Table S2 – Characteristics of studies on mortality excluded after full-text screening, and reason for exclusion.

Author, year	Country	Number of participants	Dosing regimen	Length of trial (weeks)	Category of exclusion	Reason for exclusion
Nagpal, 2009	India	71	Forth-nightly	6	Outcome missing	Missing numbers for RR calculation (no deaths occurred)
Zittermann, 2009	Germany	200	Daily	52	Outcome missing	Missing data on all-cause mortality (assessed effect on weight loss and cardiovascular disease markers)
Bizzarri, 2010	Italy	34	Daily	2 years	Sample size Outcome missing	N < 50 Missing data on all-cause mortality (assessed effect on …).
Janssen, 2010	Netherland	70	Daily	24	Outcome missing	1 death described but not to which group
Jorde, 2010	Norway	438	Weekly	52	Outcome missing	Missing numbers for RR calculation (no deaths occurred)
Cherniack, 2011	USA	34	Daily	24	Sample size	N < 50 Missing data on deaths
Mitri, 2011	USA	92	Daily	16	Outcome missing	Missing data on all-cause mortality (assessed effect on insulin sensitivity and response)
Glendenning, 2012	Australia	686	Monthly	36	Outcome missing	Missing data on all-cause mortality (assessed effect on falls, muscle strength and mobility).
Larsen, 2012	Denmark	112	Daily	20	Outcome missing	Missing data on all-cause mortality (assessed effect on 24-h BP)
Gallagher, 2013	USA	110	Daily	52	Outcome missing	Missing numbers for RR calculation (no deaths occurred)

Kane, 2013	USA	49	Daily	12	Sample size Outcome missing	Small N Missing data on all-cause mortality (assessed effect on lipid status)
Chandler, 2014	USA	105	Daily	12	Outcome missing	Missing data on all-cause mortality (assessed effect on free and total PSA)
Gallagher, 2014	USA	198	Daily	52	Outcome missing	Missing numbers for RR calculation (no deaths occurred)
Moretti, 2017	USA	40	Daily	24	Sample size Outcome missing	Small N Missing data on deaths
Vos, 2017	Belgium	87	Monthly	2 years	VD in both groups	Additional vitamin D 800IU/d in both groups as part of standard post-surgical prevention of osteoporosis

…

Supplemental Table S3 – GRADE: RESPIRATORY TRACT INFECTIONS

Li-Ng, 2009

Reference: Li-Ng M, Aloia JF, Pollack S, Cunha BA, Mikhail M, Yeh J, et al. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiology and infection. 2009;137(10):1396-404.	**Study design: RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate-High ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine whether vitamin D suppl during winter season prevents or decreases URI symptoms **Conclusion** *Vitamin D did not result in decreased incidence or severity of symptomatic URIs during winter* **Country** USA (New York) **Data collection period** 2006-2007	**Recruitment** Volunteers were recruited from local newspaper advertisements, mailing of brochures to community residents, and flyers posted at Winthrop University Hospital medical offices. **Inclusion-/exclusion criteria** • Inclusion criteria: Age 18-80 years and stable medical condition with no change in medications for 6 months prior to study entry. • Exclusion criteria: Morbid obesity (BMI >35), current tobacco use, history of hypercalcemia, nephrolithiasis or sarcoidosis, pregnancy, recent hospitalization, current liver or kidney disorders, malignancy, malabsorption and use of immunosuppressants or medications that infer with vitamin D metabolism such as phenytoin and carbamazepine. **Data** 162 participants were randomized (84 to vitamin D vs 78 to placebo). Participants were followed for 3 months, including visits at 6 and 12 weeks post-randomization. Use of calcium and vitamin D supplements was estimated by questionnaires. Baseline examination included medical history, height, weight and blood samples. Blood was collected again at the 12-week visit. **Outcome validation (i.e. diagnosis)** Incidence of URIs was recorded by a bi-weekly questionnaire. This questionnaire was modified from established validated instruments. URI was defined as the presence of two or more URI symptoms (fever, cough, productive sputum or change in sputum color and quantity, muscle aches, nausea or vomiting) and the absence of allergy symptoms (clear nasal discharge, watery eyes, and itchy nose). **Intervention variables** Vitamin D3 50 ug daily versus placebo. **Important confounding factors** Not described which factors were considered or how these were planned to be adjusted for. **Statistical methods** Chi square test was used to assess the effect of vitamin D suppl on incidence of URI, as was correlation and t-tests. Symptom severity and duration was measured both across the overall sample, and stratified by patient. The main analysis used all data. Secondary analyses ignored data for the first 4 weeks because it takes about 3 months for vitamin D levels to achieve steady state. Continous change (e.g. severity of illness was measured with a mixed-model repeated-measures ANOVA. A General Linear Model was used to analyze the incidence of URIs in repeated measures on the same patient. Means were expressed as 95% confidence intervals (CIs) and the confidence level set at P =.05.	**Main findings** There was no difference in the incidence of URIs between the vitamin D and placebo group (48/388 vs. 50/363 respectively, p=.56). There was no difference in URI duration (p=0.86) or symptom severity (p=0.4 between the vitamin D and the placebo group, effect estimates were not reported. For URI incidence the absolute risk reduction (**aRR**) was reported: 1.4% in favor of vitamin D, 95%CI [-2.4, 3.4] (not significant, p=0.56)	• **Ethics approval?** Approved by the institutional review board of Winthrop University Hospital. • **Adverse events?** Accounted for. • **Aim?** Defined, but not specified what was the primary end-point. • **Generalizability?** Not generalizable to hospitalized/severe cases of influenza • **Did the randomization work?** There were no significant differences between groups at baseline • **Procedure for randomization?** Computer-generated randomization sequence. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Yes. A modified version of the instrument was used (low risk of classification bias). • **Risk of attrition bias?** Low: 14 patients discontinued the study. • **Applicability in clinical practice?** Considering the population recruited, the results were representative in a generally healthy popylation of adults. Application in clinical situations are limited with regards to younger populations and populations with a non-stable medical condition • **All outcomes reviewed?** Yes. • **Cost/benefit:** Not evaluated. • **Findings supported by previous literature?** Findings in contrast with a finnish study by Laaksi et al, but supported by a sub-study analysis of the RECORD trial. **Strengths:** First RCT to evaluate vitamin D suppl in reducing incidence/severity of URIs during winter. High compliance rate and low drop-out rate. Objective definition of URI. High return-rate of completed questionnaires. Time between online questionnaires was 2 weeks, and so risk of recall-bias was low. Laboratory 25OHD content in capsules verified by independent laboratory. Vitamin D assessments were reliable as the laboratory participate in an international quality assessment program. **Weaknesses:** Supplementation started during, and not prior to, wintertime. It takes about 3 months for vitamin D to reach steady state, thus the effect of vitamin D suppl lagged behing the cold and influenza season. The vitamin D dose given might have been insufficient. Mean baseline 25OHD was not that low. Enrollment goals not met, thus limited power to detect effects (80% power to detect a 23% effect difference in incidence of URI between vitamin D and placebo). High influenza vaccination rate, indicating a health cautious population, and also reducing the power by reducing overall incidence of URIs **Plausible explanations for the results?** Yes.

Reference: Laaksi I, Ruohola JP, Mattila V, Auvinen A, Ylikomi T, Pihlajamäki H. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. J Infect Dis 2010; 202:809-14.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low ⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine wether vitamin D supplementation decreases the number of days absent from duty beacause of acute respiratory tract infection. **Conclusion** *Results indicate a preventive effect of vitamin D supplementation against respiratory tract infection.* **Country** Finland. **Data collection period** October 2005 – March 2006	**Recruitment** 400 young men (18-28 yrs) of an infantry unit in the Finnish Defence Forces undergoing compulsory periodic military training was invited to participate. **Inclusion-/exclusion criteria** • Inclusion criteria: No receipt of regular medication and having passed the entry medical examination as healthy. • Exclusion criteria: Exclusion criteria were the use of supplementary vitamin D, multivitamins, and cod liver oil. **Data** 164 (41%) volunteered to participate in the study and met the inclusion criteria. After randomization, blood samples were obtained from 73 subjects at the beginning of the study in October 2005 and again from 108 subjects in March 2006 to determine the serum 25(OH)D concentrations. Participants were followed for 6 months. **Outcome validation (i.e. diagnosis)** Identification of respiratory tract infections happened through review of medical records for all participants covering 6 months of military service. Any diagnosed acute respiratory tract infection (ie, sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, and laryngitis) was recorded. Secondary outcomes: were self-reported symptoms of acute respiratory tract infection and hospitalization due to acute respiratory tract infection. **Intervention variables** The subjects were randomly assigned to the intervention group, which received 400 IU (10 mg; n=80) vitamin D3 (Minisun; Verman) daily, or the placebo group (n=84) (Pharmia; a capsule identical in size and form to the active preparation). **Important confounding factors** Secondary outcomes were self-reported symptoms of acute respiratory tract infection, implementing a source of reporting bias. **Statistical methods** The power of the study were maximized by recruiting all voluntary conscripts and a priori sample size calculations were not performed. Primary analysis included all randomized subjects in accordance with the intention-to-treat principle. Differences between the groups in continuous variables were tested using the Mann-Whitney U test. x2 tests were used to assess categorical data. We set a 2-sided P value of <.05 as the alpha criterion. Hazard ratios were calculated by Cox regression analysis; the end point of the follow-up period was the first infection, with censoring at premature release from duty, or at the end of the study after a 6-month follow-up period. Cox regression analysis was adjusted for influenza vaccination and smoking at baseline.	**Main findings** The number of days absent from duty due to respiratory tract infection, did not differ between groups: Mean number of days absent (±SD) was 2.2±3.2 days in the intervention group and 3.0±4.0 days in the placebo group (P=.096). The number needed to treat, calculated from the proportion of men without any days absent from duty, was 6.4 (95% CI, 3–257). **Secondary outcomes** - Proportion of men remaining healthy throughout the 6-month study period was greater in the intervention group (41 [51.3%] of 80) than in the placebo group (30 [35.7%] of 80; P=.045). - In a Cox regression analysis with adjustments for smoking and influenza vaccination, the adjusted hazard ratio (HR) for absence from duty due to respiratory tract infection was lower in the intervention group (HR, 0.71; 95% confidence interval [CI], 0.43– 1.15). Self-reported secondary outcomes did not differ between the groups. The mean number of hospital days (±SD) was 0.31±1.21 per subject in the intervention group and 0.90±2.22 in the placebo group (P=.06).	• **Ethics approval?** Approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland. • **Adverse events?** Not accounted for. • **Aim?** Clearly defined primary outcome. • **Did the randomization work?** There were no significant differences between groups at baseline, except regarding smoking and influenza vaccination which were slightly more common in the placebo group. • **Procedure for randomization?** Random allocation was performed using computer-generated random numbers. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes. • **Primary endpoints – validated?** Primary end-points yes, secondary endpoints no. • **Risk of attrition bias?** Altogether, 60 subjects had dropped out (26% in VD group and 46% of placebo group) by the end of the study, with no specific reason given for study withdrawal. • **All outcomes reviewed?** Yes. • **Generalizability?** Limited. • **Applicability in clinical practice?** Considering the population recruited, the results were not particularly generalizable, and application in clinical situations are limited with regard to females, other age-groups and populations with co-morbidities. • **Cost/benefit:** Not evaluated. • **Findings supported by previous literature?** Findings regarding effect on serum PTH concentration contrasted with those found in a similar study, in which a winter time elevation of serum PTH concentration was inhibited by vitamin D supplementation (800 IU/day). **Strengths:** The homogeneity of the study setting and population with regard to the outcome studied. The completeness of the outcome data (respiratory infections identified) regarding absence from duty. **Weaknesses:** Effec seen only in the secondary outcome measures. A priori power calculation not performed. The power of the study was limited by the number of subjects who withdrew from the study. Vitamin D dose given was insufficient in order to achieve vitamin D sufficiency at 6 months. Only 41% of the invited agreed to participate. **Plausible explanations for the results?** Yes.

Reference: Manaseki-Holland S, Qader G, Isaq Masher M, et al. Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. Trop Med Int Health 2010;15:1148-55.		Study design: **RCT** *Double blind, placebo-controlled*	
		Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine whether - supplementation of oral 100 000 iu of vitamin D3 along with antibiotics could reduce the <u>duration</u> of illness in children with pneumonia; - supplementation could reduce the <u>risk of repeated episodes</u> **Conclusion** *A single high-dose oral vitamin D3 supplementation to young children along with antibiotic treatment for pneumonia could reduce the occurrence of repeat episodes of pneumonia* **Country** Afghanistan **Data collection period** 2006-2007	**Recruitment** At outpatient clinics if the child met the study criteria and after either the parent read the Dari consent form or it was explained to him∕her by the doctor. **Inclusion-/exclusion criteria** • <u>Inclusion criteria</u>: Children aged 1–36 months, diagnosed with non-severe or severe pneumonia at the outpatient clinic at Maywand Hospital • <u>Exclusion criteria</u>: Children who had clinical signs of rickets or were known to have received high-dose vitamin D treatment in the past 3 months (one child) had severe vomiting (one child) or pronounced wheeze (10 children). **Data** 453 children were included in the study. <u>Daily follow-up up to 10 days</u>, either at the study hospital by paediatricians or at home by medical doctors if discharged <u>to assess the resolution of signs and symptoms</u> of the first episode of pneumonia. <u>Thereafter, followed fortnightly up to 90 days</u> by trained female medical doctors <u>to assess any illness</u> and to refer to the study hospital if necessary. **Outcome validation (i.e. diagnosis)** Severity of pneumonia was categorised using WHO's IMCI criteria. All doctors involved were trained in IMCI and examination of the study signs and symptoms and their work in the clinics or follow-up were monitored through random observations by a supervisor on weekly basis. **Intervention variables** Bolus dose of vitamin D3 100,000 IU versus placebo. **Important confounding factors** The potential confounding of baseline characteristics on treatment effect was assessed in statistical analyses. **Statistical methods** Power calculations and numbers included in analyses were adequately described. Incidence rates of pneumonia were calculated by dividing the number of new episodes of pneumonia by total time at risk for all children. Hazard ratios with 95% CIs were obtained with Cox proportional-hazards models to measure time to repeat episodes between treatment groups. Kaplan–Meier plots and log-rank tests were used to compare the time to recover from the index episode of pneumonia between the vitamin D and placebo groups.	**Main findings** The mean number of days to recovery from the index episode of pneumonia was the same for both the vitamin D group and the placebo group [4.74 (SD 2.22) vs. 4.98 (SD 2.89); P = 0.17] The risk of children having a repeat episode of pneumonia during the 90-day posttreatment period was significantly lower in the vitamin D group than in the placebo group (RR 0.78; 95% CI 0.64, 0.94, P = 0.01) Children in the vitamin D group survived without experiencing a repeated episode of pneumonia for a longer period than children in the placebo group, for the first or only episode of pneumonia (HR 0.71; 95% CI 0.53–0.95, P = 0.02) There was no confounding effect of baseline measures on risk of repeat pneumonia or time to repeat episode.	• **Ethics approval?** Approved by the Ethics and Review Board of the Ministry of Public Health of Afghanistan. • **Adverse events?** Accounted for. • **Aim?** Which was the primary outcome was not clearly defined. • **Did the randomization work?** There was no statistically significant difference in any of the baseline characteristics between the groups • **Procedure for randomization?** Random number sequence generated in an Excel spreadsheet with no restrictions. • **Blinding?** Placebo (containing olive oil alone) and vitamin D syringes looked the same and the contents tasted the same. None of the investigators, staff in Kabul and caretakers of children, were aware of the study groups. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Yes, severity of pneumonia was categorised using WHO's IMCI criteria. • used ot clearly described. • **Risk of attrition bias?** The number of children lost to follow-up during the first 10 days of post-treatment follow-up was small and similar between the two groups • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Limited to those children of similar age with high risk of VD deficiency and especially to children who had an episode of pneumonia. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** In harmony with findings that vitamin D can enhance the immune function. **Strengths:** Study design. Population at high risk of vitamin D deficiency. Study outcomes were ascertained by experienced doctors and the loss to follow-up was minimal. Use of IMCI clinical definitions is comparable with other trials with pneumonia as an outcome in children. Low risk of misclassification. **Weaknesses:** Lack of x-ray confirmation of cases of pneumonia. Treatment from health care providers other than the study doctors might have occurred. Not conducted quality control of the vitamin D3 preparation (too costly and technically difficult). No measurement of vitamin D level in the serum achieved as the result of this supplementation. Bolus instead of daily supplementation. **Plausible explanations for the results?** Yes.

Reference: Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010;91:1255-60.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine the effect of vitamin D supplements on the incidence of seasonal influenza A in schoolchildren.	**Recruitment** Volunteers were asked to participate in the study by the pediatricians in charge of the outpatient clinics. Parents and children were asked to provide written informed consent after the pediatrician explained the study to them. **Inclusion-/exclusion criteria** • Inclusion criteria: Schoolchildren aged 6–15 y, with or without underlying diseases • Exclusion criteria: Children were excluded if they; had a history of stones in the urinary tract or diseases of calcium or bone metabolism; were already taking vitamin D3 or activated vitamin D as a treatment of an underlying disease; had a history of allergic reactions to ingredients in the tablets; had difficulties swallowing tablets; had been receiving immunosuppressive therapy including oral corticosteroids or chemotherapy within the past year; were considered incapable of taking part in the study by the pediatrician in charge. **Data** Parents of participants filled out questionnaires pre-study on basic data (sex, age, weight, and height), family structure, medical history (inc. atopic dermatitis, otitis media, sinusitis, asthma from age 3 y and older, and other underlying diseases), and skin reaction to sun exposure (ie, level of sunburn). Parents of participants filled out questionnaires post study on diagnosis by pediatricians of primary and secondary outcomes; adherence with study drug; frequency of outdoor activities per week; average frequency of intake of specific dietary items per week, including sun-dried or fresh shiitake mushrooms, salmon, sardines, mackerel, tuna, and egg yolk; and days absent from school. A log was completed daily that included the following information: adherence to study drug, days absent from school, times of visits to clinics or hospitals, hospital admissions, and cases of influenza, fever, asthma attack, and gastroenteritis (nausea, vomiting, and diarrhea). **Outcome validation (i.e. diagnosis)** The primary outcome was influenza A, diagnosed by medical doctors using a rapid influenza diagnostic test (RIDT) with a nasopharyngeal swab specimen, on an outpatient basis, following the manufacturer's protocol. **Intervention variables** Vitamin D3 1200 IU daily versus placebo. **Important confounding factors** See statistical methods. **Statistical methods** Power calculations were adequately described. Efficacy was assessed by using an intention-to-treat analysis. Continuous variables were compared by using Wilcoxon's ranksum test, and categorical variables were assessed with the chisquare test. The incidences of both primary and secondary outcomes were compared in the 2 groups by using relative risks (RRs) and 95% CIs, subgrouped by sex, age, and nonasthma or asthma. The null hypothesis of equality of risk ratios between the demographic groups was tested with a chi-square test. All reported P values were 2-sided. P values ,0.05 were considered statistically significant. No adjustments were made for multiple comparisons.	**Main findings** Influenza A occurred in 18 of 167 (10.8%) children receiving vitamin D3 compared with 31 of 167 (18.6%) children receiving placebo (RR: 0.58; 95% CI; 0.34, 0.99; P = 0.04). Absolute risk reduction Influenza A: (18/167))-(31/167) = 0.077 = 7.7%. NNR = 100/0.077 = 1299 The incidence of influenza A was compared based on the timing of onset of disease symptoms relative to the initiation of vitamin D intake after supplementation started: Between day 1 and day 30, the occurrence of influenza A was not significantly different between the vitamin D3 group (2/167; 1.2%) and the placebo group (4/167; 2.4%). Between day 31 and day 60, influenza A occurred significantly less often in the vitamin D3 group (9/167; 5.4%) than in the placebo group (22/167; 13.2%) (RR: 0.41; 95% CI: 0.19, 0.86; P = 0.014). Between day 61 and the end of the study, the occurrence of influenza A was not significantly different between the vitamin D3 group (7/167; 4.2%) and the placebo group (5/ 167; 3.0%).	• **Ethics approval?** The study protocol was reviewed and approved by the ethics committee of all participating hospitals. • **Adverse events?** Accounted for. • **Aim?** Primary endpoint was clearly defined. • **Did the randomization work?** There were no significant differences in baseline characteristics between the 2 groups as assessed by chi-square tests and Wilcoxon's rank-sum test. • **Procedure for randomization?** Central computerized procedure to randomly assign children in permutated blocks of 4 to receive either vitamin D3 or placebo. • **Blinding?** Both participants and investigators were blinded throughout the study. The randomization code was disclosed to the staff at the data monitoring center after labeling the number on each bottle. Staff at the data monitoring center had no contact with the patients • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Not adequaltely described. • **Risk of attrition bias?** Accounted for in section "Adherence". Loss to follow-up occurred for 50 children in the vitamin D3 group and 46 in the placebo group (P = 0.72). Low risk of attrition bias. • **Presentation of results?** Yes, see results. • **Generalizability?** Described as limited as for other populations than the one studied due to that the comorbidity ratio of the study population was relatively high, and that most participants were enrolled at outpatient clinics. • **Applicability in clinical practice?** Considering the population recruited, the application of the results in clinical situations was concluded as limited. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed • **Findings supported by previous literature?** Not described. **Strengths:** Not described. **Weaknesses:** The major limitations of the present study were a 1) small sample size; 2) lack of serum 25-hydroxyvitamin D data; 3) lack of urinary calcium data; and 4) lack of information on the presence or development of influenza A antibodies. Self-reported data. **Plausible explanations for the results?** Yes.
Conclusion			
Daily supplementation with 1200 IU vitamin D3 in school children between December and March showed a significant preventive effect against influenza A, although no significant difference was observed for influenza B.			
Country			
Japan			
Data collection period			
December 2008 – March 2009			

Reference: Bergman P, Lindh AU, Björkhem-Bergman L, Lindh JD. Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. PLoS One 2013;8:e65835.		Study design: **RCT** *Double blind, placebo-controlled*	
		Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To investigate if supplementation with vitamin D3 could reduce infectious symptoms and antibiotic consumption among patients with antibody deficiency or frequent RTIs. **Conclusion** *Supplementation with vitamin D3 may reduce disease burden in patients with frequent RTIs* **Country** Sweeden **Data collection period** 2010-2011	**Recruitment** Volunteers were recruited at the Immunodeficiency Unit, Karolinska University Hospital, Huddinge, Sweden. **Inclusion-/exclusioncriteria** • Inclusioncriteria: Age 18–75 years, increased susceptibility to respiratory tract infections (>42 days with symptoms from the respiratory tract during a 12-month period prior to study inclusion). • Exclusion criteria: Prophylactic treatment with antibiotics, history of hypercalcaemia or stones in the urinary tract, sarcoidosis, ongoing supplementation with vitamin D3 exceeding 400 IU/day, HIV-infection and pregnancy. **Data** 140 participants were randomized, 70 to vitamin D and 70 to placebo. Registration of symptom of infection in a diary on a daily basis. This was sent via regular mail to the study site every month. The following data were recorded: symptoms from the respiratory tract, ears and sinuses, treatment with antibiotics, numbers of bacterial cultures, times and reasons of visits to hospitals, frequency of travelling abroad and adherence to study drug. **Outcome validation (i.e. diagnosis)** The primary endpoint was an infectious score based on five parameters: Symptoms from respiratory tract, ears and sinuses, malaise and antibiotic consumption. Validation not described. **Intervention variables (validated)** 12 months' treatment with vitamin D3 (Vigantol, 4000 IU/day, Merck GmbH, Darmstadt, Germany) or placebo oil. One drop contained 500 IU vitamin D3 or placebo oil (Miglyol oil, Merck GmbH, Darmstadt, Germany) and the participants were asked to take eight drops daily. **Important confounding factors** Primary outcome based on self-reported information. Age differed in treatment-ams at baseline. Very heterogeneous population with regard to immune deficiency and concomitant diseases. **Statistical methods** Sample size calculation described. Log-transformation of infectious scores (due to skewed data). 124 patients were included in the main per-protocol analysis. Log-transformation of infectious scores (due to skewed data) investigated with multivariable linear regression analysis adjusting for age, gender, smoking, type of immune deficiency and significant comorbidities (respiratory or non-respiratory).Because of the transformation procedure, the adjusted effect of vitamin D3 was expressed as a ratio between the score in the vitamin D3 and the placebo group. To explore potential divergent effects on different organ systems, both adjusted and unadjusted analyses were repeated separately for each individual item of the infectious score. In addition, the temporal aspects of the vitamin D3 effect were investigated by dividing the study period into four 90-day periods (starting on the first day of treatment) and repeating the analyses separately for each time period. Intention-to-treat (ITT) analysis based on actual outcome data could not be performed. The potential impact of dropouts was addressed in an ITT analysis based on multiple imputation of missing outcome data.	**Main findings** One year of vitamin D3 treatment was associated with a significantly reduced overall infectious score for patients allocated to the vitamin D group compared with the placebo group (adjusted relative score 0.771, 95% CI 0.604 to 0.985, p=0.04). The absolute unadjusted score per patient was 202 points for the vitamin D group and 249 points for the placebo group, a significant reduction of 47 points per patient (p=0.023, Mann-Whitney U test).	• **Ethics approval?** Approved by the local Ethical Committee and the Swedish Medical Product Agency. • **Adverse events?** Accounted for. • **Aim?** Primary endpoint clearly defined. • **Did the randomization work?** The randomisation resulted in age distributions that were not entirely balanced between the two groups. • **Procedure for randomization?** Block randomisation with a block size of ten was used to ascertain equal group sizes. Staff at Karolinska Trial Alliance was responsible for randomisation procedures. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes. • **Primary endpoints – validated?** Not assessed. • **Risk of attrition bias?** During the course of the study, 16 patients left the study prematurely (8 patients from each study group). Reasons for dropout included elevated parathyroid hormone (n=2), with- drawn consent (n=5), adverse events (n=1), prescription of vitamin D outside the study (n=1), failure to complete diary (n=4) or non-compliance to study medication (n=3 • **Presentation of results?** Yes, see results. • **Generalizability?** The generalizability is limited regarding healthy populations not at increased risk of respiratory tract infection. Moreover, as the sample sizes in each sub-group were small, conclusions of effects in specific disease groups were not possible. • **Applicability in clinical practice?** Considering the population recruited, application in clinical situations are limited with regards to healthy individuals • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** ... • **Findings supported by previous literature?** Findings were supported by one study using 1200 IU/day showed a signifi- cant reduction of influenza among school children in Japan. Other RCTs using lower doses of vitamin D3, 400–2000 IU/day, had mainly been negative with regard to the prevention of infections. **Strengths:** A high daily dose of vitamin D3 was used, the study time was a full year covering all seasons and patients with an increased frequency of respiratory tract infections were studied. **Weaknesses:** A single study centre, small sample size (n=140) and a selected group of patients. **Plausible explanations for the results?** Yes.

Camargo, 2012

Reference: Camargo CA Jr, , Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. Pediatrics 2012;130:e561-7.	Study design: **RCT** *Double blind, placebo-controlled*
	Grade - quality — Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine if vitamin D supplementation in children with vitamin D deficiency would lower the risk of ARIs.	**Recruitment** 779 children were invited from 21 third- and fourth-grade classrooms of two public schools (selected for their size, proximity, and comparable socio-demographic profiles). A subset of 247 children were included in this study. **Inclusion-/exclusion criteria** • Inclusion criteria: Children were eligible to participate if they had no known allergies to milk. • Exclusion criteria: Not described. **Data** Participants were followed for 7 weeks. A blood sample was taken at baseline and at follow-up for determination of 25OHD-level. **Outcome validation (i.e. diagnosis)** The primary outcome in this study was the parent-reported number of ARIs that occurred during the preceding 3 months. Information was collected by using a survey at completion of the study, and ARIs were ascertained by using the following question: "Over the past 3 months, how many chest infections or 'colds' has your child had—counting only those infections that lasted for at least 24 hours with symptoms?" The response categories were counts from "none" to "six or more." **Intervention variables** Daily ingestion of unfortified regular milk (control; n = 104) or milk fortified with 300 IU of vitamin D3 (n = 143). **Statistical methods** Unadjusted and adjusted random-intercept negative binomial regression were used to test the association between vitamin D supplementation and the number of ARIs in the past 3 months, in an intention-to-treat analysis. Age, gender, and baseline "ever wheeze" history were defined as covariates in the multivariable analysis. To examine if the effect of vitamin D supplementation on ARI risk varied according to baseline 25(OH)D status, children were stratified according to their median baseline 25(OH)D level, and the multivariable regression analysis was repeated in the 2 subgroups. Model results were reported as rate ratios (RRs) with 95% confidence intervals (CIs). A 2-tailed P =.05 was considered statistically significant. **Important confounding factors** The confounding effect of covariates was assessed by examining the association of vitamin D supplementation with ARI before and after adding the covariates as fixed effects to the model. Moreover, because classrooms (and not individual children) were assigned the different treatment groups, our analyses assume correlation between the children of each class; random effects for each classroom were entered to account for this intraclass correlation. The models were compared by using deviance statistics.	**Main findings** Compared to controls, children receiving vitamin D reported significantly fewer ARIs during the study period (mean: 0.80 vs 0.45; P = .047), with a rate ratio of 0.52 (95% confidence interval: 0.31–0.89). Adjusting for age, gender, and history of wheezing, vitamin D continued to halve the risk of ARI (rate ratio: 0.50 [95% confidence interval: 0.28–0.88]). Similar results were found among children either below or above the median 25(OH)D level at baseline (rate ratio: 0.41 vs 0.57). Absolute risk reduction of one or more ARI: (72/141)-(55/104)= -0,0182 = 1.8% NNT = 100/1.8 ≈ 56	• **Ethics approval?** Main study approved by the institutional review boards at the Harvard School of Public Health and Mongolian Ministry of Health. • **Adverse events?** Accounted for. • **Aim?** Primary outcome well defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Randomization was based on a random number generator, with allocation concealment and off-site assignment of classrooms to a specific intervention. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Not described. Self reported information at the end of the 7 week study. Moderate risk of re-call bias and classification bias. • **Risk of attrition bias?** Very low, follow-up was 99% (Among the 247 children in the primary comparison, outcome data were missing for only 3 children (1 lost to follow-up and 2 discontinued the intervention due to changing schools)) • **Presentation of results?** Yes, see results. • **Applicability in clinical practice?** Considering the population recruited, the results were representative in a general population of Mongolian school children, and application of these findings are relevant with regards to public health and vitamin D food-fortification strategies. • **Generalizability?** The halving of ARIs has important public health implications for Mongolian children and perhaps other populations with low levels of serum 25(OH)D. However, generalizability is limited with regards to populations including infants, health care workers, and the elderly; individuals with HIV or other immunodeficiency conditions; and individuals with asthma or other chronic respiratory disorders. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed • **Findings supported by previous literature?** This study suggested that the association between vitamin D status and ARI risk is causal, at least among school-aged children with very low vitamin D status in early winter; In children, there is 1 comparable trial by Urashima et al5 in Japan. In a sample of 334 school-children, the investigators found that 1200 IU of vitamin D3 supplement daily lowered the risk of influenza A (RR: 0.58 [95% CI: 0.34–0.99]) but, for unclear reasons, did not affect the risk of influenza B (RR: 1.39 [95% CI: 0.90–2.15]) and therefore yielded an overall null result for influenza risk. **Strengths:** Double blinding of participants and investigators and very high follow-up rate 99%. **Weaknesses:** Suppl should have been initiated earlier during the trisl. Imprecision of the primary outcome (risk of misclassification was present). Funded by anonymous source, however, the project funders were stated to have no role in the design, implementation, analysis, or interpretation of data. **Plausible explanations for the results?** Yes.
Conclusion *Vitamin D suppl significantly reduced the risk of ARIs in winter among Mongolian children with vitamin D deficiency*			
Country Mongolia			
Data collection period January to mid March 2009			

Reference: Lehouck A, Mathieu C, Carremans C, et al. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. Ann Intern Med 2012;156:105-14.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate/High ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To explore whether supplementation with high doses of vitamin D could reduce the incidence of COPD exacerbations.	**Recruitment** Volunteers were recruited from single-center at the University Hospitals Leuven, over a 1.5-year recruitment period in 2008 and 2009. Screened during hospitalization for an exacerbation or before referral for respiratory rehabilitation. **Inclusion-/exclusion criteria** • Inclusion criteria: Eligible patients were current or former smokers, were older than 50 years, had a diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) definition (postbroncho- dilator FEV1–FVC ratio <0.7), and had an FEV1 less than 80% predicted. • Exclusion criteria: history of hypercalcemia, sarcoidosis, or active cancer. Treatment with vitamin D supplements for newly discovered symp- tomatic osteoporosis and long-term azithromycin treat- ment, with antibacterial and anti-inflammatory functions, were additional exclusion criteria **Data** 182 participants were followed for 1 year. Baseline characteristics included BMI, Airflow Obstruction, Dyspnea, and Exercise Capacity (BODE) Index and the Charlson comorbidity index. Follow-up visits occurred every 4 months (at 4, 8, and 12 months). To obtain data on exacerbations, participants were asked to complete diaries every 2 weeks that detailed respiratory tract symptoms, visits to health care providers, hospitalizations, and changes in medication. At each visit, diaries were reviewed in the participant's presence and the general practitioner was contacted in case of doubt, missing data, or suspicion of self-medication. **Outcome validation (i.e. diagnosis)** The primary end point was the time to first exacerbation, defined as sustained worsening of respiratory symptoms during 48 hours and requiring oral corticosteroid, antibiotic, or combination treatment that was initiated by a physician. Respiratory symptoms included at least 1 of the Anthonisen criteria (increased dyspnea, sputum volume, or sputum purulence) with or without minor symptoms, such as cough, fever, common cold, wheezing, or sore throat. Secondary end points were exacerbation rate; time to first hospitalization; time to second exacerbation; FEV1; quality of life, as measured with the Chronic Respiratory Questionnaire (CRQ) (scores for dyspnea, emotion, fatigue, and mastery); and death. In addition to these clinical end points, bacterial presence in morning sputa, plasma cathelicidin levels, serum 25-(OH)D levels, and blood monocyte capacities for phagocytosis were determined in a blinded manner **Intervention variables** 100 000 IU of vitamin D suppl or placebo every 4 weeks for 1 year **Important confounding factors** Self-reported data. Age, FEV1, GOLD stage, and smoking status did not have a statistically significant influence on the model. **Statistical methods** Power-calculations described, ITT analysis applied. Time to first or second exacerbation and time to first hospitalization were compared by using Kaplan–Meier curves and log-rank tests. Effect sizes between groups of primary or secondary outcomes are given with P values and 95% CIs. P values less than 0.05 are considered statistically significant. Subgroup analyses were thoroughly described, and in particular it was noted that: Mean number of exacerbations per patient-year calculated by dividing the total number of exacerbations by the total years of follow-up in the ITT population. Exacerbation rate in the ITT population was analyzed with a generalized linear model for a Poisson distribution, correcting for duration of treatment exposure and overdispersion.	**Main findings** The median time to first exacerbation did not significantly differ between the groups (hazard ratio, 1.1 [CI, 0.82 to 1.56]; P=0.41), nor did exacerbation rates, FEV1, hospitalization, quality of life, and death. A post hoc analysis in 30 participants with severe vitamin D deficiency (serum 25-[OH]D levels < 10 ng/mL) at baseline showed a significant reduction in exacerbations in the vitamin D group (rate ratio, 0.57 [CI, 0.33 to 0.98]; P=0.042).	• **Ethics approval?** Approved by • **Adverse events?** Not accounted for. • **Aim?** Clearly defined primary outcome. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Pharmacists of the University Hospitals Leuven, who were independent from the clinical study team, randomly assigned participants by using a computer-generated randomization list in blocs of 20 and prepared the study medication. • **Blinding?** Yes. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Unclear • **Risk of attrition bias?** Low: 150 (82%) subjects completed the study, 15 (8%) died, and 17 (9%) were classified as withdrawals with no differential dropout between the 2 groups. • **Presentation of results?** Yes, see results. • **Generalizability?** Generalizabe to patients with COPD. • **Applicability in clinical practice?** Considering the population recruited, the results were representative in a COPD subjects, and results relevant in clinical situations including management of COPDexacerbation. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** The absence of a vitamin D–mediated effect in this study sample contrasts with indirect evidence from most association studies in COPD, but is consistent with recent data from the Lung Health Study, which showed that vitamin levels did not determine the rate of decline in FEV1 in a limited subgroup. **Strengths:** First of its kind. The findings may help to guide the design of and dosage in future trials. **Weaknesses:** Single center. Small sample. **Plausible explanations for the results?** Yes.

Aim

Conclusion

High-dose vitamin D supplementation in a sample of patients with COPD did not reduce the incidence of exacerbations. In participants with severe vitamin D deficiency at baseline, supplementation may reduce exacerbations

Country

Belgium

Data collection period

2008-2009

Manaseki-Holland, 2012

Reference: Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. Lancet 2012; 379: 1419-27.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To assess whether oral supplementation of vitamin D3 (cholecalciferol) will reduce the incidence and severity of pneumonia in a high-risk infant population	**Recruitment** Volunteers were recruited from five of the 18 socioeconomically deprived inner-city districts; identified households with young children with detailed maps and advice from a non-governmental organisation working in the region. The study field-supervisors mapped the region independently to verify the accuracy of the maps. 20 pairs of female fieldworkers visited every home starting from streets closest to the hospital and radiating out until required sample size were reached. **Inclusion-/exclusion criteria** • Inclusion criteria: Infants aged 1–11 months and living in the study region • Exclusion criteria: Families expecting to move to another town within 18 months, diagnosis of rickets or treatment with vitamin D in the previous 3 months, and clinical diagnosis of Kwashiorkor or Marasmus. **Data** 3046 children (1524 children were assigned to receive vitamin D3 and 1522 placebo) followed up every 2 weeks to obtain background information, assess illness (symptom history and examination of chest in-drawing, body temperature, signs of dehydration by skin pinching, respiratory rate count over 1 min with a stopwatch), and to refer to the study hospital if needed. Venous blood samples collected at baseline. Respiratory rate and anthropomorphic data were collected twice, children clinically diagnosed with pneumonia were offered free chest radiographs. Causes of death ascertained through scrutiny of hospital notes, and verbal autopsy interviews with the WHO standard questionnaire and review of the interview data by two physicians independently. **Outcome validation (i.e. diagnosis)** Primary endpoint was the first episode of pneumonia from the time of enrolment confirmed by chest radiograph (consolidation or infiltrates). A new episode of pneumonia defined as an episode happening 15 days or longer after the first. We judged an episode happening within 14 days to be continuation of the previous episode. **Intervention variables** Vitamin D3 100,000 IU versus placebo every third month for 1 year. **Important confounding factors** Nutritional factors, accounted for in diet questionnaire, weight at recreuitment and growth comparison. **Statistical methods** Power-calculations adequately described. Compared baseline characteristics and the distribution of prestated confounders for intervention and placebo groups. ITT analysis included all children randomly assigned to study groups. PP analysis included children who in both groups received all doses with an interval between the doses of 60 and 120 days and had not violated the randomisation codes. Pearsons correlation coefficient or Cramers V (for paired categorical variables) to assess the potential problem of multicollinearity. Calculated person-time at risk for each child up to the date a child reached the primary endpoint, was last seen at the end of the study, or when censored because they were lost to follow-up. Initial comparisons of time-to-an-episode between the two groups with log-rank tests and Kaplan–Meier plots. Estimated the incidence rate ratio (RR) for the episodes of pneumonia with Cox proportional hazard models.	**Main findings** There was no significant difference between the incidence of first or only pneumonia between the vitamin D (0·145 per child per year, 95% CI 0·129–0·164) and the placebo group (0.137, 0·121–0·155) Incidence rate ratio: 1·06 (95% CI 0·89–1·27).	• **Ethics approval?** Approved by the ethics and review board of the Ministry of Public Health of Afghanistan and the ethics committee of the London School of Hygiene and Tropical Medicine. • **Adverse events?** Accounted for. • **Aim?** Clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** An independent statistician randomised unique identification numbers individually in fixed blocks of 20 to the vitamin D3 or placebo group by use of a random number generator with the SAS routine. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Applied WHO standards. • **Risk of attrition bias?** Low: Low loss to follow up • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a the high-risk, thus the generalizability to populations with low-to-moderate risk of vitamin deficiency is unknown • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Findings at odds with smaller case-control hospital studies that show an enhanced rate of vitamin D deficiency or rickets in children with pneumonia and the increasing evidence suggesting that calcitriol, the biologically active metabolite of vitamin D, has an important role in the human immune system. A systematic review of the role of vitamin D supplementation in infectious diseases had mixed findings, concluding that more rigorously designed clinical trials are needed. No studies report the effect of vitamin D on radiologically confirmed pneumonias. Other trials assessing infections of the upper respiratory tract also had mixed findings. **Strengths:** Large sample size. Low attrition bias. Robust assertaintion of outcomes and low risk of misclassification. Blinding of participants and **Weaknesses:** Limited generalizability. Not assessed genotyping. Steady state VD not reached in all patients. **Plausible explanations for the results?** Yes.

Conclusion
Quarterly bolus doses of oral vitamin D3 supplementation to infants are not an effective intervention to reduce the incidence of pneumonia in infants in this setting.

Country
Afghanistan

Data collection period
2008-2009

Reference: Murdoch DR, Slow S, Chambers ST, et al. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. JAMA 2012; 308: 1333- 9.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	High ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine the effect of vitamin D supplementation on incidence and severity of URTIs in healthy adults.	**Recruitment** Participants were staff or students of the Canterbury District Health Board, the regional publicly funded health care organization, or the University of Otago, Christchurch. Following an advertising campaign, we screened and enrolled volunteers during February through April 2010 **Inclusion-/exclusion criteria** • Inclusion criteria: Aged 18 years or older, were able to give written informed consent, and who anticipated that they would be a resident of the Christchurch region for the study period. • Exclusion criteria: use of vitamin D supplements other than as part of a daily multivitamin preparation (in which the daily intake was >400 IU). Use of immunosuppressants or medications that interfere with vitamin D metabolism (eg, thiazide diuretics, phenytoin, carbamazepine, primidone, phenobarbital, doses of prednisone >10 mg/d, methotrexate, azathioprine, cyclosporin), history of hypercalcemia or nephrolithiasis, sarcoidosis, kidney disorders requiring dialysis or polycystic kidney disease, cirrhosis, current malignancy diagnosis in which the cancer was aggressive and prognosis was poor, baseline plasma calcium (corrected for plasma albumin concentration) greater than 10.4 mg/dL or less than 8.4 mg/dL, enrollment or planned enrollment in other research that would conflict with full participation in the study or confound the observation or interpretation of the study findings and pregnancy or planned pregnancy during the study period.	**Main findings** There were 593 URTI episodes in the vitamin D group and 611 in the placebo group, with no statistically significant differences in the - number of URTIs per participant (mean, 3.7 per person in the vitamin D group and 3.8 per person in the placebo group; risk ratio, 0.97; 95% CI, 0.85-1.11) No statistically significant differences between VD and placebo group in the - number of days of missed work as a result of URTIs (mean, 0.76 days in each group; risk ratio, 1.03; 95% CI, 0.81-1.30), - duration of symptoms per episode (mean, 12 days in each group; risk ratio, 0.96; 95% CI, 0.73-1.25) or severity of URTI episodes.	• **Ethics approval?** Approved by by the Upper South B Regional Ethics Committee • **Adverse events?** Accounted for. • **Aim?** Clearly defined primary end point. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Computer-generated, not further described. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Yes, low risk of classification bias. • **Risk of attrition bias?** Low. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Not considered. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** ... • **Findings supported by previous literature?** Findings were consistent with 2 other randomized controlled trials that were specifically designed to assess whether vitamin D supplementation prevents acute respiratory infections in adults.
Conclusion *Vitamin D by monthly administration of 100000 IU of vitamin D did not reduce the incidence or severity of URTIs in healthy adults.*	**Data** Participants were followed for 1.5 years. Interviewer adm questionnaire at the screening visit (inc. data on demographics, occupation, medical history, smoking, current medications, and supplement use). At each monthly visit filled out questionnaire on episodes of respiratory tract illness during the preceding month that had not already been reported to study personnel and also noted any changes in medications or supplement use and adverse events. Monthly collection of nasal swabs, tested for respiratory viruses by real-time PCR (Fast Track Diagnostics). Plasma calcium and serum 25-OHD levels were measured at baseline and at 2, 6, 12, and 18 months after enrollment.	These findings remained unchanged when the analysis was repeated by season and by baseline 25-OHD levels.	**Strengths:** Relatively large sample size, the 18- month duration, and the high dose of vitamin D administered (with a loading dose). Dosing regimen. Rigorous efforts to capture URTI episodes and the collection of virological data.
Country New Zealand	**Outcome validation (i.e. diagnosis)** The primary end point was number of URTI episodes. Secondary end points were duration of URTI episodes, severity of URTI episodes, and number of days of missed work due to URTI episodes. **Intervention variables** Participants were randomly assigned to receive an initial dose of 200 000 IU oral vitamin D3, then 200 000 IU 1 month later, then 100 000 IU monthly (n = 161), or placebo administered in an identical dosing regimen (n = 161), for a total of 18 months. **Important confounding factors** Influenza vaccination and loss to follow-up.		**Weaknesses:** Low prevalence of vitamin D deficiency. Unable to assess the effect of vitamin D suppl on prevention of infection caused by individual viruses. No genotyping.
Data collection period 2010-2011	**Statistical methods** Power calculations described. The numbers of URTI events for each participant were summed and then compared between the treatment and placebo groups using a negative binomial model that included a dispersion parameter. Analysis of the number of days of missed work also used a negative binomial model but because there were multiple events for many participants, a generalized estimating equation model with an exchangeable correlation matrix was used. A comparison of the sum of the WURSS-24 scores in the first 7 days of the URTI event was made using a general linear mixed model and modeling the participants as random effects. For participants who had incomplete data on the WURSS-24, missing observations were estimated using multiple imputation (5 imputations) using the Markov chain Monte Carlo method on natural log scores (with a constant of 1 added). Duration of URTI events was assessed using the Cox proportional hazard model with multiple events per par- ticipant bing treated as clustered events and using robust sandwich covariance matrix estimates. Hypothesis testing was 2-sided with statistical significance set at P<.05.		**Plausible explanations for the results?** Yes.

Reference: Marchisio P, Consonni D, Baggi E, et al. Vitamin D supplementation reduces the risk of acute otitis media in otitis-prone children. Pediatr Infect Dis J 2013;32:1055-60.	**Study design: RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	**Material and methods**	**Results**	**Discussion/comments/checklist**
To evaluate whether a deficit in vitamin D (VD) is associated with an increased risk of recurrent acute otitis media (AOM) and whether VD supplementation is effective in reducing the number of AOM episodes in otitis-prone children. **Conclusion** *The administration of VD in a dos- age of 1000 IU/d restores serum values of ≥30ng/mL in most cases and is associated with a significant reduction in the risk of uncomplicated AOM.* **Country** Italy **Data collection period** 2011-2012	**Recruitment** Volunteers were recruited among children who were regularly followed by the outpatient section of Pediatric Clinic 1, (…) Policlinico, University of Milan, Italy. **Inclusion-/exclusion criteria** · Inclusion criteria: Children 1–5 years of age with a history of rAOM (defined as at least 3 episodes in the preceding 6 months or at least 4 episodes in the preceding 12 months, with the most recent episode in the previous 2–8 weeks), who were regularly followed by an outpatient clinic · Exclusion criteria: All the factors that can favor the development of AOM, including severe atopy, acquired or congenital immunodeficiency, cleft pal- ate, a chronically ruptured eardrum, craniofacial abnormalities or obstructive adenoids, sleep apnea syndrome or the placement of tympanostomy tubes. **Data** Participants were followed for 6 months. Monthly control visits to monitor the incidence of new episodes of AOM, diary recording of children's clinical problems and the daily administration of VD. Blood sample obtained from each child at the time of enrollment and within 2 days of the discontinuation of supplementation to determine VD level. At each visit, the details of any medical event occurring since the previous visit were recorded, and the children underwent a complete physical examination and pneumatic otoscopy. **Outcome validation (i.e. diagnosis)** All the medical examinations carried out at the center carried out by trained investigators using standardized questionnaires. AOM was diagnosed on the basis of the presence of spontaneous otorrhea from an acute TMP or any combination of fever, ear ache, irritability and hyperemia or opacity accompanied by the bulging or immobility of the tympanic membrane; in doubtful cases, tympanometry was used to establish the presence of effusion or perforation. 25(OH)D measured using a DiaSorin quantitative chemiluminescence immunoassay (LIAISON 25 OH Vitamin D Total Assay; DiaSorin, San Francisco, CA), which has an analytic sensitivity of 4ng/mL and a coefficient of variation of 3–4%. **Intervention variables** Oral VD 1000 IU/d (10 drops of Pédiatre, Vitamin D3, Pediatrica, Livorno, Italy) or placebo for 4 months. **Important confounding factors** Underreporting of AOM episodes (overcome by weekly phonecall to verify status). **Statistical methods** The $\chi 2$ and Mann-Whitney tests were, respectively, used to compare the categorical and continuous variables in the treatment and placebo group at baseline and follow-up. Frailty Cox models fitted to calculate the hazard ratios and 95% confidence intervals of the occurrence of AOM during the study period, while taking into account the intraindividual correlations. The same models were used to assess whether serum VD levels at the end of supplementation influenced the occurrence of AOM. For all the 3 outcomes additional multiple Cox frailty models were performed, adjusting simultaneously for sex, breast-feeding, pacifier use, day-care attendance, history of allergy and previous antipneumococcal vaccination.	**Main findings** The number of children experiencing ≥1 AOM episode during the study period was significantly lower in the treat- ment group (26 versus 38; P = 0.03). There was a marked difference in the number of children who developed uncomplicated AOM (P < 0.001), but no difference in the number of children with ≥1 episode of spontaneous otorrhea. The likelihood of AOM was significantly reduced in the patients whose serum VD concentrations were ≥30 ng/mL.	· **Ethics approval?** Approved by the Ethics Committee of the University of Milan, Italy, and conducted in accordance with the standards of Good Clinical Practice for trials of medicinal prod- ucts in humans · **Adverse events?** Accounted for. · **Aim?** Primary outcome not clearly defined. · **Did the randomization work?** Yes, there were no significant differences between groups at baseline. · **Procedure for randomization?** Random number generator · **Blinding?** Both participants and investigators were blinded throughout the study. · **Did the groups receive the same co-intervention/treatments?** Yes · **Primary endpoints – validated?** Not described. · **Risk of attrition bias?** Low. · **Presentation of results?** Yes, see results. · **Generalizability/Applicability in clinical practice?** Not discussed. · **Did authors review all outcomes?** Yes. · **Cost/benefit effectiveness** "*From an economical point of view, the advantage is obtained with an expense not sig- nificantly different from that needed for treating a single AOM episode.*" · **Findings supported by previous literature?** Yes. **Strengths:** Study design. **Weaknesses:** Low sample size. The mean baseline 25(OH)D levels in the children enrolled were only slightly lower than 30 ng/mL **Plausible explanations for the results?** "*We cannot offer any definite reason for these findings, but some data concerning the bacterial etiology of AOM with TMP, the role of VD in conditioning the human defenses and the relation- ships between VD and bacterial virulence may provide some clues*"

Rees, 2013

Reference: Rees JR, Hendricks K, Barry EL, Peacock JL, Mott LA, Sandler RS, et al. Vitamin D3 supplementation and upper respiratory tract infections in a randomized, controlled trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;57(10):1384-92.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
Tested whether 1000 IU/day vitamin D3 supplementation reduced winter episodesand duration of URTI and its composite syndromes, influenza-like illness (ILI; fever and ≥2 of sore throat, cough,muscle ache, or headache) and colds (no fever, and ≥2 of runny nose, nasal congestion, sneezing, sore throat,cough, swollen or tender neck glands)	**Recruitment** Volunteers were recruited at 11 clinical centers. Recruitment method not described. **Inclusion-/exclusion criteria** • Inclusion criteria: Had at least 1 colorectal adenoma recently removed and none remaining after complete colonoscopy. Aged 45–75 years old, in good general health with no contraindications to study treatment, and had no familial colorectal cancer syndromes or history of serious intestinal disease • Exclusion criteria: Individuals with serum vitamin D levels<12 ng/mL were excluded. **Data** 2259 participants were followed for … and filled out a detailed health questionnaire and had blood samples drawn at enrollment. Daily health diaries regarding fever, headache, muscle aches, chills, cough, runny nose and allergies. Information was also collected each month on influenza and pneumococcal vaccines, antibiotics and antiviral medications, and medical care sought for URTI symptoms. Initially, all diaries were completed on paper while a web-based application was being programmed. Because not reached target enrollment of 800 participants, existing participants summer 2010 were asked to continue completing health diaries for a second winter season through March 2011 or until 2 months after the end of treatment. Participants were compensated $5 for reviewing informational materials and $5 for each completed diary. **Outcome validation (i.e. diagnosis)** URTI was defined as either a cold or ILI. ILI was any episode with at least 1 day of fever (≥100°F [37.8°C] or participants reported feeling hot) and ≥2 of sore throat, cough, muscle ache, or headache. A cold required absence of ILI and ≥2 of the following on a single day: runny nose, nasal congestion, sneezing, sore throat, cough, and swollen or tender neck glands. **Intervention variables** Vitamin D3 1000 IU versus placebo daily. Modified 2 × 2 factorial design to identical-looking pills containing vitamin D3, calcium carbonate, both, or placebo ("4-arm study") **Important confounding factors** See statistical methods. **Statistical methods** Power calculation described. Rate ratios computed with 95% CIs for illness episodes among participants randomized to vitamin D vs placebo, using generalized estimating equations (GEEs) with robust Poisson errors and exchangeable correlation matrices to adjust for overdispersion. Season-specific rates were estimated using person-years of observation during that season. Days of illness were frequently zero, so GEEs with negative binomial errors were used for these rate ratios. Analyses were adjusted for calcium treatment and the stratifying variables center, colonoscopy surveillance interval (3 or 5 years), a 3-category term for sex and participation in the 4- or 2-arm study, and season. Associations between symptoms analyzed and serum 25(OH)D on treatment, independent of randomized treatment assignment, adjusting for variables that were significant predictors of baseline serum level. Analyses were conducted using Stata version 12 [25] according to ITT except as indicated. Sensitivity analyses to explore effects of missing data and episode definitions were in Supplementary Appendix.	**Main findings** Among those who completed symptom diaries, supplementation did not significantly reduce winter episodes of URTI (rate ratio [RR], 0.93; 95% confidence interval [CI], .79–1.09) including colds (RR, 0.93; 95% CI,.78–1.10) or ILI (RR, 0.95; 95% CI, .62–1.46), nor did it reduce winter days of illness (RR, 1.13; 95% CI, .90–1.43). There was no significant benefit according to adherence, influenza vaccination, body mass index, or baseline vitamin D status. Semiannual surveys of all participants (N = 2228) identified no benefit of supplementation on ILI (odds ratio [OR],1.14; 95% CI, .84–1.54) or colds (OR, 1.03; 95% CI, .87–1.23)	• **Ethics approval?** Approved by the institutional review boards at each clinical center. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups. • **Procedure for randomization?** A web-based, random number generator assigned treatment within blocks, stratified by study center, sex, and colonoscopy interval (3 or 5 years) • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a general population aged 45 or older, thus findings not generalizable to younger populations. Application in clinical situations with counselling regarding vitamin D supplementation. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, 3 previous, high-quality RCTs of vitamin D supplementationthat collected URTI symptom data prospectively from healthy adults. None has shown a significant benefit. **Strengths:** Large sample size; detailed reporting of daily symptoms using health diaries in a large subgroup during an average of 13 months' observation including 2 winter seasons. 25(OH)D measurements. Excellent adherence to regimen and use of a common route and dose of supplementation. All participants were randomized for at least 12 months before completing symptom diaries, so failure to attain a steady state did not account for our negative findings. Extensive sensitivity analyses and other secondary data to support primary results, including semiannual surveys of 2228 randomized participants over multiple seasons, **Weaknesses:** Economic compensation. Self-reported data through collection of health diaries. Health diarys from only one-third of the participants. Self-selection of participants from the parent study was influenced by any early effect (or lack of effect) of study treatmenton URTI symptoms. Use of semiannual self-reported adherence to pilltaking (recall bias). Lack of laboratory confirmation of URTI and potential misclassification of colds and ILI by symptom-based case definitions. **Plausible explanations for the results?** Yes.
Conclusion *Vitamin D3 did not significantly reduce the incidence or durationof URTI in adults with a baseline serum 25-OHD level ≥12 ng/mL*			
Country USA			
Data collection period 2009-2011			

Goodall, 2014

Reference: Goodall EC, Granados AC, Luinstra K, Pullenayegum E, Coleman BL, Loeb M, et al. Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial. BMC infectious diseases. 2014;14:273.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To assess whether vitamin D3 supplementation (10,000 international units per week) versus placebo and gargling versus no gargling could prevent viral, clinical upper respiratory tract infection (URTI) in university students	**Recruitment** Volunteers were enrolled at McMaster University. Recruitment not further described. **Inclusion-/exclusion criteria** - Inclusion criteria: Age ≥17 years, and lived with at least one student housemate. - Exclusion criteria: Medical conditions (hypercalcemia, parathyroid disorder, chronic kidney disease, use of anticonvulsants, malabsorption syndromes, sarcoidosis), who were currently or planning to become pregnant, who were taking ≥1000 international units (IU)/day vitamin D3, or who were unable to swallow capsules **Data** 600 participants were followed for 13 months on average. Participants completed a baseline questionnaire that collected demographic, health and lifestyle information and submitted a self-collected mid-turbinate flocked nasal swab. Students completed weekly electronic surveys. Symptomatic students also completed an electronic symptom diary. The primary and secondary outcomes were the occurrence of symptomatic clinical URTI and laboratory confirmed URTI respectively. **Outcome validation (i.e. diagnosis)** The primary outcome was the incidence of clinical URTI defined as the participant's perception of a "cold" in conjunction with two or more symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, or fever). When participants reported symptoms but were uncertain if they were ill, two clinicians reviewed and deemed adjudicated events if 1) at least two symptoms were reported and included one of nasal congestion, sneezing, cough, sore throat, and wheezing, and 2) no additional information was provided that attributed the symptoms to another cause. Secondary outcomes included laboratory confirmed illness, viral load, and symptom duration and severity, all outcomes were described adequately. **Intervention variables** 4 treatment arms: 1) vitamin D3 and gargling, 2) placebo and gargling, 3) vitamin D3 and no gargling, and 4) placebo and no gargling. Weekly administration. **Important confounding factors** Se statistical methods. **Statistical methods** Power calculation were adequately described. Poisson regression with robust standard errors was used to assess our primary question of whether vitamin D3 or gargling could reduce the number of clinical URTIs experienced in those groups. This analysis was chosen in place of logistic regression since odds ratios overestimate treatment effects when incorrectly interpreted as risk ratios. Robust standard errors were calculated in place of model based standard errors which are typically too large. Multiple imputation, using the Markov chain MonteCarlo method, was conducted to address missing data. Information collected at baseline and through weekly surveys was used to predict missing values for independent and dependent variables. The pooled imputed data was used to conduct an ITT analysis adjusted for randomization strata: housing, trial year, vitamin D3 and gargling allocation. Interaction between vitamin D3 use and gargling was investigated using a cross product term. A complete case analysis, adjusted for the same variables, was performed as a sensitivity analysis. An identical complete-case analysis was conducted to assess the secondary outcome of laboratory confirmed infections. Symptom severity and viral load (log viral copies/mL) were compared by t-test. Cox regression was used to assess time to symptom resolution adjusted for the variables listed above. All analyses were planned a priori. Results were considered statistically significant with p < 0.05.	**Main findings** Of 600 participants, 471 (78.5%) completed all surveys while 43 (7.2%) completed none; 150 (25.0%) reported clinical URTI. Seventy participants (23.3%) randomized to vitamin D3 reported clinical URTI compared to 80 (26.7%) randomized to placebo (RR:0.79, CI95:0.61-1.03, p = 0.09). Eighty-five participants (28.3%) randomized to gargling reported clinical URTI compared to 65 participants (21.7%) randomized to the no gargling arm (RR:1.3, CI95:0.92-1.57, p = 0.19). Laboratory testing identified 70 infections (46.7 per 100 URTIs). Vitamin D3 treatment was associated with a significantly lower risk for laboratory confirmed URTI (RR: 0.54, CI95:0.34-0.84, p = 0.007) and with a significantly lower mean viral load measured as log10 viral copies/mL (mean difference: −0.89, CI95: -1.7, −0.06,p = 0.04). Fewer students assigned to gargling experienced laboratory confirmed URTI, however this was not statistically significant (RR:0.82, CI95:0.53-1.26, p = 0.36).	**Ethics approval?** The study protocol was approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board. **Adverse events?** Accounted for. **Aim?** Primary outcome clearly defined. **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. **Procedure for randomization?** The study sample was stratified based on housing (in residence versus off-campus) and block randomization occurred within each stratum using a 1:1:1:1 allocation ratio. **Blinding?** Both participants and investigators were blinded (except in the gargling part of the study) **Did the groups receive the same co-intervention/treatments?** Yes **Primary endpoints – validated?** Low risk of classification bias. **Risk of attrition bias?** Low risk. **Presentation of results?** Yes, see results. **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a younger population, thus findings not generalizable to older populations. Application in clinical situations with counselling regarding vitamin D supplementation. **Did authors review all outcomes?** Yes. **Cost/benefit effectiveness** Not assessed. **Findings supported by previous literature?** Yes, but the study also differed in several potentially important ways from previous studies, and this was well elucidated. **Strengths:** Laboratory confirmation of the primary outcome. **Weaknesses:** Self reported data and self-collected nasal swabs. Definition of clinical URTI may have been excessively broad and insufficiently specific. Study was underpowered as the observed event rate was lower than expected. Short intervention period. Not measured 25OHD. **Plausible explanations for the results?** Yes.
Conclusion *These results suggest that vitamin D3 is a promising intervention for the prevention of URTI. Vitamin D3 significantly reduced the risk of laboratory confirmed URTI and may reduce the risk of clinical infections*			
Country Canada			
Data collection period 2010-2011			

Reference: Tran B, Armstrong BK, Ebeling PR, English DR, Kimlin MG, van der Pols JC, et al. Effect of vitamin D supplementation on antibiotic use: a randomized controlled trial. The American journal of clinical nutrition. 2014;99(1):156-61.	Study design: Post hoc analysis of a previously performed **RCT** *(Double blind, placebo-controlled)*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
Aim: to examine the effect of oral vitamin D supplementation on antibiotic use	**Recruitment** Recruited 644 people aged between 60 and 84 y. We used the Australian Electoral Roll as the sampling frame, and the recruitment proportion was 10%. **Inclusion-/exclusion criteria** • Inclusion criteria: Aged between 60 and 84 y. • Exclusion criteria: People who were taking .400 IU vitamin D/d or those who had a history of kidney stones, hyperparathyroidism, osteomalacia, osteoporosis, or sarcoidosis. **Data** Participants were planned to be followed for 12 mo of intervention, but because of some delays in recruitment, 22% of participants (n = 148) were required to complete the study in 9–11 mo to comply with the expiry date of the investigational product. Concentrations of serum 25(OH)D before and after the intervention were measured in nonfasting blood samples by using a commercial chemiluminescent immunoassay [LIAISON25(OH)DVitamin D TOTAL Assay; DiaSorin Inc]. Intraassay and interassay variances were 3–6% and 6–9%, respectively. Information about demographic characteristics and lifestyle factors (time outdoors, physical activity, smoking, alcohol consumption, and vitamin D intake) from a self-reported questionnaire at study entry. We asked participants to consent to linkage with pharmacy records held by the national health insurance scheme (Medicare Australia). This enabled us to capture information about antibiotics prescribed that qualified for a subsidy through the Australian Pharmaceutical Benefits Scheme (PBS). **Outcome validation (i.e. diagnosis).** Validation were described for all outcomes. **Intervention variables** Monthly Vitamin D3 30,000 IU, 60,000 IU or placebo. **Important confounding factors** See statistical methods. **Statistical methods.** Power calculations not described. Used ITT analyses to assess the effect of vitamin D supplementation on antibiotic use. Conducted analyses restricted to subjects who both consented to linkage and completed the study (n = 601). To assess the effect of vitamin D supplementation on whether or not participants were prescribed antibiotics at least once, calculated RRs and 95% CIs by using a log-binomial model and taking into account person time. The total number of times antibiotics were prescribed in the supplement compared with placebo groups was assessed by using negative binomial regression. In addition to analyzing all antibiotics as a single medication group, the following subgroups were analyzed: penicillins, cephalosporins, and other antibiotics to determine whether results varied by the subclass of antibiotics. First, all analyses were conducted for all participants, and second, analyses were stratified by age (≥70 compared with <70 y) to address the hypothesis that vitamin D supplementation may have a greater effect in older people whose immune systems are likely to be less effective. The significance of the difference in effect sizes according to age analyzed by including a multiplicative term (age group x randomization group) in the models adjusted for baseline 25(OH)D. Also investigated the effect of stratifying by the baseline 25(OH)D concentration at the median and 50 nmol/L.	**Main findings** People who were randomly assigned 60,000 IU cholecalciferol had non-significant 28% lower risk of having antibiotics prescribed at least once than did people in the placebo group (RR: 0.72; 95% CI: 0.48, 1.07). In analyses stratified by age, in subjects aged ≥70 y, there was a significant reduction in antibiotic use in the high dose vitamin D compared with placebo groups (RR: 0.53; 95% CI: 0.32, 0.90), whereas there was no effect in participants aged <70 y (RR: 1.07; 95% CI: 0.58, 1.97) (P-interaction = 0.1).	• **Ethics approval?** Approved by the Human Research Ethics Committee at the Queensland Institute of Medical Research • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Participants were randomly assigned by the National Health and Medical Research Council Clinical Trials Centre by using computer-generated stratified permuted blocks • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability?** Not generalizable to • **Applicability in clinical practice?** Considering that the proportion of participants with insufficient serum 25(OH)D was higher than has been reported for other Australian population, it is possible that these results are not generalizable to all Australian adults. Thus, the confirmation of these results in other trials would be advisable before assuming that our findings can be extrapolated to all adults in this age range. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, **Strengths:** Use of linkage to national administrative health data sets to capture prescriptions of antibiotics, which avoided the need to rely on self-report for the key outcome. The follow-up and compliance in this study were extremely high and not different between the 3 study arms. No difference in the proportion of people who had visited a general practitioner at least once during the trial or in the number of general practitioner visits, suggesting that all participants had an equal opportunity to be prescribed antibiotics. In addition, the PBS does not capture medications prescribed for hospital inpatients, but restriction of the analysis to subjects who had not reported being hospitalized during the trial (n = 490; no difference between groups) generated the same trends, although CIs were wider. **Weaknesses:** The randomization rate was only 10%, % of participants w/low s-25(OH)D was higher than prev reported for Australian populations. Unable to assess the influence of vitamin D on specific infections. Approx. 1/4 of participants did not receive the full 12 mo suppl, and although this amount did not differ according to the study arm, it is possible that it may have limited the power of the D-Health Trial and increased the likelihood of the occurrence of type 2 error. The post hoc nature (potential for type 1 error). **Plausible explanations for the results?** Yes.
Conclusion			
Although this study was a post hoc analysis and statistically non significant, this trial lends some support to the hypothesis that supplementation with 60,000 IU vitamin D/mo is associated with lower risk of infection, particularly in older adults			
Country			
Australia			
Data collection period			
2010-2012			

Urashima, 2014

Reference: Urashima M, Mezawa H, Noya M, Camargo CA, Jr. Effects of vitamin D supplements on influenza A illness during the 2009 H1N1 pandemic: a randomized controlled trial. Food & function. 2014;5(9):2365-70.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To elucidate whether vitamin D3 has preventiveactions against influenza A	**Recruitment** Volunteers were recruited by letter and an assembly at the Seisoku High School in Minato-ku. The background,aims, methods, and possible risks/benefits of this study wereexplained to 895 Seisoku High School students aged 15 to 18years and their parents, first by a letter and then via talks andcommunication by the first author (M.U.) at the school. **Inclusion-/exclusion criteria** • Inclusion criteria: Students aged 15 to 18years not fulfilling exclusion criteria • Exclusion criteria: students who had already been infected with an inuenza-like illness after May 2009; (2) those who had a history of urinary tract stones or diseases of calcium/bone metabolism; (3) those who had a bone fracture; (4) those who were already taking vitamin D supplements or activatedvitamin D; (5) those who had asthma, as asthma may be an exacerbating factor in the pandemic inuenza; and (6) those who had serious allergies, in order to avoid severe reactions to ingredients in the study supplement **Data** 247 participants were followed for 2 months. The students were asked to visit a doctor's clinic if they developed a fever (defined as body temperature higher than 37.0 degrees C) during the pandemic phase. As a school rule in Japan, students or parents are required to inform homeroom teachers of a doctor's diagnosis. Then, the homeroom teacher was asked to send a fax to the data monitoring center providing a detailed description from thestudents/parents as told to them and/or a certificate provided by the doctor regarding the diagnosis of or recovery from influenza A. Participants were also asked to complete a daily log during the study period to: (1) reconfirm the diagnosis of influenza by a medical doctor,(2) assess adherence with the study supplement, and (3) assessother subjective symptoms, such as fever, runny nose, cough,sore throat, and arthralgia **Outcome validation (i.e. diagnosis)** The primary outcome was the occurrence of influenza A, diagnosed by medical doctors with RIDT usingnasopharyngeal swabs. The sensitivity of the RIDT used in Japan for 2009 pandemic influenzaA (H1N1) virus infection confirmed by polymerase chainreaction is approximately 77%. Secondary outcomes were: (1)doctor-diagnosed influenza-like illness, including not only RIDT-positive but also RIDT-negative influenza cases suspected by doctors due to clinical signs (e.g., fever, headache, arthralgia,runny nose, and coughing) and close contact with patients with influenza; and (2) school absence and the reason for absence. **Intervention variables** Vitamin D3 2000 IU versus placebo daily. **Important confounding factors** Not described. **Statistical methods** Power calculation was described. Efficacy was assessed using anintention-to-treat analysis, which includes all students in thestudy, regardless of whether they were taking supplement after randomization. The incidence of both primary and secondary outcomes in the two groups was compared using a RR and 95%CI. All reported P values are two-sided and P < 0.05 was considered statistically significant. No adjustments were madefor multiple comparisons.	**Main findings** Influenza A was equally likely in the vitamin D3 group (20/148:13.5%) compared with the placebo group (12/99: 12.1%). By post hoc analysis, influenza A occurredsignificantly less in the vitamin D3 group (2/148: 1.4%) compared with the placebo group (8/99: 8.1%) (risk ratio, 0.17; 95% confidence interval, 0.04 to 0.77; P ¼ 0.009) in the first month. During the second month, the vitamin D3 group experienced more events and effectively caught up with the placebo group	• **Ethics approval?** The study protocol wasreviewed and approved by the institutional review board of Seisoku High School. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Used a central computerized procedure torandomly assign students in permutated blocks of five to receive either vitamin D3 or placebo in a 3 : 2 ratio. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk, no loss to follow-up. • **Presentation of results?** Yes, see results. • **Generalizability?** Trial was performed at a single Japanese high school during the 2009H1N1 pandemic and not in a more diverse population during amore typical infuenza season, a study design that reduces generalizability • **Applicability in clinical practice?** Considering the homogenous population recruited, extrapolation of the results are limited with regards to older, younger and populations with comorbid conditions. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, similarity of results in two previous RCTs **Strengths:** Study design. No loss to follow-up. **Weaknesses:** Single center. Did not measure 25OHD. Small numbers of RIDT positive. Investigators did not perform RITD directly or consult medical records. Incidence of RIDT-positive influenza A was 13% which was far less than the expected 25%. Small sample size. Individual UVB/sun exposure per day and diet was not measured/assessed. Diagnosis was made by means of a RIDT and not polymerasechain reaction at the primary care setting in Japan **Plausible explanations for the results?** Yes.

Conclusion

Vitamin D3 supplementation did not lower the overall incidence of influenza A duringthe 2009 H1N1 pandemic. A post hoc analysis suggests that the initial benefit during the first month oftreatment was lost during the second month.

Country

Japan

Data collection period

2009

Reference: Dubnov-Raz G, Livne N, Raz R, Cohen AH, Constantini NW. Vitamin D Supplementation and Physical Performance in Adolescent Swimmers. International journal of sport nutrition and exercise metabolism. 2015;25(4):317-25.	Study design: **RCT** *Double blind, placebo-controlled*
	Grade - quality Low ⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
The aim of this study was to examine if vitamin D3 supplementation reduces URI burden in vitamin D-insufficient swimmers	**Recruitment** 82 adolescent competitive swimmers from four swimming teams in Israel were tested for serum 25(OH)D concentrations. Swimmers with insufficiency were invited to participate. **Inclusion-/exclusion criteria** • Inclusion criteria: Age range 12–21 years, and being a swimmer in the selected teams • Exclusion criteria: Swimmers were excluded from testing if they refused to undergo any or all of the testing procedures, if they had a history of chronic health conditions, or if they were taking any chronic medications or dietary supplements, including multivitamins. **Data** 55 competitive adolescent swimmers with vitamin D insufficiency participants were followed for 12 winter weeks. A URI symptom questionnaire was completed weekly. Serum vitamin D concentrations were measured at supplementation beginning and end by drawing 5 ml of blood, which was immediately transferred to the endocrine laboratory at the Sheba Medical Center, centrifuged and stored at 4 °C for analysis. Serum 25(OH)D was measured by radio-immunoassay (Diasorin, Stillwater, Minnesota, USA. Intra-assay CV 12%; Interassay CV 10%). **Outcome validation (i.e. diagnosis).** The primary outcomes were the number of URIs, their duration, and their severity. When a participant had more than one URI during the trial, in the analysis of duration and severity we used the mean value over the episodes. As vitamin D is fat soluble, we allowed serum vitamin D concentrations to rise before beginning URI event recording. URI data were collected for 12 weeks, starting one month after supplementation began and ending one month after suppl ended. During the data collection period, participants filled out a respiratory symptoms questionnaire based on the Wisconsin Upper Respiratory Symptom Survey (WURSS). A URI event was defined as having at least one URI symptom for at least one day, and at least three days apart from a prior event. The duration of each URI event was the number of days that symptoms were present. The severity of URI events was calculated as the average self-rated score. **Intervention variables** Randomized to receive vitamin D3 2,000 IU or placebo daily. **Important confounding factors** See statistical methods. **Statistical methods** The duration and severity of URI in the intervention groups was analyzed by unpaired t test, and changes in 25(OH)D concentrations were analyzed using a paired t test. Proportions were compared using Fisher's exact test. The relationship between the change in 25(OH)D concentrations during the study and the duration of colds was analyzed by linear regression. A linear regression model was constructed explaining the duration of colds by the change in 25(OH)D concentration and the intervention. Interaction term were added between those two explanatory variables. The improvement of the model fit was calculated from the change in $-2 \times \log$ (likelihood), which follows the χ^2 (1 df) distribution. Also examined whether the baseline 25(OH)D concentration or the change in 25(OH)D concentration during the study better explained the duration of colds in the placebo group by constructing a model in which the duration of colds was first explained by the BL 25(OH)D concentration. Addition of the change in 25(OH)D concentration improved the model fit by χ^2 (1 df) = 14.2, corresponding to p =.0002. Performed similar analyses for the severity of colds.	**Main findings** There were no between-group differences in the frequency, severity, or duration of URIs. Exploratory analyses revealed that in the placebo group only, the change in 25(OH)D concentrations during the trial was highly associated with the duration of URIs (r =−0.90, p < .001), and moderately associated with the severity of URIs (r = −0.65, p = .043). The between-group differences for duration were highly significant.	• **Ethics approval?** Approved by the Institutional Review Board of Sheba Medical Center, Tel Hashomer, Israel, and conducted according to the Declaration of Helsinki • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** The randomization process was conducted for males and females separately, arranging each group in order of their baseline serum 25(OH)D concentrations. Each two contiguous participants were randomized as a pair, one to the intervention arm and the other to the placebo arm, using a computer software program. This technique ensured an equal number of males and females in each group, and similar mean baseline 25(OH)D concentrations in both study arms. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited in which few participants with vitamin D deficiency, the results are applicable mainly to young swimmers with vitamin D insufficiency. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Findings of no overall effect of vitamin D supplementation on URI burden are in concert with four previously published randomized-controlled trials in the general adult population and one conducted in children. **Strengths:** Study design. Used vitamin D3. High adherence to the vitamin supplementation. Relatively high number of URI events, which overlapped the timing of maximal influenza-like activity in Israel, and allowed for a significant amount of data to be collected. **Weaknesses:** Small sample size, and smaller than expected thus limited power. Self reported data. Low response rate in questionnaire filling. Few participants with vitamin D deficiency. 25OHD at trial end were above 30 ng/ml in only 50% the supplemented participants. **Plausible explanations for the results?** Yes.

Conclusion

Vitamin D3 supplementation in adolescent swimmers with vitamin D insufficiency did not reduce URI burden. However, larger decreases in serum 25(OH)D concentrations were associated with significantly longer and more severe URI episodes

Country

Israel

Data collection period

2010-2011

Martineau, 2015 (ViDiCO)

Reference: Martineau AR, James WY, Hooper RL, Barnes NC, Jolliffe DA, Greiller CL, et al. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. The Lancet Respiratory medicine. 2015;3(2):120-30	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate-High ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To investigate whether vitamin D3 (colecalciferol) supplementation would reduce the incidence of moderate or severe COPD exacerbations and upper respiratory infections	**Recruitment** Individuals with a medical record diagnosis of COPD, emphysema, or chronic bronchitis were identified in 60 general practices and at COPD clinics in four Acute National Health Service Trusts in London, UK. They were invited to attend a screening visit. **Inclusion-/exclusion criteria** • Inclusion criteria: In appendix. • Exclusion criteria: Age younger than 40 years, ratio of forced expiratory volume in 1 s (FEV1) to forced vital capacity (FVC) or slow vital capacity of more than 70% after inhalation of 400 μg salbutamol, and medical record diagnosis of asthma. Vitamin D supplements taken at doses of up to 10 μg (400 IU) per day were permitted during the trial. **Data** 240 participants were followed for 1 year. Individuals who met the eligibility criteria entered a run-in period of at least 2 weeks during which they completed a daily study diary, recording details of respiratory symptoms, medication use, health-care use, time off work, and out- of-pocket expenses incurred as a result of COPD exacerbations or upper respiratory infections. Face- to-face follow-up visits were at 2 months, 6 months, and 12 months. Blood samples for the assessment of vitamin D status and PTH were taken at 2 months and 12 months. **Outcome validation (i.e. diagnosis)** Coprimary endpoints for the trial were time to first moderate or severe COPD exacerbation and time to first upper respiratory infection. Prespecified secondary endpoints were well described. Exacerbation of COPD was defined as the occurrence of at least two major COPD symptoms, or one major COPD symptom and at least one minor COPD symptom, during at least 2 days consecutively. Upper respiratory infection was defined as an influenza-like illness (indicated by the presence of cough, feeling of fever or chills, and muscle pain) or a cold, defined with the Jackson criteria, and this was a priori validated with PCR detection of 11 respiratory viruses in nasopharyngeal swabs. **Intervention variables** Randomly assigned in a 1:1 ratio to receive six 2-monthly oral doses of 6 mL Vigantol oil (Merck Serono, Darmstadt, Germany) containing 3 mg (120,000 IU) vitamin D3 or 6 mL identical placebo (Miglyol oil, Caesar and Loretz, Hilden, Germany). Administration of doses at 2 months and 6 months was directly observed, and doses at 4 months, 8 months, and 10 months were taken during a telephone call scheduled with a member of the study team. **Important confounding factors** None described. **Statistical methods** Analysis was by ITT, and significance was tested at the 5% level. Time-to-event outcomes were analysed with Cox regression adjusted for stratification factors. Methods for statistical analysis of secondary outcomes were in the appendix. Interim analyses described.	**Main findings** Vitamin D 33 compared with placebo did not affect time to first moderate or severe exacerbation (adjusted HR 0·86, 95% CI 0·60–1·24, p=0·42) or time to first upper respiratory infection (0·95, 0·69–1·31, p=0·75). Prespecified subgroup analysis showed that vitamin D3 was protective against moderate or severe exacerbation in participants with baseline serum 25-hydroxyvitamin D concentrations of less than 50 nmol/L (0·57, 0·35–0·92, p=0·021), but not in those with baseline 25-OH D levels of at least 50 nmol/L (1·45, 0·81–2·62, p=0·21; p=0·021 for interaction between allocation and BL serum 25-OH D status). BL vitamin D status did not modify the effect of the intervention on risk of upper respiratory infection ($p_{interaction}$=0.41).	• **Ethics approval?** Approved by the East London and the City Research Ethics Committee 1. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Generated randomisation sequence using a computer program that assigned the term active or placebo to the numbers 1 to 300 with permuted blocks of ten. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Moderate risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative for a population with a wide spectrum of disease severity, recruited from several urban community and hospital centres. Applicable in clinical recommendations regarding vitamin D supplementation. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, findings support and extend those from the trial by Lehouck et al who investigated the effects of a monthly oral dose of 2·5 mg vitamin D3 on exacerbation risk in a cohort of patients with moderate to very severe COPD. Findings in previous literature otherwise well elucidated in the discussion. **Strengths:** Inadequate vitamin D status was highly prevalent in the study population at BL, bolus regimen with high compliance. The use of prospectively completed daily symptom diaries allowed detection of unreported exacerbations and to characterize participants' symptoms with precision, allowing ascertainment of the effect of the intervention on symptom severity as well as incidence of exacerbations and URTIs. **Weaknesses:** Median time by first moderate or severe exacerbation and URTI were longer than anticipated in the power calculation, whereby completion of the study diary improved disease control through enhanced compliance with inhaled corticosteroids. Intermittent bolus dosing might be less effective than daily dosing for inducing the non-classical actions of vitamin D. **Plausible explanations for the results?** Yes.
Conclusion *Vitamin D3 suppl protected against moderate or severe exacerbation, but not URTI, in patients with COPD with baseline 25-OH D levels < 50 nmol/L. Findings suggest that correction of vitamin D deficiency in patients with COPD reduces the risk of moderate or severe exacerbation.*			
Country UK			
Data collection period 2009-2012			

Martineau, 2015 (ViDiAs)

Reference: Martineau AR, MacLaughlin BD, Hooper RL, Barnes NC, Jolliffe DA, Greiller CL, et al. Double-blind randomised placebo-controlled trial of bolus-dose vitamin D3 supplementation in adults with asthma (ViDiAs). Thorax. 2015;70(5):451-7.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate-High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
Does vitamin D3 supplementation prevent asthma exacerbation or upper respiratory infection (URI) in adults with inhaled corticosteroid-treated asthma?	**Recruitment** Participants were identified by searching databases at 60 general practices and at asthma clinics in two Acute National Health Service Trusts in London, UK, and invited for screening **Inclusion-/exclusion criteria** • <u>Inclusion criteria</u>: Adult patients with a medical record diagnosis of asthma treated with ICS • <u>Exclusion criteria</u>: Age <16 years or >80 years; tobacco smoking history >15 pack-years; medical record diagnosis of COPD; and failure to exhibit significant variability/ reversibility in airway obstruction **Data** 250 participants were followed for 1 year. Participants attending screening visits completed the St George's Respiratory Questionnaire (SGRQ), the EuroQoL-5D questionnaire and the Asthma Control Test (ACT) and underwent a baseline clinical assessment incorporating spirometry, measurement of FENO and collection of a blood sample. A subset of 50 participants was invited to undergo sputum induction with hypertonic saline. Participants fulfilling eligibility criteria entered a run-in period of at least 2 weeks, during which they were asked to complete a symptom diary on a daily basis for 12 months. Face-to-face follow-up visits were performed at 2 months, 6 months and 12 months of follow-up. **Outcome validation (i.e. diagnosis)** Coprimary end points for the trial were time to first severe asthma exacerbation and time to first URI. Severe asthma exacerbation was defined (see text). URI was defined as influenza-like illness or as a cold with symptom scores meeting modified Jackson criteria. Secondary end points were peak values and areas under the curve for symptom scores during severe exacerbation/URI; proportion of days with poor asthma control; proportion of nights with awakenings due to asthma symptoms; time to unscheduled healthcare attendance and use of antibiotics for exacerbation/URI; ACT and SGRQ scores, FENO concentration, daily ICS doses, % predicted FEV1, PEFR, use of inhaled relief medication and induced sputum differential cell count and supernatant inflammatory profiles at 2 - 6 - and 12 months; through serum concentrations of 25(OH)D and parathyroid hormone (PTH) at 2 months and 12 months; and health economic outcomes (costs of exacerbations and URI, quality-adjusted life years and incremental net benefit over 1 year). **Intervention variables** Randomly assigned to receive six 2-monthly oral doses of 6 mL Vigantol oil (Merck Serono, Darmstadt, Germany) containing 3 mg (120 000 IU) vitamin D3, or 6 mL organoleptically identical placebo (Miglyol oil, Caesar and Loretz, Hilden, Germany) with allocation ratio 1:1. **Important confounding factors** Not described. **Statistical methods** Analysis was by intention-to-treat: all participants who took at least one dose of study medication were included in efficacy and safety analyses. Significance was tested at the 5% level. Time-to-event outcomes were analysed using Cox regression adjusted for stratification factors. Subgroup analyses were conducted to determine whether the effect of vitamin D3 supplementation on coprimary outcomes was modified by baseline vitamin D status (using serum 25(OH)D thresholds of 50 nmol/L and 75 nmol/L) or genotype.	**Main findings** Vitamin D3 did not influence time to first severe exacerbation (adjusted HR 1.02, 95% CI 0.69 to 1.53, p=0.91) or first URI (adjusted HR 0.87, 95% CI 0.64 to 1.16, p=0.34). No clinically important effect of vitamin D3 was seen on any of the secondary outcomes listed above. The influence of vitamin D3 on coprimary outcomes was not modified by baseline vitamin D status or genotype. 206/250 participants (82%) were vitamin D insufficient at baseline.	• **Ethics approval?** Approved by the East London and The City Research Ethics Committee 1 • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Randomisation was assigned by permuted blocks of 10 and stratified according to (A) British Thoracic Society treatment step (2–3 vs 4–5) and (B) inclusion in versus exclusion from the induced sputum substudy. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Not described. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Assessed health economic outcomes as part of secondary outcomes. • **Findings supported by previous literature?** Yes, the results of this trial support and extend the findings of the VIDA trial, recently reported by Castro et al. **Strengths:** Inadequate vitamin D status was highly prevalent. Significant potential for improvement in asthma control (high FENO at baseline). Generous dose of vitamin D3 (more than three times the recommended dietary allowance for adults proposed by the US IOM). High degree of compliance with intervention (3/6 doses were directly observed and 3/6 were supervised telephonically). Daily symptom diaries allowed to characterise participants' symptoms in fine detail. **Weaknesses:** Patients with URI symptoms were not sampled for detection of pathogen. A minority (32%) of asthma exacerbations in our trial were associated with URI (a somewhat lower proportion than the 44% reported elsewhere). Urban setting and participants had a relatively high prevalence of allergic rhinitis and eczema at BL. Study may have lacked power to detect small or moderate effects of the intervention on exacerbation risk. Intermittent bolus dosing regimen; the interarm difference in serum 25(OH)D concentrations at 12months was modest (22nmol/L); some have proposed that intermittent dosing may be less effective than daily for inducing non-classical actions of vitamin D. **Plausible explanations for the results?** Yes.
Conclusion			
In patients with a high prevalence of vitamin D insufficiency at baseline, vitamin D3 supplementation did not influence time to exacerbation or URI or concentrations of inflammatory markers in induced sputum; effects of the intervention were not modified by baseline vitamin D status or by polymorphisms in the vitamin D pathway			
Country			
UK			
Data collection period			
2009-2012			

Martineau, 2015 (ViDiFlu)

Reference: Martineau AR, Hanifa Y, Witt KD, Barnes NC, Hooper RL, Patel M, et al. Double-blind randomised controlled trial of vitamin D3 supplementation for the prevention of acute respiratory infection in older adults and their carers (ViDiFlu). Thorax. 2015;70(10):953-60.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
Does addition of intermittent bolus-dose vitamin D3 supplementation to a daily low-dose regimen enhance protection against acute respiratory infection in older adults and their carers?	**Recruitment** Sheltered accommodation schemes in London were identified by searching http://www.housingcare. org/. 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 were sent a letter inviting them to attend a screening visit. **Inclusion-/exclusion criteria** • Inclusion criteria: Provided in online supplemental material. • Exclusion criteria: 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 contain- ing >10 µg (400 IU) vitamin D per day up to 2 months before first dose of study medication **Data** Participants attending the screening visit completed the EuroQoL EQ-5D questionnaire. 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. 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. **Outcome validation (i.e. diagnosis)** Definition and validation of the primary outcome was well described. The primary outcome was time to first ARI; secondary outcomes included time to first upper/lower respiratory infection, and symptom duration. During the 2 weeks run-in period, a study diary was completed daily, and 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. Participants completing follow-up filled six diaries in total (12 weeks of data/diary). **Intervention variables** 240 participants were randomized. 54 schemes (137 individual participants) were allocated to the active intervention (vitamin D3 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 D3 10 µg daily for residents, placebo once every 2 months for carers) for 1 year. **Statistical methods** Analysis was by ITT, and significance was tested at the 5% level. Time-to-event outcomes were analysed using a Cox regression adjusted for minimisation variables and participant study group, allowing for a shared frailty within the same unit with frailty following a gamma distribution. Effects of allocation on time-to-event outcomes were presented as HRs. Prespecified secondary endpoints and subgroup analyses were well described.	**Main findings** 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. Inadequate vitamin D status was common at baseline: 220/240 (92%) participants had serum 25(OH) D concentration <75 nmol/L. The probability that the active intervention was cost-effective for prevention of ARI was less than 60% at a realistic willingness to pay (£20 000) for a QALY gain.	• **Ethics approval?** Approved by East London and The City Research Ethics Committee 1. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Assigned to active or control arms of the trial with a 1:1 ratio. Details provided in supplemental material. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a vitamin D deficient population. Application in clinical situations are relevant regarding vitamin D recommendations. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Also collected data on quality of life and ARI-associated costs, allowing us to conduct a health economic evaluation of the intervention. • **Findings supported by previous literature?** Findings in previous studies were well elucidated in the discussion. **Strengths:** High prevalence of inadequate vitamin D status, and adherence to bolus doses of study medication was high as administration of all such doses was directly supervised by study staff. Collected detailed prospective data on outcomes by using a PCR-validated case definition. This allowed detection of 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. **Weaknesses:** Study design: The proportion of residents/ carers enrolling in the trial at each scheme was lower than expected. 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 characterized if vitamin D status had additionally been measured at 3–7 days post dose when it would have been expected to peak. **Plausible explanations for the results?** Yes.
Conclusion			
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			
Country			
UK			
Data collection period			
2010-2012			

Denlinger, 2016

Reference: Denlinger LC, King TS, Cardet JC, Craig T, Holguin F, Jackson DJ, et al. Vitamin D Supplementation and the Risk of Colds in Patients with Asthma. American journal of respiratory and critical care medicine. 2016;193(6):634-41.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine whether vitamin D supplementationreduces cold symptom occurrence and severity in adults withmild to moderate asthma and vitamin D insufficiency	**Recruitment** Not described. **Inclusion-/exclusion criteria** • Inclusion criteria: Mild tomoderate asthma, baseline serum levels of25(OH)D3 less than 30 ng/ml, and asthma symptoms despite low-dose ICS therapy • Exclusion criteria: Not described. **Data** 408 adult patients were followed for 28 weeks. Participants underwent assessment of treatment failure, exacerbation, lung function,airway hyperresponsiveness, asthma symptoms, asthma control (measured usingthe Asthma Control Test [ACT] score through surveys at baseline and Instructions on how to complete these surveys and their distribution occurred at the randomization visit, with reinforcement of its use at all subsequent visits, which occurred at 4- to 6-week intervals. Electronic diaries were used to assess asthma symptoms. Melanin-dependent skin pigmentation as a measure of sun exposure were estimated with a SmartProbe 400 spectrophotometer and validation was described. Skin pigmentation was measured before and after the 28 weeks of the trial. **Outcome validation (i.e. diagnosis)** Cold symptoms were assessed using the 21-item Wisconsin UpperRespiratory Symptom Survey (WURSS-21), a validated instrument with a range of 0–140 points and a minimal important difference of 18.5. **Intervention variables** Randomized to receive placebo or cholecalciferol (100,000 IU once, then 4,000 IU/d for 28 wk) as add-on therapy in the background of a tapering ICS protocol **Important confounding factors** Se statistical methods. **Statistical methods** Power calculation thoroughly described. Testing the primary hypothesis regarding the effect of vitamin D supplementationon the severity of colds was addressed by fitting a repeated-measures analysis ofcovariance (RM-ANCOVA) model tothe peak WURSS-21 cold scores during each cold, allowing for multiple colds for each participant. In addition to evaluating the average peak cold score from each cold, the average scores on Day 1 and Day 2 were also compared between the intervention and control groups. The rates of colds were compared between the treatment groups using Poisson regression. Each model included adjustment for center, African American race, and BMI>25 kg/m2. Exploratory analyses were also well elucidated. All tests were two-sided, with a P value less than 0.05 denoting significance.	**Main findings** Vitamin D supplementation had no effect on the primary outcome: the average peak WURSS-21 scores (62.0 [95% CI, 55.1–68.9; placebo] and 58.7 [95% CI, 52.4–65.0; vitamin D]; P = 0.39). The rate of colds did not differ between groups (rate ratio [RR], 1.2;95% CI, 0.9–1.5); however, among African Americans, those receiving vitamin D versus placebo had an increased rate of colds (RR, 1.7; 95% CI, 1.1–2.7; P = 0.02). This was also observed in a responder analysis of all subjects achieving vitamin D sufficiency, regardless of treatment assignment (RR, 1.4; 95% CI, 1.1–1.7; P = 0.009). A total of 203 participants experienced at least one cold. During the trial, 25-hydroxyvitamin D levels of 41.9 ng/ml (95% confidence interval [CI], 40.1–43.7 ng/ml) were achieved by 12 weeks,	• **Ethics approval?** Approved by • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups. • **Procedure for randomization?** Not described. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability?** Not generalizable to • **Applicability in clinical practice?** Considering the population recruited (including subjects with mild to moderate asthma), the results might not be representative among patients with more severe asthma who had a history of frequent RTI-induced exacerbations. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Not described. **Strengths:** Adequately powered (90% power toobserve an effect size that was smaller thanthe minimal clinically important differencefor the survey instrument used), observation period longer than 6 months **Weaknesses:** No formal adjustment for the number of secondary analyses that were performed, thus secondary results should be considered exploratory. Population studied might not have been ideal to evaluate respiratory exacerbation during the course of the cold to confirmvirus-associated events. The ICS tapering protocol may have had unanticipated effects. Possible that the change in ICS doses during the protocol influenced vitamin D metabolism and/or the expression of the vitamin D receptor and binding protein. **Plausible explanations for the results?** Yes.

Conclusion
Our findings in patients with mild tomoderate asthma undergoing an inhaled corticosteroiddose reduction do not support the use of vitamin Dsupplementation for the purpose of reducing cold severity orfrequency.

Country
USA

Data collection period

Gupta, 2016

Reference: Gupta P, Dewan P, Shah D, Sharma N, Bedi N, Kaur IR, et al. Vitamin D Supplementation for Treatment and Prevention of Pneumonia in Under-five Children: A Randomized Double-blind Placebo Controlled Trial. Indian pediatrics. 2016;53(11):967-76.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To evaluate the efficacy of single oral mega-dose of Vitamin D3 for treatment and prevention of pneumonia in under-five children.	**Recruitment** Volunteers were recruited from hospital tertiary-care. **Inclusion-/exclusion criteria** • Inclusion criteria: age between 6 mo-5 yage with WHO-defined severe pneumonia (presence of lower chest indrawing in children presenting with cough or difficult breathing), and family staying within 10 km radius of the hospital. • Exclusion criteria: Children having a history or clinical features suggestive of rickets, severe acute malnutrition, asthma, hypertension,complicated pneumonia or illness severe enough to require ventilation, chronic respiratory disease, heart disease, renal or hepatic insufficiency, neurological illness resulting in abnormalities of muscle tone/power, and known immunodeficiency.	**Main findings** 126 (39%) were vitamin D deficient (serum25(OH)D <12 ng/mL). Median (95% CI) time for resolution of severe pneumonia was 30 (29, 31) in the vitamin D group as compared to 31 (29,33) in the placebo group [adjusted hazard ratio (95%CI): 1·39 (1·11, 1·76); P=0·005].	• **Ethics approval?** Approval was obtainedfrom the institutional ethical committee of the UniversityCollege of Medical Sciences, Delhi. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups. • **Procedure for randomization?** Computer-generated block randomization
Conclusion	**Data** 324 children (of 980 assessed) were followed for 6 months. Details were recorded for socio-demographic variables, immunization status, nature and duration of presenting symptoms, and past history of similar episodes/nebulization. All children were examined for vital signs, pallor, cyanosis, nasal flaring, grunt, and mental status. Measurement method for these factors were well described. Chest was auscultated for presence of any added sounds (wheeze and/or crepitations). Weight, length/height, mid-upperarm circumference, and head circumference were recorded for all participants as per standard techniques.	The risk of recurrence ofpneumonia in next 6 months was comparable in the two groups [placebo: 36/158 (22·8%); vitamin D: 39/156 (25%); RR (95% CI):1·13 (0·67,1·90); P=0·69].	• **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Moderate risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results.
There is no robust evidence of a definite biological benefit, either for therapy or prevention, to suggest a routine megadose supplement of vitamin D3 for under-five children with severe pneumonia	**Outcome validation (i.e. diagnosis)** At home, participants were followed for 180 days (from day of enrolment) to document the recurrence of episodes of pneumonia. An episode was regarded as 'recurrence' if the child remained free of symptoms of cough or fast breathing for at least seven days followingcompletion of the course of antibiotic therapy as per protocol for the previous episode of pneumonia. Field workers made home visits every fortnight to assess recurrent episodes of pneumonia. If available, the records of hospitalization/treatment were reviewed.	Proportion of vitamin D deficient children declined from 38% to 4% in the supplementation group, and from 41% to 33% in the placebo group, two weeks after supplementation.	• **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a population of children under five. Application in clinical situations in which routine suppl of vitamin D is considered.
Country	**Intervention variables** Single dose of 100,000 IU of oral D3 or placebo **Important confounding factors** See statistical methods.	There was no significant effect of vitamin D supplementation on serum levels of cathelicidin, IgA and IgG.	• **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed • **Findings supported by previous literature?** Findings from
India	**Statistical methods**. Sample size calculations described. Cox proportional hazards regression model was constructed to create the time to event curves and estimate the HRs with 95% CI between the treated/control groups for time to resolution of severe pneumonia, and adjusted for co-variates (age, sex, nutritional status (WHZ score), severity of illness (respiratory rate), and BL s-25(OH)D levels). Incidence of pneumonia during follow up was calculated by dividing the total number of new episodes of pneumonia by total time at risk, for all children in each group. Relative risk for incidence of recurrence of pneumonia (the second primary outcome variable) was compared between the groups. Changes (pre-post) in biochemical and immunological markers between the groups were compared byunpaired Student t test. Parameters which did not follow anormal distribution were log-transformed. Non-parametric (Mann Whitney U) test was used to compare groups if the applied transformation did not result in normal distribution. Within group means at BL and follow-up were compared with paired t-test; or Wilcoxon signed rank test, if the data were not normally distributed. Kaplan-Meier survival function plots were constructed to compare the median duration for time to complete recovery from pneumonia, fever clearance time, and duration of hospitalization, between the two groups (placebo and vitamin D supplemented) by using the logrank test. P<0·05 was considered as significant. Used Bonferroni correction. The above analyses were also conducted for the subgroup of vitamin D deficient participants. The effect of vitamin D supplementation on outcome variables was analyzed by ITT basis.	The time taken for complete recovery from pneumonia, duration of hospitalization, and fever clearance time were comparable for the two groups.	previous studies were well elucidated in the discussion. **Strengths:** Study design. **Weaknesses:** WHO criteria to identify severe pneumonia are highly sensitive but have a low specificity. Clinically, most of these children had a wheezy illness and very fewhad consolidation/bacterial pneumonia; a few may have been suffering from allergic respiratory illness. Did not attempt a microbiological diagnosis of pneumonia by lung tap or bronchoalveolar lavage. No outcome related to cell-mediated immunity. Flow-cytometry and evaluation of cytokines could not be undertaken. Workup for parameters of humoral immunity was also not holistic. Excluded clinical rickets at enrolment but not severe vitamin D deficiency based on 25OHD levels. **Plausible explanations for the results?** Yes.
Data collection period			

Jorde, 2016

Reference: Jorde R, Sollid ST, Svartberg J, Joakimsen RM, Grimnes G, Hutchinson MY. Prevention of urinary tract infections with vitamin D supplementation 20,000 IU per week for five years. Results from an RCT including 511 subjects. Infectious diseases (London, England). 2016;48(11-12):823-8.		Study design: Post hoc analysis of a previously performed *double blind, placebo-controlled RCT*	
		Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To evaluate the effect of supplementation with vitamin D on upper respiratory infections (common cold, bronchitis, influenza) and urinary tract infections(UTI) in a post hoc analysis	**Recruitment** Volunteers were recruited among participants who underwent an oral glucose tolerance test as part of the Tromsø Study 2007–2008. **Inclusion-/exclusion criteria** • Inclusioncriteria: Prediabetes (IFG (serum glucose 6.0–6.9 mmol/L) and/or IGT (fasting serum glucose<7.0 mmol/L and 2-h value 7.8–11.0 mmol/L at OGT test with 75 g glucose) • Exclusion criteria: Primary hyperparathyroidism, granulomatousdisease, history of urolithiasis, cancer diagnosed in the pastfive years, unstable angina pectoris, myocardial infarction orstroke in the past year were excluded. Pregnant or lactatingwomen, or women of fertile age with no use of contraception **Data** 511 participants were followed for 5 years. All visits were performed at the Clinical Research Unit at theUniversity Hospital of North Norway. At the first visit, a brief clinical examination was performed, and questionnaires onmedical history including infections, medication and vitamin D supplementation were filled in. Height and weight were measuredwearing light clothing. Fasting blood samples had beencollected at the OGTT, and supplementary non-fasting bloodsamples were drawn at this visit. **Outcome validation (i.e. diagnosis)** For the next five years, the subjects met every sixth month and filled in questionnaires on infections. The questions regarding infections were: 1) have you the last six months had a common cold, and in that case how many times? 2) have you the last six months had bronchitis, and in that case how many times? 3) have you the last six months had influenza or ILI (with fever), and in that case how many times? 4) have you the last six months had a UTI, and in that case how many times? **Intervention variables** Subjects were randomized (non-stratified) in a 1:1 ratio to one capsule vitamin D (cholecalciferol 20,000 IU (Dekristol; Mibe, Jena, Germany)) per week or an identical looking placebo capsule containing arachis oil (Hasco-Lek, Wroclaw, Poland). New medication was supplied every sixth month and unused capsules returned and counted. The subjects were not allowed to take vitamin D supplements (including cod liver oil) exceeding 400 IU per day. 256 subjects received vitamin D and 255 placebo. **Important confounding factors** Se statistical methods. **Statistical methods.** Normal distribution was evaluated with visual inspection ofhistograms and by kurtosis and skewness. Comparisons between the two groups at BL and during the study were performed with Student's t-test or chi-square tests. Occurrence of RTI or UTI in the two groups was evaluated with Cox regression with gender and age as covariates. p<0.05 (two-tailed) was considered statically significant. Data are presented as mean ± SD for normally distributed values and as median (5th, 95th percentiles) for serum PTH that had a non-normal distribution. The power calculation of the study was made for the main endpoint (development of T2DM) and a separate power calculation for the infection questionnaire was not made.	**Main findings** Mean baseline serum 25OHD level was 60 nmol/L. Eighteen subjects in the vitamin Dgroup and 34 subjects in the placebo group reported UTI during the study (p<0.02), whereas no significant differences were seen for RTI. The effect on UTI was most pronounced in males. The effect of vitamin D on UTI was unrelated to baseline serum 25(OH)D level.	• **Ethics approval?** The study was approved by the Regional Committee for Medical and Health Research Ethics and by the Norwegian Medicines Agency • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups. • **Procedure for randomization?** Not described. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Moderate risk. One hundred and sixteen subjects in the vitamin D and 111 in the placebo group completed the five-year study. • **Presentation of results?** Yes, see results. • **Generalizability?** Considering the population recruited, the results were representative in a well fed, general population with pre-diabetes. Extrapolation to populations with children or very old subjects are not appropriate. • **Applicability in clinical practice?** The effect of vitamin D supplementation was not related to BL serum 25(OH)D levels. Thus, the protective effect of vitamin D was significant also in those with BL serum 25(OH)D > 50 nmol/L (which is consider as sufficient at least for bone health). If this result is not a chance finding, this may indicate that the threshold for vitamin D effects is different for the urinarytract than for the skeleton. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Findings in previous studie swere well elucidated in the discussion. **Strengths:** The study was performed according to strict RCT rules, the questionnaire was administered and checked by highly trained nurses. Included a large number of subjects. Used sufficient vitamin D doses for a long period of time. **Weaknesses:** Study not designed to assess effect of vitamin D on RTI (not the primary endpoint). Used questionnaires with self-reported occurrence of infections without any bacteriological, virological or serological verification. A separate power calculation was not made. **Plausible explanations for the results?** Yes.
Conclusion			
Supplementation with vitamin D might prevent UTI, but confirmatory studies are needed			
Country			
Norway			
Data collection period			
2008-2015			

Supplemental Table S4 – GRADE: ALL-CAUSE MORTALITY

Wejse, 2009

Reference: Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo- controlled trial. American Journal of Respiratory and Critical Care Medicine 2009;179(9):843–50.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Moderate ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To test whether vitamin D supplementation of patients with tuberculosis (TB) improved clinical outcome and reduced mortality.	**Recruitment** Volunteers were recruited by field assistants daily identifying new patients with TB initiating tb chemotherapy at the three health centers and at the national TB hospital situated in the study area, inviting patients to be included in the trial the next day. **Inclusion-/exclusion criteria** - Inclusion criteria: Either a diagnosis of TB by sputum examination (smear microscopy; no culture was available) or by World Health Organization (WHO, Geneva, Switzerland) clinical criteria, age 15 years or more, and residence in the study area. - Exclusion criteria: There were no exclusion criteria. **Data** Participants were followed for 6 months. Measurements: BMI, height and weight, severity of TB assessed by TB score (see outcome), and serum sampling. **Outcome validation (i.e. diagnosis)** The primary outcome was reduction in a clinical severity score (TBscore). The TBscore is a newly developed tool aimed at assessment of change in clinical state in patients with TB. It is based on points assigned to signs and symptoms, including cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, low body mass index, and low mid–upper arm circumference, giving patients a TBscore from 0 to 13. Change in TBscore has been shown to detect clinical change well; a high TBscore correlates well with mortality and low TBscores correlate with favorable outcomes, cure, and completed treatment. The secondary outcome was all-cause mortality at 12 months of follow-up. A verbal autopsy was conducted on all deaths, with a physician using a standardized questionnaire to obtain information from the nearest relative. No traumatic deaths were recorded; all died of causes that may be related to TB or HIV. We further assessed sputum conversion in smear-positive patients, weight gain and changes in immunologic response by changes in CD41 T-lymphocyte count. The primary end point was available only for the patients completing 8 months of treatment. Mortality was analyzed by ''intention to treat,'' that is, for all included patients regardless of number of follow-ups and study drug treatments. **Intervention variables** Vitamin D3 100,000 IU of cholecalciferol or identical placebo ampoules at inclusion, and this was repeated 5 and 8 months after inclusion. Hence patients completing treatment received in total 300,000 IU of cholecalciferol or three placebo doses. **Important confounding factors** HIV positive subjects. **Statistical methods** The Pearson chi-square (x2) was used to assess statistical differences in proportions between groups (P , 0.05); the Student t test to assess differences in means between two groups when a normal distribution was present; and the Wilcoxon rank-sum test when non- parametric analysis was needed. Linear and logistic regression analyses were used as multivariate models to adjust clinical outcomes for effects of other factors. Cox regression and the Wilcoxon-Breslow-Gehan log-rank test for equality of survivor functions were used to analyze mortality, and Kaplan-Meier survival graphs were used to estimate the survival function. A two-sided P = 0.05 was considered significant for the primary outcome. For mortality P = 0.03 was considered significant because of interim analyses. A false discovery rate was used to correct for multiple testing: test P value 5 [(no. of tests 1 1)/(2 3 no. of tests)] 3 crude P value, hence for exploratory subgroup analyses a P value of 0.03 were considered significant. Therefore 97% confidence intervals were used in estimates in all subgroup analysis.	**Main findings** Overall mortality was 15% (54 of 365) at 1 year of follow-up and similar in both arms (30 of 187 for vitamin D treated and 24 of 178 for placebo). Relative risk, 1.19 [0.58–1.95]. Reduction in TB score and sputum smear conversion rates did not differ among patients treated with vitamin D or placebo. HIV infection was seen in 36% (131 of 359): 21% (76 of 359) HIV-1, 10% (36 of 359) HIV-2, and 5% (19 of 357) HIV-112.	**Ethics approval?** Approved by the National Science and Ethics Committee as well as the Danish National Committee on Biomedical Research Ethics. **Adverse events?** Accounted for. **Aim?** Primary outcome clearly defined. **Did the randomization work?** Yes, there were no significant differences between groups at baseline. **Procedure for randomization?** A list of continuous study numbers was generated with a random allocation to treatment 1 or 2. Study numbers were consecutive and given to patients by the field assistant at inclusion, and patients were recorded in a book with prewritten study numbers and allocation sequence numbers 1 or 2. **Blinding?** Both participants and investigators were blinded throughout the study. **Did the groups receive the same co-intervention/treatments?** Yes **Primary endpoints – validated?** Low risk of classification bias. **Risk of attrition bias?** Low risk **Presentation of results?** Yes, see results. **Generalizability/Applicability in clinical practice?** Not generalizable to HIV-positive patients treated with antiretroviral therapy. **Did authors review all outcomes?** Yes. **Cost/benefit effectiveness** Not assessed. **Findings supported by previous literature?** Findings of lack of clinical effect are contradictory to what Brincourt, as well as numerous studies from the preantibiotic era, have reported. However, they all used much higher dosages than in this trial, and all of these studies were uncontrolled. Findings also contrast with those of Range and colleagues, who reported a 50% reduction in mortality among HIV- infected patients with TB treated with multivitamin supplemen- tation including vitamin D in a randomized clinical trial in Tanzania. **Strengths:** Addresses the controversy of hypercalcemia in patients with TB **Weaknesses:** Insufficient dose. Included HIV positive subjects. **Plausible explanations for the results?** Yes.
Conclusion			
Vitamin D does not improve clinical outcome among patients with TB and the trial showed no overall effect on mortality in patients with TB; it is possible that the dose used was insufficient.			
Country			
Guinea-Bissau			
Data collection period			
2006			

Reference: Lips P, Binkley N, Pfeifer M, Recker R, Samanta S, Cohn DA, et al. Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. American Journal of Clinical Nutrition 2010;91(4):985–91.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
We examined the effects of a weekly dose of 8400 IU vitamin D3 on postural stability, muscle strength, and safety. **Conclusion** *Weekly treatment with 8400 IU vitamin D3 raised 25(OH)D concentrations in elderly, vitamin D–insufficient individuals. Treatment with 8400 IU vitamin D3 did not reduce medio- lateral sway significantly compared with treatment with placebo in this population, although in post hoc analysis, treatment with 8400 IU vitamin D3 reduced sway in the subgroup of patients who had elevated sway at baseline. Weekly treatment with 8400 IU vitamin D3 was well tolerated* **Country** Multinational **Data collection period** 2005-2006	**Recruitment**. **Inclusion-/exclusion criteria** • Inclusion criteria:. Both sex aged ≥70y who were vitamin D insufficient [serum 25(OH)D concentrations ≤ 20 but ≥6 ng/mL]. All study participants were required to be ambulatory (able to walk 10 ft without a walking aid) and mentally competent [obtaining a score of ≥24 on the Folstein's Mini-Mental State Examination]. If patients had serum 25(OH)D concentrations ≥6 but ≤ 9 ng/mL, they needed to have 24-h urine calcium concentrations ≥50 mg/d and bone-specific alkaline phosphatase concentrations not higher than the upper limit of normal to be eligible for the study. • Exclusion criteria: primary hyperparathyroidism, active thyroid disease, impaired renal function, osteomalacia, neurologic impairment, peripheral neuropathy, myocardial infarction within 6 mo of screening, uncontrolled hypertension, postural hypotension, malabsorption syndrome, alcohol abuse (ie, .2 drinks/d), or cancer. Treatment with oral glucocorticoids, anabolic steroids, or a growth hor-mone within 12 mo of screening; treatment with .800 IU vi- tamin D/d or with active metabolites of vitamin D within 6 mo of screening; or treatment with any drug that might affect vi- tamin D metabolism or interfere with postural stability at screening were also reasons for exclusion **Data** 226 patients were followed for 16 weeks. Measurements included laboratory analyses, measurement of mediolateral body sway (measured with eyes open with the AccuSwayPLUS platform (Advanced Medical Technol- ogy Inc) at baseline and after 16 wk of treatment. Secondary endpoints included change in functional status assessed with the short physical performance battery (SPPB) as well as mean serum 25(OH)D, calcium, and phosphate concentrations. Safety and tolerability were also assessed. **Outcome validation (i.e. diagnosis)** Outcome validation and assays for laboratory analyses were thorougfly described. **Intervention variables** Once-weekly dose of 8400 IU vitamin D(3) compared with placebo. **Important confounding factors** Unclear. **Statistical methods**. Power calculation well described. The all-patients-treated population was used for efficacy analyses. Randomized participants who took at least one treat- ment dose were included in the all-patients-treated analyses, provided that the necessary baseline and at least one post- randomization data point were available. A parametric analysis of covariance model with terms for baseline body sway, baseline vitamin D stratum, and treatment group was used to analyze data and estimate the within- and between-treatment differences for the primary endpoint of mediolateral body sway after treatment for 16 wk. A prespecified subgroup analysis on the basis of a baseline 25(OH)D concentration (15 or .15 ng/mL) and a post hoc subgroup analysis on the basis of baseline mediolateral sway with eyes open were performed for the primary endpoint. For the analysis of urine calcium and serum PTH, a log transformation was used. The least-squares means were back transformed for the presentation of results.	**Main findings** After 16 wk, neither mediolateral sway nor SPPB differed significantly between treatment groups. Post hoc analysis of patients subgrouped by baseline sway (0.46 compared with 0.46 cm), treatment with 8400 IU vitamin D3 significantly reduced sway compared with treatment with placebo (P = 0.047) in patients with elevated baseline sway but not in patients with normal baseline sway. Adverse experiences and incidences of hypercalcemia, hypercalciuria, and elevated creatinine were similar with both treatments. In patients treated with 8400 IU vitamin D3, but not in placebo treated patients, parathyroid hormone decreased significantly.	• **Ethics approval?** Yes. • **Adverse events?** Accounted for. • **Aim?** Primary outcome not clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** After a 2-wk placebo run-in period, participants were randomly assigned 1:1 to receive a once-weekly dose of 8400 IU vitamin D3 or a placebo. Participants were stratified (2:1) at randomization according to baseline serum 25(OH)D concentration (15 ng/mL:.15 ng/mL). Patients were assigned a unique allocation number according to their appropriate stratification block. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Unclear. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** These results of neuromuscular function do not confirm the results of a number of studies that showed higher serum 25(OH)D concentrations to be associated with better physical performance. **Strengths:** Study design, validated outcome measures. Not discussed in particular. **Weaknesses:** The main limitation of this study was that a substantial number of participants had mediolateral sway values at baseline that were consistent with participants who did not fall, suggesting that their balance as measured by sway was adequate. In these patients, there may have been little room for improvement of sway and physical performance with treatment. Small size. The primary endpoint did not provide a clear answer because of the unusually healthy condition of the elderly patients enrolled in this trial and perhaps to the low number of patients enrolled. Neuromuscular efficacy was only observed by post hoc analysis in a subset with greater mediolateral sway at baseline. **Plausible explanations for the results?** Yes. 1. All-cause mortality not assessed by authors

Sanders, 2010

Reference: Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 2010;303(18): 1815–22.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To determine whether a single annual dose of 500,000 IU of cholecalciferol administered orally to older women in autumn or winter would improve adherence and reduce the risk of falls and fracture.	**Recruitment** The study recruited community-dwelling women as previously described. Invitation letters were sent to all age-eligible women listed on the elec- toral roll of the region surrounding the study center. **Inclusion-/exclusion criteria** • Inclusion criteria: Women were included in the study if they were at higher risk of hip fracture, defined by criteria such as maternal hip fracture, past fracture, or self-reported faller. • Exclusion criteria: Excluded if they could not provide informed consent or information about falls or fractures; permanently resided at a high- level care facility; had an albumin- corrected calcium level higher than 2.65 mmol/L; or had a creatinine level higher than 150 µmol/L, or currently took vitamin D doses of 400 IU or more, cal- citriol, or antifracture therapy	**Main findings** Women in the cholecalciferol (vitamin D) group had 171 fractures vs 135 in the placebo group; 837 women in the vitamin D group fell 2892 times (rate, 83.4 per 100 person-years) while 769 women in the placebo group fell 2512 times, rate 72.7 per 100 person-years. Incidence rate ratio [RR], 1.15; 95% confidence interval [CI], 1.02-1.30; P=.03	**Ethics approval?** Approved by the institutional review boards of Barwon Health and the University of Melbourne and carried out in compliance with the Helsinki Declaration. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Allocation was performed by an independent statistician using computer-generated randomization of numbers performed in blocks of 500.
Conclusion *Among older community-dwelling women, annual oral administration of high-dose cholecalciferol resulted in an increased risk of falls and fractures*	**Data** 2317 participants were followed for 3-5 years. Age, calcium intake, and fracture-risk profile were collected at baseline by questionnaire. Falls and fractures were recorded using postcard calendars completed daily by writing F if they had a fall, fracture, or both and N if they did not and were returned monthly by prepaid post. **Outcome validation (i.e. diagnosis)** Falls were defined as "an event reported either by the faller or a witness, resulting in a person inadvertently coming to rest on the ground or another lower level, with or without loss of consciousness or injury." This definition was explained to participants and reinforced twice yearly via newsletter. Only fractures radiologically confirmed were included in the analyses.	The incidence RR for fracture in the vitamin D group was 1.26 (95% CI, 1.00-1.59; P=.047) vs the placebo group (rates per 100 person-years, 4.9 vitamin D vs 3.9 placebo). A temporal pattern was observed in a post hoc analysis of falls. The incidence RR of falling in the vitamin D group vs the placebo group was 1.31 in the first 3 months after dosing and 1.13 during the following 9 months (test for homogeneity; P=.02).	• **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Yes, low risk of classification bias. • **Risk of attrition bias?** Low risk • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Given the pragmatic design the study provides high potential for translation into public health policy and clinical practice.
Country Australia	**Intervention variables** A single oral dose of D3 500 000 IU or matched placebo each year for 3 to 5 years (in autumn or winter). **Important confounding factors** No adjustment was made for multiple testing.	In the substudy, the median baseline serum 25- hydroxycholecalciferol was 49 nmol/L. Less than 3% of the substudy participants had 25-OHD levels lower than 25 nmol/L. In the vitamin D group, 25- hydroxycholecalciferol levels increased at 1 month after dosing to approximately 120 nmol/L, were approximately 90 nmol/L at 3 months, and remained higher than the placebo group 12 months after dosing.	• **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** No, but the opposing outcomes of 2 studies that used the same total annual dose (300 000 IU intramuscularly) suggest that the dosing regimen (ie, 4 monthly vs annually) rather than the total dose might determine the outcome.
Data collection period 2003-2007	**Statistical methods.** Power calculation described. All analyses were ITT. Initial comparisons of outcome measures between treatment groups were performed using chi2 tests or Wilcoxon rank-sum tests. The primary outcome measures, numbers of falls and fractures, were analyzed using Poisson regression models with robust standard errors to allow for nonindependence of multiple events for the same participant. The models included only treatment group but were also fitted adjusting for baseline calcium intake and age. The data were also analyzed using negative binomial regression models that explicitly allow for overdispersion. For comparison with similar studies, time to first fracture and fall was analyzed using Cox proportional hazards models. Kaplan-Meier plots of cumulative incidence are presented. Post hoc analyses were described. No adjustment was made for multiple testing. All P values are 2-sided to detect differences, P <.05.		**Strengths:** The major strength of our study is that it was a large randomized, double-blind, placebo-controlled trial. Falls and fracture ascertainment were robust, although. **Weaknesses:** The main weaknesses of the study are also related to its pragmatic design—the participants were not evaluated at the study center so that baseline clinical information may have been missed. Biochemical assessment of all participants was not possible. Nonclinical vertebral fractures would have been missed. No adjustment was made for multiple testing. **Plausible explanations for the results?** Yes. 1. All-cause mortality not assessed by authors

Grimnes, 2011

Reference: Grimnes G, Figenschau Y, Almås B, Jorde R. Vitamin D, insulin secretion, sensitivity, and lipids: results from a case-control study and a randomized controlled trial using hyperglycemic clamp technique. Diabetes 2011;60(11): 2748–57.	**Study design: RCT** (and nested case control; not evaluated) *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To compare insulin sensitivity (primary end- point) and secretion and lipids in subjects with low and high serum 25(OH)D levels and to assess the effect of vitamin D supplementation on the same outcomes among the participants with low serum 25(OH)D levels.	**Recruitment**. Participants were recruited by mail from a population-based study (the 6th Tromsø Study) based on their serum 25(OH)D measurements. The participants received a gift card valued at $90 for the baseline as well as the 6-month visits. **Inclusion-/exclusion criteria** • Inclusion criteria: Subjects 30-75 yrs with 25OHD between the 5th and the 10th percentiles. • Exclusion criteria: Diabetes, acute myocardial infarction or stroke during the past 12 months, cancer during the past 5 years, steroid use, serum creatinine >130 mmol/L (males) or >110 mmol/L (females), possible primary hyperparathyroidism (plasma parathyroid hormone [PTH] .5.0 pmol/L combined with serum calcium .2.50 mmol/L), sarcoidosis, systolic blood pressure .175 mmHg or diastolic blood pressure .105 mmHg, and specifically for women, pregnancy, lactation, or fertile age and no contraception use	**Main findings** At the end of the study, there were no statistically significant differences in the outcome variables between the two groups.	• **Ethics approval?** Approved by the Regional Committee for Medical Research Ethics and the Norwegian Medicines Agency • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline regarding measures of insulin secretion, insulin sensitivity, or lipids. • **Procedure for randomization?** The randomization was performed by the central randomization unit at the University Hospital of North Norway, using block randomization with various block sizes.
Conclusion	**Data** 108 participated in the randomized controlled trial. Those who had accepted the study invitation attended a screening examination where medical history, blood pressure, and blood samples for PTH, calcium, creatinine, HbA1c, and 25(OH)D were obtained. Fasting blood samples for serum lipids. Questionnaire self reported info on physical activity, fish-intake, cod liver oil, sunbed use etc.	The 52 participants with high serum 25(OH)D levels (85.6 6 13.5 nmol/L [mean 6 SD]) had significantly higher insulin sensitivity index (ISI) and lower HbA1c and triglycerides (TGs) than the 108 participants with low serum 25(OH)D (40.3 6 12.8 nmol/L), but the differences in ISI and TGs were not significant after adjustments.	• **Blinding?** Neither the participants, the staff performing the examinations, nor the researchers knew the randomization status of the participants during the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk • **Presentation of results?** Yes, see results.
Vitamin D suopl to apparently healthy subjects with insufficient serum 25(OH)D levels does not improve insulin sensitivity or secretion or serum lipid profile	**Outcome validation (i.e. diagnosis)**. Primary outcome was insulin sensitivity. Validation of methods described. **Intervention variables** Capsules of 20,000 IU vitamin D3 versus identical looking placebo twice weekly for 6 months. **Important confounding factors** Finally, we had the opportunity to adjust for possible confounding factors, such as physical activity and fat fish intake..	After supplementation, serum 25(OH)D was 142.7 6 25.7 and 42.9 6 17.3 nmol/L in 49 of 51 completing participants randomized to vitamin D and 45 of 53 randomized to placebo, respectively.	• **Generalizability/Applicability in clinical practice?** Considering the population recruited, findings are only representable and generalizable to Caucasians, and does not apply in other ethnic groups. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?**.Administration of a similar dose (4,000 IU/day) of vitamin D3 for 6 months lowered fasting insulin and increased insulin sensitivity in South-Asian women, and an increase in oral glucose insulin sensitivity in vitamin D–supplemented (120,000 IU three times, 2 weeks apart) Indian men after only 6 weeks has also been reported. The participants included in these studies were more vitamin D deficient and insulin resistant at baseline than in this study.
Country	**Statistical methods**. Power calculation described. The data were checked for normal distribution using visual inspection of histograms, and skewed variables were log transformed before statistical analyses when appropriate. For between-group comparisons of baseline values and D-values (6 months minus baseline) of the two treatment groups in the intervention study, Student t test or chi2 tests were used. Paired t tests were used to analyze changes from baseline to 6 months within each treatment group. To control for possible confounders, general linear models were used to compare case and control subjects at baseline. Because use of statin affects serum lipid and CRP levels, the analyses were also performed with the statin users excluded. To compare the effect of vitamin D and placebo on the outcome variables, we also used ANCOVA models adjusting for the baseline value, presenting the relative effect of vitamin D to the effect of placebo (set to 1 as reference). The results from the intervention study were analyzed both as intention to treat analyses (with last observation carried forward) and per-protocol analyses. Tests for interactions between treatment group and sex, above or below median of HOMA-IR, BMI, or dairy servings per week, were performed for the primary outcome variable ISI. Data are presented as mean 6 SD for normally distributed variables and as median (5th to 95th percentiles) for non normally distributed variables, unless otherwise indicated. P , 0.05 was considered a significant finding.		**Strengths:** Methodology used to measure insulin secretion and sensitivity. Low and high serum 25(OH)D levels were confirmed through two different measurements, some of which were > 2 years apart. The doses used were high enough to achieve a substantial increase in serum 25(OH)D levels, which was verified in serum measurements. Good compliance and high retention increased the validity of the findings. **Weaknesses:** Statistical power was limited in detection of a small difference. Short intervention. Homogenous sample (Caucasians), **Plausible explanations for the results?** Yes.
Norway			
Data collection period			1. All-cause mortality not assessed by authors
6 months in 2008			

Reference: Manaseki-Holland S, Qader G, Isaq Masher M, et al. Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. Trop Med Int Health 2010;15:1148-55.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	**Material and methods**	**Results**	**Discussion/comments/checklist**
To determine whether - supplementation of oral 100 000 iu of vitamin D3 along with antibiotics could reduce the <u>duration</u> of illness in children with pneumonia; - supplementation could reduce the <u>risk of repeated episodes</u> **Conclusion** *A single high-dose oral vitamin D3 supplementation to young children along with antibiotic treatment for pneumonia could reduce the occurrence of repeat episodes of pneumonia* **Country** Afghanistan **Data collection period** 2006-2007	**Recruitment** At outpatient clinics if the child met the study criteria and after either the parent read the Dari consent form or it was explained to him ⁄ her by the doctor. **Inclusion-/exclusion criteria** • Inclusion criteria: Children aged 1–36 months, diagnosed with non-severe or severe pneumonia at the outpatient clinic at Maywand Hospital • Exclusion criteria: Children who had clinical signs of rickets or were known to have received high-dose vitamin D treatment in the past 3 months (one child) had severe vomiting (one child) or pronounced wheeze (10 children). Thirteen children with very severe pneumonias and nine children with other severe illnesses (meningitis, heart or renal disorders, measles, severe malnutrition and suspected tuberculosis) were also excluded from the study. 1 child was excluded because parents were likely to move during the study. **Data** <u>Daily follow-up up to 10 days</u>, either at the study hospital by paediatricians or at home by medical doctors if discharged <u>to assess the resolution of signs and symptoms</u> of the first episode of pneumonia. <u>Thereafter, followed fortnightly up to 90 days</u> by trained female medical doctors <u>to assess any illness</u> and to refer to the study hospital if necessary. **Outcome validation (i.e. diagnosis)** Severity of pneumonia was categorised using WHO's IMCI criteria (Box 1). All doctors involved were trained in IMCI and examination of the study signs and symptoms and their work in the clinics or follow-up were monitored through random observations by a supervisor on weekly basis. **Intervention variables** Bolus dose of vitamin D3 100,000 IU versus placebo. **Important confounding factors** The potential confounding of baseline characteristics on treatment effect was assessed in statistical analyses. **Statistical methods** Power calculations and numbers included in analyses were adequately described. Incidence rates of pneumonia were calculated by dividing the number of new episodes of pneumonia by total time at risk for all children. Hazard ratios with 95% CIs were obtained with Cox proportional-hazards models to measure time to repeat episodes between treatment groups. Kaplan–Meier plots and log-rank tests were used to compare the time to recover from the index episode of pneumonia between the vitamin D and placebo groups.	**Main findings** The mean number of days to recovery from the index episode of pneumonia was the same for both the vitamin D group and the placebo group [4.74 (SD 2.22) vs. 4.98 (SD 2.89); P = 0.17] The risk of children having a repeat episode of pneumonia during the 90-day posttreatment period was significantly lower in the vitamin D group than in the placebo group (RR 0.78; 95% CI 0.64, 0.94, P = 0.01) Children in the vitamin D group survived without experiencing a repeat episode of pneumonia for a longer period than children in the placebo group, for the first or only episode of pneumonia (HR 0.71; 95% CI 0.53–0.95, P = 0.02) There was no confounding effect of baseline measures on risk of repeat pneumonia or time to repeat episode.	• **Ethics approval?** Approved by the Ethics and Review Board of the Ministry of Public Health of Afghanistan. • **Adverse events?** Accounted for. No adverse events related to vitamin D3 were observed. • **Aim?** Which was the primary outcome was not clearly defined. • **Did the randomization work?** There was no statistically significant difference in any of the baseline characteristics between the groups • **Procedure for randomization?** Random number sequence generated in an Excel spreadsheet with no restrictions. • **Blinding?** Placebo (containing olive oil alone) and vitamin D syringes looked the same and the contents tasted the same. None of the investigators, staff in Kabul and caretakers of children, were aware of the study groups. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Yes, severity of pneumonia was categorised using WHO's IMCI criteria. • used ot clearly described. • **Risk of attrition bias?** The number of children lost to follow-up during the first 10 days of post-treatment follow-up was small and similar between the two groups • **Presentation of results?** Yes, see results. • **Generalizability?** Limited to those children of similar age with high risk of VD deficiency and especially to children who had an episode of pneumonia. • **Applicability in clinical practice?** Considering the population recruited, the results were representative in a limited sample of the overall population. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** In harmony with findings that vitamin D can enhance the immune function. **Strengths:** Study design. Population at high risk of vitamin D deficiency. Study outcomes were ascertained by experienced doctors and the loss to follow-up was minimal. Use of IMCI clinical definitions is comparable with other trials with pneumonia as an outcome in children. Low risk of misclassification. **Weaknesses:** Lack of x-ray confirmation of cases of pneumonia. Treatment from health care providers other than the study doctors might have occurred. Not conducted quality control of the vitamin D3 preparation (too costly and technically difficult). No measurement of vitamin D level in the serum achieved as the result of this supplementation. Bolus instead of daily supplementation. **Plausible explanations for the results?** Yes. 1. All-cause mortality not assessed by authors

Reference: Witham MD, Crighton LJ, Gillespie ND, Struthers AD, McMurdo ME. The effects of vitamin D supplementation on physical function and quality of life in older heart failure patients: a randomised controlled trial. Circulation Heart Failure 2010;3(2):195–201.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To test whether vitamin D supplementation of patients with heart failure and vitamin D insufficiency can improve physical function and quality of life. **Conclusion** *Vitamin D supplementation did not improve functional capacity or quality of life in older patients with heart failure with vitamin D insufficiency* **Country** UK, Scotland **Data collection period** …	**Recruitment** Recruited from primary and secondary care in Tayside and Fife health board areas in Scotland. Participants were patients discharged from medical, cardiology, and Medicine for the Elderly wards; Medicine for the Elderly, cardiology, and heart failure clinics; primary care patient lists; and community dwelling patients following local media publicity **Inclusion-/exclusion criteria** • Inclusion criteria: Eligible for inclusion if they were aged ≥70 years with a previously recorded clinical diagnosis of chronic heart failure, previously documented left ventricular systolic dysfunction by echocardiography, radionuclide ventriculography, or angiography as part of their usual clinical care and had New York Heart Association class II or III symptoms. Participants were required to have a screening 25OHD of <50nmol/L (20 ng/mL) • Exclusion criteria: Clinical diagnosis of osteomalacia, under investigation for recurrent falls, taking vitamin D supplements, moderate to severe cognitive impairment, s-creatinine >200 umol/L, liver function tests >3 times the upper limit of the local reference range, systolic BP <90 mm Hg, albumin-adj calcium (>2.55 mmol/L or <2.20 mmol/L), and metastatic malignancy. Wheelchair bound and unable to perform the primary outcome. Unwilling or unable to give informed consent. **Data** 105 participants were randomized. Measured 6-min walk test, timed up and go test, daily physical activity levels, health status and health related quality of life, blood samples for cardiovascular and inflammatory markers. **Outcome validation (i.e. diagnosis)**. Measured at BL, 10 and 20 weeks. The primary outcome measure was the 6-minute walk test, 15 a measure of submaximal exercise capacity. The test has been validated for use in older patients with heart failure. Tests were performed using a flat, straight, indoor 25-m course. Participants used their usual walking aids and received standardized encouragement at regular intervals. **Intervention variables** An oral dose (100 000 U D2 or placebo) was administered after baseline outcome measures were performed and was repeated at 10 weeks, observed ingestion to ensure 100% adherence. **Important confounding factors** Se statistical methods. **Statistical methods**. Power calculation described. Diff between groups at BL were compared w/Student t test for cont var or M-W U test for non-normally distributed cont var. Pearson chi2 test to compare cat var. Changes analyzed using 2-sample Student t test. For 6-minute walk and daily activity, percentage change from baseline was calculated to compensate for the wide ranges of values seen in these outcomes and to adjust for differences in BL values in a similar way to covariate analysis. To compensate for differences in the groups at BL, the difference in change in the primary outcome at 20 weeks was adjusted for BL variables exhibiting between-group differences at a significance level of P<0.2. Sensitivity analyses described	**Main findings** 100,000 IU D2 given at BL and 10 weeks did not improve physical function as measured by the 6-minute walk or timed up and go tests despite an increase of >100% (20 nmol/L) in 25OHD at 10 weeks, which was sustained at 20 weeks. Secondary outcomes measuring muscle function (timed up and go), self-reported physical and psychosocial function (Functional Limitations Profile), and daily activity (accelerometry) also showed no improvement with vitamin D supplementation. Paradoxically, there was a small, but significant worsening in disease-specific quality of life (Minnesota score) in the treatment group compared with the placebo group. BNP level fell in the treatment group by 10 weeks compared with placebo.	• **Ethics approval?** The study conformed to the principles of the Declaration of Helsinki and was approved by Fife and Forth Valley Research Ethics Committee • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Performed using computer-generated random number tables by DHP Pharmaceuticals (Gwent, United Kingdom), who over encapsulated the study medication to render it identical to placebo. Code allocation was concealed from the research nurse and investigators until after data analysis was complete. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk: 88% in the placebo group completed the 10-week 6-minute walk test compared with 85% in the treatment group. At 20 weeks numers were 81% placebo group versus 79% treatment group. Reasons for failure to perform the walk test included death, illness, and poor health. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** The enrollment of typical older, frail patients with heart failure means that the results should be generalizable to a significant proportion of patients with left ventricular systolic dysfunction heart failure but may not be generalizable to the 50% of patients with heart failure with preserved systolic function. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, Schleithoff et al published a RCT examining vitamin D sppl in younger patients with heart failure, showing that vitamin D3 sppl at a dose of 2000 IU per day reduced tumor necrosis factor-alpha levels and increased interleukin-10 levels. There was no effect seen on BNP levels, left ventricular ejection fraction, blood pressure, or maximal oxygen uptake, concurring with the findings of this study. **Strengths:** The tight CIs around the primary, the final evaluable sample size reached the required target, despite a lower-than-anticipated recruitment rate. Large, intermittent doses of vitamin D ensured that adherence to therapy was 100% **Weaknesses:** Used vitamin D2, vitamin D3 would be preferable. A longer time period is necessary to elicit changes in physical function. **Plausible explanations for the results?** Yes. The minimum clinically important diff for the 6-min walk distance is 30 m, and the narrow CIs surrounding the change make it highly unlikely that a clinically significant effect of this vitamin D dose was missed. 1. All-cause mortality not assessed by authors

Reference: Avenell A, MacLennan GS, Jenkinson DJ, McPhersonGC, McDonald AM, Pant PR, et al. Long-term follow-upfor mortality and cancer in a randomized placebo-controlledtrial of vitamin D(3) and/or calcium (RECORD trial).Journal of Clinical Endocrinology and Metabolism 2012;97(2):614–22.		**Study design: RCT** *Double blind, placebo-controlled*	
		Grade - quality	Moderate ⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
Our objective was to investigate whether vitamin D or calcium supplementation affectsmortality, vascular disease, and cancer in older people	**Recruitment** Volunteers were recruited from fracture clinics or orthopedic wards. **Inclusion-/exclusion criteria** • Inclusion criteria: Fragility fracture within the last 10 yr and aged at least70 yr • Exclusion criteria: Cancer likely to metastasize to bonewithin the previous 10 yr, bed- or chair-bound before fracture,abbreviated mental test below 7 (16), fracture associated withpreexisting local bone abnormality, known hypercalcemia, renalstone in the last 10 yr, life expectancy less than 6 months, knownto be leaving the United Kingdom, taking more than 200 IU (5g) vitamin D or more than 500 mg calcium in supplementsdaily, treatment with fluoride, bisphosphonates, calcitonin, tibolone,hormone replacement therapy, selective estrogen receptormodulators, or any vitamin D metabolite (such as calcitriol)in the last 5 yr or vitamin D by injection in the last year **Data** 5292 prticipants were followed for a median of 6,2 yrs. Data were derived only from the main cause of death for death registrations, and registrations of new cancers for all trial participants were collected only through the national United Kingdom databases of the General Register of Scotland; the National Health Service Medical Research Information Service, England; and the United Kingdom Association of Cancer Registries. **Outcome validation (i.e. diagnosis)** All-cause mortality, mortality due to vascular disease and cancer, and cancer registrations were prespecified outcomes in the main trial protocol. This paper reports follow-up mortality data that had been notified during the trial and within 3 yr of trial closure as well as cancer notifications relevant to this period. **Intervention variables** Participants were randomized into four equal groups to receive two tablets daily with meals containing a total of 800 IU (20 ug) vitamin D3, 1000 mg elemental calcium (as carbonate), both vitamin D3 and calcium, or placebo. **Important confounding factors** The explanatory variables in the models were the treatment group and the variables used for minimization at randomization (age, gender, time since fracture, andtype of fracture). Post hoc analysis to explore the effects of compliance with the treatment regimen on outcome. **Statistical methods** Power calculation described. Survival time was modeled using Cox proportional hazards regression models. Four outcome survival measures were explored: time to death, time to death from vascular disease and cancer (censoring those dying from other causes in each case), and time to cancer incidence. The main analyses focused on main effects reflecting the factorial design: vitamin D3 vs. no vitamin D3 and calcium vs. no calcium. Interaction between calcium and vitamin D3 was also tested for. Two methods of handling the non compliance were used. First, intention-to-treat (ITT, patients analyzed as per the treatment they were randomized to) was used on the complete dataset. For each model, hazard ratios (HR) with 95% confidence intervals (CI) were calculated.	**Main findings** In intention-to-treat analyses, mortality [hazard ratio (HR) 0.93; 95% confidence interval(CI) 0.85–1.02], vascular disease mortality (HR 0.91; 95% CI 0.79–1.05), cancer mortality (HR 0.85; 95% CI 0.68–1.06), and cancer incidence (HR 1.07; 95% CI 0.92–1.25) did not differ significantly between participants allocated vitamin and those not. All-cause mortality (HR 1.03; 95%CI 0.94–1.13), vascular disease mortality (HR 1.07; 95%CI 0.92–1.24), cancer mortality (HR 1.13; 95% CI 0.91–1.40), and cancer incidence (HR 1.06; 95% CI 0.91–1.23) also did not differ significantly between participants allocated calcium and those not. In a post hoc statistical analysis adjusting for compliance, thus with fewer participants, trends for reduced mortality with vitamin D and increased mortality with calcium were accentuated, although all results remain non-significant.	• **Ethics approval?** Ethical approval was obtained from the Multicenter ResearchEthics Committee for Scotland and each center's Local ResearchEthics Committee • **Adverse events?** Not accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Randomization was centralized, computer generated, stratifiedby center, and minimized by age (under 80 yr or 80 yr andover), gender, time since fracture (previous 3 months or longer),and type of enrolling fracture (proximal femur, distal forearm,clinical vertebral, and other). • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/ treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability?** We do not know whether the results reported here would be found in younger populations, older people without a history of previous fragility fracture, or very high-risk populations in nursing homes. • **Applicability in clinical practice?** The pragmatic nature of the RECORD Trial, without frequent follow-up visits and tablet counting reflects real-world practice. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Yes, results consistent with findings in a previous meta-analysis by Bolland et al. **Strengths:** Pragmatic nature of the study, reflecting real world practice. Participants were older and had poorer vitamin D status at recruitment than many other trials. **Weaknesses:** Compliance with trial medication was limited. The analyses adjusted for compliance had reduced statisticalpower, and we were unable to provide CI for calciumand cancer mortality. Low 25OHD levels seen in a very small subgroup. **Plausible explanations for the results?** Yes.
Conclusion			
In this older age group at high risk of refracture, daily 800 IU vitamin D3 supplementation was not found to significantly reduce all-cause and vascular disease mortality or cancer incidence or mortality in the ITT analyses.			
Country			
UK, Scotland			
Data collection period			

Lehouck, 2012

Reference: Lehouck A, Mathieu C, Carremans C, et al. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. Ann Intern Med 2012;156:105-14.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To explore whether supplementation with high doses of vitamin D could reduce the incidence of COPD exacerbations.	**Recruitment** Volunteers were recruited from single-center at the University Hospitals Leuven, over a 1.5-year recruitment period in 2008 and 2009. Screened during hospitalization for an exacerbation or before referral for respiratory rehabilitation. **Inclusion-/exclusion criteria** • Inclusion criteria: Eligible patients were current or former smokers, were older than 50 years, had a diagnosis of COPD according to the Global Initiative for Chronic Ob- structive Lung Disease (GOLD) definition (postbroncho- dilator FEV1– FVC ratio <0.7), and had an FEV1 less than 80% predicted. • Exclusion criteria: history of hypercalcemia, sarcoidosis, or active cancer. Treatment with vitamin D supplements for newly discovered symp- tomatic osteoporosis and long-term azithromycin treat- ment, with antibacterial and anti-inflammatory functions, were additional exclusion criteria	**Main findings** The median time to first exacerbation did not significantly differ between the groups (hazard ratio, 1.1 [CI, 0.82 to 1.56]; P=0.41), nor did exacerbation rates, FEV1, hospitalization, quality of life, and death.

A post hoc analysis in 30 participants with severe vitamin D deficiency (serum 25-[OH]D levels < 10 ng/mL) at baseline showed a significant reduction in exacerbations in the vitamin D group (rate ratio, 0.57 [CI, 0.33 to 0.98]; P=0.042). | • **Ethics approval?** Approved by • **Adverse events?** Not accounted for. • **Aim?** Clearly defined primary outcome. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Pharmacists of the University Hospitals Leuven, who were independent from the clinical study team, randomly assigned participants by using a computer-generated randomization list in blocs of 20 and prepared the study medication. • **Blinding?** Yes. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Unclear • **Risk of attrition bias?** Low: 150 (82%) subjects completed the study, 15 (8%) died, and 17 (9%) were classified as withdrawals with no differential dropout between the 2 groups. • **Presentation of results?** Yes, see results. • **Generalizability?** Generalizabe to patients with COPD. • **Applicability in clinical practice?** Considering the population recruited, the results are representative in a COPD subjects, and results relevant in clinical situations including management of COPDexacerbation. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** The absence of a vitamin D–mediated effect in this study sample contrasts with indirect evidence from most association studies in COPD, but is consistent with recent data from the Lung Health Study, which showed that vitamin levels did not determine the rate of decline in FEV1 in a limited subgroup. **Strengths:** First of its kind. The findings may help to guide the design of and dosage in future trials. **Weaknesses:** Single center. Small sample. **Plausible explanations for the results?** Yes.

1. All-cause mortality not assessed by authors |

(Conclusion) *High-dose vitamin D supplementation in a sample of patients with COPD did not reduce the incidence of exacerbations. In participants with severe vitamin D deficiency at baseline, supplementation may reduce exacerbations*

Country: Belgium

Data collection period: 2008-2009

Data 182 participants were followed for 1 year. Baseline characteristics included BMI, Airflow Obstruction, Dyspnea, and Exercise Capacity (BODE) Index and the Charlson comorbidity index. Follow-up visits occurred every 4 months (at 4, 8, and 12 months). To obtain data on exacerbations, participants were asked to complete diaries every 2 weeks that detailed respiratory tract symptoms, visits to health care providers, hospitalizations, and changes in medication. At each visit, diaries were reviewed in the participant's presence and the general practitioner was contacted in case of doubt, missing data, or suspicion of self-medication. **Outcome validation (i.e. diagnosis)** The primary end point was the time to first exacerbation, defined as sustained worsening of respiratory symptoms during 48 hours and requiring oral corticosteroid, antibiotic, or combination treatment that was initiated by a physician. Respiratory symptoms included at least 1 of the Anthonisen criteria (increased dyspnea, sputum volume, or sputum purulence) with or without minor symptoms, such as cough, fever, common cold, wheezing, or sore throat. Secondary end points were exacerbation rate; time to first hospitalization; time to second exacerbation; FEV1; quality of life, as measured with the Chronic Respiratory Questionnaire (CRQ) (scores for dyspnea, emotion, fatigue, and mastery); and death. In addition to these clinical end points, bacterial presence in morning sputa, plasma cathelicidin levels, serum 25-(OH)D levels, and blood monocyte capacities for phagocytosis were de- termined in a blinded manner **Intervention variables** 100 000 IU of vitamin D supplementation or placebo every 4 weeks for 1 year **Important confounding factors** Self-reported data. Age, FEV1, GOLD stage, and smoking status did not have a statistically significant influence on the model. **Statistical methods** Power-calculations described, ITT analysis applied. Time to first or second exacerbation and time to first hospitalization were compared by using Kaplan–Meier curves and log-rank tests. Effect sizes between groups of primary or secondary outcomes are given with P values and 95% CIs. P values less than 0.05 are considered statistically significant. Subgroup analyses were thoroughly described, and in particular it was noted that: Mean number of exacerbations per patient-year calculated by dividing the total number of exacerbations by the total years of follow-up in the ITT population. Exacerbation rate in the ITT population was analyzed with a generalized linear model for a Poisson distribution, correcting for duration of treatment exposure and overdispersion.

Reference: Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. Lancet 2012; 379: 1419-27.		Study design: **RCT** *Double blind, placebo-controlled*	
		Grade - quality	Low-Moderate[1] ⊗⊗⊗
Aim	**Material and methods**	**Results**	**Discussion/comments/checklist**
To assess whether oral supplementation of vitamin D3 (cholecalciferol) will reduce the incidence and severity of pneumonia in a high-risk infant population **Conclusion** *Quarterly bolus doses of oral vitamin D3 supplementation to infants are not an effective intervention to reduce the incidence of pneumonia in infants in this setting.* **Country** Afghanistan **Data collection period** 2008-2009	**Recruitment** Volunteers were recruited from five of the 18 socioeconomically deprived inner-city districts; identified households with young children with detailed maps and advice from a non-governmental organisation working in the region. The study field-supervisors mapped the region independently to verify the accuracy of the maps. 20 pairs of female fieldworkers visited every home starting from streets closest to the hospital and radiating out until required sample size were reached. **Inclusion-/exclusion criteria** • Inclusion criteria: Infants aged 1–11 months and living in the study region • Exclusion criteria: Families expecting to move to another town within 18 months, diagnosis of rickets or treatment with vitamin D in the previous 3 months, and clinical diagnosis of Kwashiorkor or Marasmus. **Data** 3046 children (1524 children were assigned to receive vitamin D3 and 1522 placebo) followed up every 2 weeks to obtain background information, assess illness (symptom history and examination of chest in-drawing, body temperature, signs of dehydration by skin pinching, respiratory rate count over 1 min with a stopwatch), and to refer to the study hospital if needed. Venous blood samples collected at baseline. Respiratory rate and anthropomorphic data were collected twice, children clinically diagnosed with pneumonia were offered free chest radiographs. Causes of death ascertained through scrutiny of hospital notes, and verbal autopsy interviews with the WHO standard questionnaire and review of the interview data by two physicians independently. **Outcome validation (i.e. diagnosis)** Primary endpoint was the first episode of pneumonia from the time of enrolment confirmed by chest radiograph (consolidation or infiltrates). A new episode of pneumonia defined as an episode happening 15 days or longer after the first. We judged an episode happening within 14 days to be continuation of the previous episode. **Intervention variables** Vitamin D3 100,000 IU versus placebo every third month during 1. **Important confounding factors** Nutritional factors, accounted for in diet questionnaire, weight at recreuitment and growth comparison. **Statistical methods** Power-calculations adequately described. Compared baseline characteristics and the distribution of prestated confounders for intervention and placebo groups. ITT analysis included all children randomly assigned to study groups. PP analysis included children who in both groups received all doses with an interval between the doses of 60 and 120 days and had not violated the randomisation codes. Pearsons correlation coefficient or Cramers V (for paired categorical variables) to assess the potential problem of multicollinearity. Calculated person-time at risk for each child up to the date a child reached the primary endpoint, was last seen at the end of the study, or when censored because they were lost to follow-up. Initial comparisons of time-to-an-episode between the two groups with log-rank tests and Kaplan–Meier plots. Estimated the incidence rate ratio (RR) for the episodes of pneumonia with Cox proportional hazard models.	**Main findings** There was no significant difference between the incidence of first or only pneumonia between the vitamin D (0·145 per child per year, 95% CI 0·129–0·164) and the placebo group (0.137, 0·121–0·155). Incidence rate ratio: 1·06 (95% CI 0·89–1·27)	• **Ethics approval?** Approved by the ethics and review board of the Ministry of Public Health of Afghanistan and the ethics committee of the London School of Hygiene and Tropical Medicine. • **Adverse events?** Accounted for. • **Aim?** Clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** An independent statistician randomised unique identification numbers individually in fixed blocks of 20 to the vitamin D3 or placebo group by use of a random number generator with the SAS routine. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Applied WHO standards. • **Risk of attrition bias?** Low: Low loss to follow up • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a the high-risk, thus the generalizability to populations with low-to-moderate risk of vitamin deficiency is unknown • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Findings at odds with smaller case-control hospital studies that show an enhanced rate of vitamin D deficiency or rickets in children with pneumonia and the increasing evidence suggesting that calcitriol, the biologically active metabolite of vitamin D, has an important role in the human immune system. A systematic review of the role of vitamin D supplementation in infectious diseases had mixed findings, concluding that more rigorously designed clinical trials are needed. No studies report the effect of vitamin D on radiologically confirmed pneumonias. Other trials assessing infections of the upper respiratory tract also had mixed findings. **Strengths:** Large sample size. Low attrition bias. Robust assertainment of outcomes and low risk of misclassification. Study design. **Weaknesses:** Limited generalizability. Not assessed genotyping. Steady state VD not reached in all patients. **Plausible explanations for the results?** Yes. 1. All-cause mortality not assessed by authors

Punthakee, 2012

Reference: Punthakee Z, Bosch J, Dagenais G, Diaz R, Holman R, Probstfield J, et al. Design, history and results of the Thiazolidinedione Intervention with vitamin D Evaluation (TIDE) randomised controlled trial. Diabetologia. 2012;55(1):36-45.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	Low ⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To assess the effects of TZDs (rosiglitazone and pioglitazone) on cardiovascular outcomes and the effects of vitamin D (cholecalciferol) on cancers and mortality	**Recruitment** Volunteers were recruited from outpatient primary care, diabetes and cardiology clinics in 33 countries **Inclusion-/exclusion criteria** • Inclusion criteria: T2DM and an HbA1c level ranging from 6.5% to 9.5%), were drug-naive or taking up to two non-insulin glucose-lowering medications, and were at risk of cardiovascular disease on the basis of: (1) age at least 50 years with a prior cardiovascular event; (2) age at least 55 years with documented arterial stenosis, albuminuria, ankle brachial index <0.9 or left ventricular hypertrophy; or (3) age at least 60 years with at least two risk factors (tobacco use, high LDL-cholesterol, low HDL-cholesterol or high triacylglycerols, hypertension or obesity) • Exclusion criteria: A cardiovascular event within 30 days before randomisation, history of pulmonary oedema, symptomatic heart failure (New York Heart Association class II–IV), known left ventricular ejection fraction below 40% or use of a loop diuretic, cancer diagnosed in the prior 3 years or active treatment for cancer (other than non-melanoma skin cancer or cervical carcinoma in situ), fracture in the prior year, known osteomalacia or hypercalcemia. **Data** From the study design, 16,000 people were to be followed for approximately 5.5 years. However, the trial was stopped prematurely because of regulatory concerns after a mean of 162 days without consideration of the accrued data. **Outcome validation (i.e. diagnosis)** The primary outcome measure for the for the vitamin D arm was all-cause death or cancers requiring hospitalisation, chemotherapy or surgery. Other outcomes included a composite microvascular outcome (the first occurrence of retinopathy requiring laser therapy or vitrectomy, or a 30% decline in estimated GFR [eGFR], or need for renal replacement therapy) for the TZD arm, and hospitalisation for heart failure, pneumonia or shortness of breath, hospitalisation for any reason, revascularisation and fractures for both study arms. **Intervention variables** To placebo, pioglitazone 30 mg daily or rosiglitazone 4 mg daily at a 4:3:3 ratio. Independent of their TZD allocation, participants were also randomised to placebo or vitamin D, 1,000 IU daily, in a 1:1 ratio **Statistical methods** Sample size calculations thoroughly described. All group comparisons were done according to the ITT approach. Kaplan–Meier and Cox regression analyses were planned. However, following early termination of the trial and prior to unblinding, the scientific committee determined that such analyses would be inappropriate because of the small numbers of outcomes. Characteristics of participants at baseline and at the end of the study are tabulated as means ± SD or number (percentage) and end-of-study comparisons were made by Student's t tests or χ2 tests. Incidence rates of primary outcomes, secondary outcomes and any safety events were calculated by dividing the number of individuals experiencing an event by the person-years of follow-up (from randomisation to the time of an event or censoring).	**Main findings** In the TZD arm, the cardiovascular outcome occurred in five participants (0.9%) in the placebo groups and three participants (0.4%) in the TZD groups (two allocated to pioglitazone, one to rosiglitazone). In the vitamin D arm, the primary outcome occurred in three participants (0.5%) in the placebo group and in two participants (0.3%) receiving vitamin D.	• **Ethics approval?** Approved by the US Food and Drug Administration (FDA), and ethics committees at all recruiting centres. • **Adverse events?** Adverse events were comparable in all groups. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** By a central phone-in computer system • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Not appropriate to conclude considering the premature end of the study • **Did authors review all outcomes?** Yes • **Cost/benefit effectiveness** Not assessed **Strengths:** Placebo-controlled design, large size, inclusion of a research question regarding vitamin D in addition to the TZD questions, safety oversight by an independent data monitoring committee and independent scientific leadership. **Weaknesses:** Timing. Stopped prematurely due to regulatory concerns. **Plausible explanations for the results?** Yes.
Conclusion *Uncertainty persists regarding the clinically relevant risks and benefits of TZDs and vitamin D because of the early cancellation of this comprehensive trial.*			
Country Multicenter			
Data collection period 2009-2010			

Witham, 2013

		Study design: **RCT**
Reference: Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, et al. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. JAMA internal medicine. 2013;173(18):1672-9.		*Double blind, placebo-controlled*
		Grade - quality — Low-Moderate[1] ⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To test whether high-dose, intermittent D3 supplementation lowers blood pressure in older patients with isolated systolic HT.	**Recruitment** Patients were recruited via 3 routes: from the community via primary care practices, via an article in the local newspaper about the research study, and via secondary care clinics (cardiovascular and medicine for the elderly). **Inclusion-/exclusion criteria** • Inclusion criteria: Age of 70 years or older, 25OHD level less than 30 ng/mL (to convert to nanomoles per liter, multiply by 2.496), and office systolic blood pressure greater than 140 mm Hg. • Exclusion criteria: Supplemental material. **Data** 159 participants were followed for 12 months **Outcome validation (i.e. diagnosis)** Difference in office blood pressure, 24-hour blood pressure, arterial stiffness, endothelial function, cholesterol level, insulin resistance, and b-type natriuretic peptide level. **Intervention variables** A total of 100,000 IU of oral cholecalciferol or matching placebo every 3 months for 1 year. Validation not described. **Important confounding factors** See statistical methods. **Statistical methods** Power calculation described. A 2-sided P < .05 was considered significant for all analyses. For each outcome measure, repeated-measures, mixed-effects analyses were conducted, with estimation and testing of main effects of treatment allocation (t test) and group × time interaction (F tests). Analyses for outcome measures at individual time points, including the primary outcome measures, were based on analysis of covariance, adjusting for baseline values of the response variable and levels of the stratification variables. Analyses were based on a modified intent-to-treat population (all participants with baseline and follow-up values of the response variable being analyzed). We calculated unadjusted models and models adjusted for baseline values of the outcome measure under study, along with baseline 25OHD level, baseline blood pressure, the presence of diabetes, and age. We also adjusted for use of thiazide diuretics given the known effects of these medications on calcium metabolism and use of statins given their known interaction with vascular health and vitamin D metabolism. Sensitivity analyses were conducted using multiple imputation to account for missing data and excluding patients with changes in antihypertensive medication type or dose for blood pressure analyses.	**Main findings** No significant treatment effect was seen for mean (95% CI) office blood pressure (−1 [−6 to 4]/−2 [−4 to 1] mm Hg at 3 months and 1 [−2 to 4]/0 [−2 to 2] mm Hg overall treatment effect). **Other findings** - Mean baseline office systolic blood pressure was 163/78 mm Hg. - Mean baseline 25-hydroxyvitamin D level was 18 ng/mL. - 25-Hydroxyvitamin D levels increased in the treatment group compared with the placebo group (+8 ng/mL at 1 year, P < .001). - No significant treatment effect was evident for any of the secondary outcomes (24-hour blood pressure, arterial stiffness, endothelial function, cholesterol level, glucose level, and walking distance).	• **Ethics approval?** Obtained from the Fife and Forth Valley National Health Service Research Ethics Committee. Clinical trials authorization was obtained from the UK Medicines and Healthcare Regulatory Authority. • **Adverse events?** Accounted for. There was no excess of adverse events in the treatment group, and the total number of falls was non-significantly lower in the group receiving vitamin D (36 vs 46, P = .24). • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, there were no significant differences between groups at baseline. • **Procedure for randomization?** Allocated to intervention or placebo in a 1:1 ratio. Stratified randomization was performed using a minimization algorithm, adminis- tered by the Robertson Centre for Biostatistics (Glasgow Clinical Trials Unit, University of Glasgow, United Kingdom) using a telephone-based system to conceal study allocation from investigators and participants. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Not described. • **Risk of attrition bias?** Low risk, dropout rate 11% in 12 months • **Presentation of results?** Yes, see results. • **Generalizability?** Wide range of comorbidity and concomitant medication use and, thus, the study population reflects that seen in the real world. Limitation: All being of white ethnicity • **Applicability in clinical practice?** The size of the trial means that a small beneficial effect on blood pressure still cannot be excluded, but the clinical relevance of such small improvements, at least at an individual patient level, is questionable. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed • **Findings supported by previous literature?** 4-month administration of 100 000 U of cholecalciferol was effective at increasing 25OHD levels and reducing fractures in a previous large osteoporosis trial, a finding that underpinned the choice of dose timing for the current trial. **Strengths:** The projected final evaluable sample size was reached, and the mean age (77 years) was considerably older than that seen in most hypertension trials. Despite enrolling older and sometimes frail patients, the dropout rate was only 11% in 12 months. Base- line patient characteristics were well balanced between the 2 groups, and a substantial increase in 25OHD levels was achieved with the intervention. **Weaknesses:** Insufficient dose. Intermittent dosing. Permissive factors, i.e. calcium intake. Recruitment from a single health board area and limited generalizability. Ambulatory blood pressure was somewhat lower than expected, which may have limited the ability to demonstrate reductions in ambulatory blood pressure. **Plausible explanations for the results?** Yes. 1. All-cause mortality not assessed by authors

Aim: To test whether high-dose, intermittent D3 supplementation lowers blood pressure in older patients with isolated systolic HT.

Conclusion: *Vitamin D supplementation did not improve blood pressure or markers of vascular health in older patients with isolated systolic hypertension.*

Country: UK, Scotland

Data collection period: 2009-2011

Reference: Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. Jama. 2014;312(15):1520-30.	Study design: **RCT** *Double blind, placebo-controlled*
	Grade - quality	High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To investigate whether a vitamin D3 treatment regimen intended to restore and maintain normal vitamin D status over 6 months is of health benefit for patients in ICUs.

Conclusion
Among critically ill patients with vitamin D deficiency, administration of high-dose vitamin D3 compared with placebo did not reduce hospital length of stay, hospital mortality, or 6-month mortality.

Country
Austria

Data collection period
2010-2012 | **Recruitment** Volunteers were recruited from 5 ICUs: medical, neurological, cardiothoracic surgery, and 2 mixed-surgery units.
Inclusion-/exclusion criteria
• Inclusion criteria: 18 years or older, expected to stay in the ICU for 48 hours or more, and found to have a 25OHD level of ≤20 ng/mL
• Exclusion criteria: severely impaired GI function; other trial participation, including previous participation in the pilot trial; pregnant or lactating women; hypercalcemia (total calcium of >10.6 mg/dL or ionized s-calcium of >5.4 mg/dL); tuberculosis; sarcoidosis; nephrolithiasis within the prior year; and patients not deemed suitable for study participation (ie, psychiatric disease, living remotely from the clinic, or prisoner status)
Data A total of 475 patients were included in the final analysis (237 in the vitamin D3 group and 238 in the placebo group). Participants were followed for 5 months
Outcome validation (i.e. diagnosis) The primary outcome was hospital length of stay. Secondary outcomes included, among others, length of ICU stay, the percentage of patients with 25-hydroxyvitamin D levels higher than 30 ng/mL at day 7, hospital mortality, and 6-month mortality. A predefined severe vitamin D deficiency (≤12 ng/mL) subgroup analysis was specified before data unblinding and analysis.
Intervention variables Vitamin D3 or placebo was given orally or via nasogastric tube once at a dose of 540 000 IU followed by monthly maintenance doses of 90 000 IU for 5 months.
Important confounding factors See statistics.
Statistical methods Power calculations thoroughly described. Analyses were conducted ITT. For the primary analysis comparing length of hospital stay between the 2 groups, used the Mann-Whitney test. Sensitivity analysis considered time to hospital discharge as the survival end point with death as a competing event according to Fine and Gray. For secondary end points, used t test or the Mann-Whitney test for continuous variables and the χ2 or Fisher exact test for categorical variables. Laboratory parameters were analyzed by means of analysis of covariance, taking into account the BL values. Kaplan-Meier estimates of survival functions were used and compared with the use of the log-rank test. HR and corresponding 2-sided 95% CIs were estimated with an unadjusted Cox regression model. The same analysis was performed for the predefined subgroup and extended by a formal test for interaction, including the interaction term between the treatment and the predefined subgroups in the models. Furthermore, Cox regression models were applied adjusting for age, sex, Simplified Acute Physiology Score (SAPS) II, degree of comorbidity, and serum calcium, albumin, procalcitonin, and parathyroid hormone levels at BL. Colinearity among confounding variables was investigated by correlation analysis and further assessed between these variables using the variance inflation factors (>4) and the tolerance statistic (<0.2). Used 2-sided test and P value <.05. No adjustments were made for multiple comparisons. | **Main findings**
The median (IQR) length of hospital stay was not significantly different between groups (20.1 days [IQR, 11.1-33.3] for vitamin D3 vs 19.3 days [IQR, 11.1-34.9] for placebo; P = .98).

Other findings
- Hospital mortality and 6-month mortality were also not significantly different (hospital mortality: 28.3% [95% CI, 22.6%-34.5%] for vitamin D3 vs 35.3% [95% CI, 29.2%-41.7%] for placebo; hazard ratio [HR], 0.81 [95% CI, 0.58-1.11]; P = .18;
- 6-month mortality: 35.0% [95% CI, 29.0%-41.5%] for vitamin D3 vs 42.9% [95% CI, 36.5%-49.4%] for placebo; HR, 0.78 [95% CI, 0.58-1.04]; P = .09).
- For the severe vitamin D deficiency subgroup analysis (n = 200), length of hospital stay was not significantly different between the 2 study groups: 20.1 days (IQR, 12.9-39.1) for vitamin D3 vs 19.0 days (IQR, 11.6-33.8) for placebo.
- Hospital mortality was significantly lower with 28 deaths among 98 patients (28.6% [95% CI, 19.9%-38.6%]) for vitamin D3 compared with 47 deaths among 102 patients (46.1% [95% CI, 36.2%-56.2%]) for placebo (HR, 0.56 [95% CI, 0.35-0.90], P for interaction = .04), but not 6-month mortality (34.7% [95% CI, 25.4%-45.0%] for vitamin D3 vs 50.0% [95% CI, 39.9%-60.1%] for placebo; HR, 0.60 [95% CI, 0.39-0.93], P for interaction = .12). | • **Ethics approval?** This trial was approved by the institu- tional ethical committee of the Medical University of Graz and the Austrian Agency for Health and Food Safety.
• **Adverse events?** Accounted for.
• **Aim?** Primary outcome clearly defined.
• **Did the randomization work?** Yes, there were no significant differences between groups at baseline.
• **Procedure for randomization?** Randomly assigned to placebo or vitamin D3 in a 1:1 ratio (Figure 1), using the Randomizer for Clinical Trials tool developed at the Medical University of Graz. The randomization block size was 8 for patients stratified according to ICU type and sex.
• **Blinding?** Both participants and investigators were blinded throughout the study.
• **Did the groups receive the same co-intervention/treatments?** Yes
• **Primary endpoints – validated?** Low risk of classification bias.
• **Risk of attrition bias?** Low risk. There was less than 1% missing data.
• **Presentation of results?** Yes, see results.
• **Generalizability/Applicability in clinical practice?** Lack of external validity, the single-center design and the lack of non-white or pediatric patients limits generalizability. Considering the population recruited, the results were representative in a mixed population of adult patients who were critically ill without restriction of age, sex, or admission diagnosis.
• **Did authors review all outcomes?** Yes.
• **Cost/benefit effectiveness** Not assessed.
• **Findings supported by previous literature?**
Strengths: Statistical power.
Weaknesses: No adjustments were made for multiple comparisons. Length of stay and not mortality as the primary end point. External validity, the single-center design and the lack of non-white or pediatric patients. No positive effect found in the primary outcome, only in subsequent subgroup analyses. Sample size might not allow for the identification of rare adverse effects of high-dose vitamin D3. Utilization of an immunoassay for determination of 25-hydroxyvitamin D. Did not assess hospital infection rates and the analysis was limited to known study drug–specific adverse events.
Plausible explanations for the results? Yes. |

Reference: Scragg R, Stewart AW, Waayer D, Lawes CMM, Toop L, Sluyter J, et al. Effect of Monthly High-Dose Vitamin D Supplementation on Cardiovascular Disease in the Vitamin D Assessment Study : A Randomized Clinical Trial. JAMA cardiology. 2017;2(6):608-16.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To examine whether monthly high-dose vitamin D supplementation prevents CVD in the general population	**Recruitment** Volunteers were recruited from family practices in Auckland, mostly from 55 practices, by a personalized letter mailed to their homes. **Inclusion-/exclusion criteria** • Inclusion criteria: Age of 50 to 84 years, ability to give written informed consent, resident in Auckland, New Zealand, at recruitment, and anticipated residence in New Zealand for the 4-year study period. • Exclusion criteria: Current use of vitamin D supplements, including cod-liver oil; diagnosis of psychiatric disorders that would limitability to comply with the study protocol; history of hypercalcemia, nephrolithiasis, sarcoidosis, parathyroid disease, orgastric bypass surgery; enrolled in another study, which could affect participation; or baseline corrected serum calcium level > 10.0 mg/dL	**Main findings** The primary outcome of CVD occurred in 303 participants (11.8%) in the vitamin D group and 293 participants (11.5%) in the placebo group, yielding an adjusted hazard ratio of 1.02 (95% CI, 0.87-1.20). Similar results were seen for participants with baseline vitamin D deficiency and for secondary outcomes.	• **Ethics approval?** Approved by the New Zealand Multi-region Ethics Committee in Wellington • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups. • **Procedure for randomization?** Random assignment was made to 1 of the 2 treatment groups in random block sizes of 8, 10 or 12, within race/ethnic group and 5-year age strata.
Conclusion	**Data** 5108 participants were followed for a median of 3.3 years. Basline interview including sociodemographic status, lifestyle, intake of vitamin D or calcium supplements, current medication prescribed by a physician, and medical history told by a physician. Measurement of height and weight. Brachial blood pressure. A non fasting blood sample was collected to screen for hypercalcemia, with the remaining serum aliquoted and stored at −80°C for later measurement of 25hydroxyvitamin D [25(OH)D] and lipid levels.	Of the 5108 participants included in the analysis, the mean (SD) age was 65.9 (8.3) years, 2969 (58.1%) were male, and 4253 (83.3%) were of European or other ethnicity, with the remainder being Polynesian or South Asian.	• **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative for the general population. Results cannot be generalized with regards to long.term effects. .
Monthly high-dose vitamin D supplementation does not prevent CVD. This result does not support the use of monthly vitamin D supplementation for this purpose	**Outcome validation (i.e. diagnosis)** NationalHealth Index number used to track deaths and hospital discharges and dispensed prescriptions during the follow-up period. These data were used to define CVD outcomes, alone orin combination with data about prior CVD from the baseline interview. **Intervention variables** Vitamin D3 100,000 IU versus placebo soft gel oral capsules monthly. **Important confounding factors** See statistics.	Mean (SD) baseline deseasonalized 25(OH)D concentration was 26.5 (9.0) ng/mL, with 1270 participants (24.9%) being vitamin D deficient.	• **Did authors review all outcomes?** Yes. • **Findings supported by previous literature?** Yes, findings are consistent with previous RCTs of vitamin D supplementation and Mendelian randomization studies.
Country	**Statistical methods** Power calculation described thoroughly. Analysis of the primary outcome (CVD) was conducted ITT made possible by the National Health Index number to identify CVD events regardless of whether participants continued to participate actively in the study by returning the home questionnaire. The Cox proportional hazards regression model, with robust sandwich variance estimates, was used to compare the time to first CVD event in the 2 treatment groups and to calculate CVD HRs in the placebo group. Deaths from non-CVD causes were censored. The treatment group differences in 25(OH)D levels were tested for change over time with a general linear model with repeated time using an unstructured correlation matrix. Where indicated, season-adjusted (deseasonalized) values were calculated for each participant from their individual baseline 25(OH)D concentration and date of blood sample collection by using a sinusoidal model with values derived from baseline values for all participants. Vitamin D deficiency was defined as having a deseasonalized 25(OH)D level less than 20 ng/mL. All statistical testswere 2-tailed with a 5% level of significance.	In a random sample of 438 participants, the mean follow-up 25(OH)D level was greater than 20 ng/mL higher in the vitamin D group than in the placebo group.	**Strengths:** High retention rate, 87% still participating actively in the final follow-up period. High adherence, with 84% of capsules reported taken in questionnaires, confirmed by the high serum 25(OH)D concentrations in the vitamin D group of the measured subgroup. The mean 25(OH)D concentration greater than 40 ng/mL in the vitamin D group confirmed the adequacy of the vitamin D dose, particularly the greater than 20-ng/mL difference compared with the placebo arm.
New Zealand			**Weaknesses:** Both the event-rate and the follow-up time lower than expected, thus power was lower than calculated (75%). Much less power to detect benefits for the subgroup with vitamin D deficiency and for preventing specific CVD outcomes, such as heart failure, as reported in previous RCTs. Possible longer-term beneficial effects from suppl > 3.3. years cannot be excluded. The outcome measures were not adjudicated. Unable to identify participants who emigrated during the study.
Data collection period			**Plausible explanations for the results?** Yes.
2011-2015			

Reference: Zittermann A, Ernst JB, Prokop S, Fuchs U, Dreier J, Kuhn J, et al. Effect of vitamin D on all-cause mortality in heart failure (EVITA): a 3-year randomized clinical trial with 4000 IU vitamin D daily. European heart journal. 2017.	Study design: **RCT** *Double blind, placebo-controlled*	
	Grade - quality	High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To examine whether oral vitamin D supplementation reduces mortality in patients with advanced HF	**Recruitment** Volunteers were recruited from the Clinic for Thoracic and Cardiovascular Surgery of the Heart and Diabetes Center North Rhine-Westphalia, Germany. Included patients were either in along-term program for heart transplantation or were already listed as 'elective'for heart transplantation. **Inclusion-/exclusion criteria** • Inclusion criteria: 18–79 years of age and if they were classified as having New York Heart Association functional class II or higher. • Exclusion criteria: 'high urgent' listingfor heart transplantation, hypercalcaemia (plasma calcium >2.75 mmol/L), supplemental vitamin D intake >800 IU/d, and baseline 25-hydroxyvitaminD levels >_75 nmol/L.	**Main findings** Mortality was not different in patients receiving vitamin D (19.6%; n = 39) or placebo (17.9%; n = 36) with a hazard ratio (HR) of 1.09 [95% confidence interval (CI): 0.69–1.71; P = 0.726].	• **Ethics approval?** The study protocol was approved by the ethics committee of the Medical Council of Westfalen-Lippe, Germany • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between thevitamin D and placebo groups.
	Data 400 prticipants were followed for 3 years. Blood specimens were collected every 6 months between 8 and 11 AM after an overnight fast	The need for MCS implant was however greater in patients assigned to vitamin D (15.4%, n = 28) vs. placebo [9.0%, n = 15; HR: 1.96 (95% CI: 1.04–3.66); P = 0.031]. Other secondary clinical endpoints were similar between groups.	• **Procedure for randomization?** Randomization was computer based in blocks of six and stratified by sex. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias.
Conclusion *A daily vitamin D dose of 4000 IU did not reduce mortality in patients with advanced HF but was associated with agreater need for MCS implants. Data indicate caution regarding long-term supplementation with moderately high vitamin D doses.*	**Outcome validation (i.e. diagnosis)** Primary endpoint was all-cause mortality. 4 sources of information was used: repeated contacts with the participants, contacts with family physicians, a regular review of medical records, and consultation of the respective registration office. Causes of death were assessed from the medical records or by contacting the family physicians. Secondary endpoints were pre-specified. In case of hospitalization, the underlying cause was alsoassessed (routine, cardiac-related, or other cause). Decisions for high urgent listing for heart transplantation or MCS implantation were made in weekly institutional and interdisciplinary expert conferences. Secondary clinical endpoints were assessed by the same sources used to identify the primary endpoint (exception: registration office). Regarding hypercalcaemia and hypervitaminosis D, assessment was exclusively based on plasma levels.	Initial 25OHD levels were on average <40 nmol/L, remained around 40 nmol/L in patients assigned to placebo and plateaued around 100 nmol/L in patients assigned to vitamin D. The incidence of hypercalcaemia was 6.2% (n = 10) and 3.1% (n = 5) in patients receiving vitamin D or placebo (P = 0.192).	• **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability/Applicability in clinical practice?** Considering the population recruited, the results were representative in a population with heart disease. Generalizability to healthy individuals, children or other disease groups are limited. Application in clinical situations was suggested in reconsideration of upper limits for daily vitamin D supplementation. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness?** ... • **Findings supported by previous literature?** Yes, a meta-analysis of observational data indicates a statistically positive association between plasma calcium and cardiovascular disease. With respect to the present study, the Atherosclerosis Risk in Communities (ARIC) study reported that high-plasma calcium was independently associated with greater risk of incident HF.
Country Germany	**Intervention variables** Vitamin D3 4000 IU versus placebo daily. **Important confounding factors** Because recent data indicate a potential interaction between baseline 25OHD levels and mortality, also performed pre-specified analyses in subgroups with initial 25OHD levels <30nmol/L and >_30nmol/L. Tests for interaction were based on the Wald test for the interaction term (25OHD subgroup x study group), with both the 25OHD subgroup and study group in the model as categorical variables. Because the pre-specified primary analysis of the mortality rate over time was limited to the single P-value for treatment interaction, we did not adjust for multiple testing.		**Strengths:** The study design, the homogenous group ofpatients, the variety of assessed clinical and safety parameters, the study duration of 3 years, and the 100% completeness of follow-up data for the ITT analysis of the primary endpoint.
Data collection period 2010-2016	**Statistical methods**...Pwer calculation described thoroughly. Cumulative incidence of the primary and secondary endpoints was calculated using the Kaplan–Meier method. HRs and 95% CIs were estimated using Cox proportional hazards models. The proportionality of hazard assumption was evaluated by the Schoenfeld test. All statistical analyses regarding primary and secondary endpoints were prespecified, unless otherwise stated, and were conducted according to the ITT principle. To avoid elimination of subjects with missing biochemical data, theinfluence of time (trend) on plasma calcium, 25OHD, phosphate, and kidneyfunction was analysed using linear mixed models. Fixed effects were treatment, time (month), and the interaction of treatment x time.Categorical variables are summarized as numbers and as a percentage of observations. Non normally distributed data, as checked by quantile–quantile plots and the Kolmogorov–Smirnov test, were normalized by logarithmic transformation before use in parametric statistical analysis. The Mann–Whitney Utest was used for group comparisons. We considered P-values <0.05 (two-sided) as statistically significant.		**Weaknesses:** Low statistical power due to a lower annual mortality than expected. Nevertheless, the trial was able to provide significant results in exploratory clinical endpoints/biochemical parameters. While these data analyses should be interpreted with caution since they do not prove causality, they do indicate concern regarding long-term vitaminD supplementation with moderately high-daily doses. **Plausible explanations for the results?** Yes.

Reference: Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. The New England journal of medicine. 2019;380(1):23-32.	Study design: **RCT** *Double blind, placebo-controlled, 2x2 factorial design*	
	Grade - quality	High ⊗⊗⊗⊗

Aim	Material and methods	Results	Discussion/comments/checklist
To assess if vitamin D reduces the risk of cancer or cardiovascular disease.	**Recruitment** Participants were recruited throughout the United States, and the groups were balanced according to sex and with a goal to include at least 5000 black participants. **Inclusion-/exclusion criteria** • Inclusion criteria: No history of cancer (except nonmelanoma skincancer) or cardiovascular disease at trial entry, and they were required to agree to limit the use of vitamin D from all supplemental sources, including multivitamins, to 800 IU per day and to complete a 3-month placebo run-in phase • Exclusion criteria: Renal failure or dialysis, cirrhosis, history of hypercalcemia, or other serious conditions that would preclude participation **Data** 25,871 participants, including 5106 black participants, were followed for a median of 5.3 yrs. Baseline questionnaires collected data on risk factors for cancer, cardiovascular disease, and other conditions and included a food frequency questionnaire. Participants received follow-up questionnaires at 6 months and 1 year after randomization and annually thereafter to collect information on adherence to trial regimens, outside use of vitamin D supplements, development of major illnesses, updates on risk factors, and potential side effects of the trial agents. Blood samples obtained at BL and at follow-up. **Outcome validation (i.e. diagnosis)** The primary end-points were invasive cancer of any type and major cardiovascular events (composite of myocardial infarction, stroke and death from cardiovascular causes). Secondary cancer end-points were described. Medical records were reviewed for confirmation by an end-points committee of physicians who were unaware of the trial-group assignments. Cancer was confirmed on the basis of histologic or cytologic data. MI and stroke were confirmed with the use of established criteria, coronary revascularization was confirmed by medical record view, and death from cardiovascular causes was confirmed if there was convincing evidence of a cardiovascular event from all available sources. If records were unavailable (or participants were lost to follow-up), the NDI was searched for cause of death according to the death-certificate information. Deaths were defined with the use of all these sources; a secondary analysis of cause-specific deaths required medical records or other adjudication of cause of death beyond NDI coding. **Intervention variables** Vitamin D3 2000 IU versus placebo daily. Calendar packs containing the trial capsules of vitamin D or corresponding placebo (and n−3 fatty acids or corresponding placebo) were mailed with questionnaires to the participants. **Important confounding factors** Se statistical methods. **Statistical methods** Power calculation described. Analyses of effect were based on the ITT-principle. Initial analyses compared BL characteristics of participants according to trial regimen with the use of t-tests or chi square tests. Primary analyses compared the main effects of vitamin D on cancer and cardvasc dis w/the use of Cox proportional-hazards models controlled for age, sex, and randomization group in the n−3 fatty acid portion of the trial (n−3 fatty acid group or placebo group). Person-time was counted from rand to the end-point, to death or to the end of the trial. Cumulative-incidence plots and interactions with time were used to examine if effects varied over time. Prespecified analyses of the primary outcomes excluding events that occurred during the first year and the first 2 years of follow-up assessed latent effects. Possible variations in the effect according to race or ethnic group, age, sex, BMI, BL 25OHD level, concurrent randomization to the n−3 group, outside use of vitamin D suppl, and BL risk factors for cancer and CVD were specified a priori.	**Main findings** Cancer was diagnosed in 1617 participants (793 in the vitamin D group and 824 in the placebo group; HR, 0.96; 95% CI [0.88 to 1.06]; P = 0.47). A major cardiovascular event occurred in 805 participants (396 in the vitamin D group and 409 in the placebo group; HR 0.97; 95% CI, 0.85 to 1.12; P = 0.69). In the analyses of secondary end points, the hazard ratios were as follows: - for death from cancer (341 deaths), 0.83 (95% CI, 0.67 to 1.02); for breast cancer, 1.02 (95% CI, 0.79 to 1.31); - for prostate cancer, 0.88 (95% CI, 0.72 to 1.07); - for colorectal cancer, 1.09 (95% CI, 0.73 to 1.62); - for the expanded composite end point of major cardiovascular events plus coronary revascularization, 0.96 (95% CI, 0.86 to 1.08); - for MI, 0.96 (95% CI, 0.78 to 1.19); for stroke, 0.95 (95% CI, 0.76 to 1.20); and for death from cardiovascular causes, 1.11 (95% CI, 0.88 to 1.40). In the analysis of death from any cause (978 deaths), the hazard ratio was 0.99 (95% CI, 0.87 to 1.12). No excess risks of hypercalcemia or other adverse events were identified.	• **Ethics approval?** The trial was approved by the institutional review board of Partners HealthCare–Brigham and Women's Hospital and was monitored by an external data and safety monitoring board. • **Adverse events?** Accounted for. • **Aim?** Primary outcome clearly defined. • **Did the randomization work?** Yes, baseline characteristics were similar between the vitamin D and placebo groups. • **Procedure for randomization?** Randomization was computer generated within sex, race, and 5-year age groups in blocks of eight. • **Blinding?** Both participants and investigators were blinded throughout the study. • **Did the groups receive the same co-intervention/treatments?** Yes • **Primary endpoints – validated?** Low risk of classification bias. • **Risk of attrition bias?** Low risk. • **Presentation of results?** Yes, see results. • **Generalizability?** Not generalizable to • **Applicability in clinical practice?** Considering the population recruited, the results were representative in a large general population sample with racial, ethnic, and geographic diversity. Application in clinical situations are limited with regards to children and excluded disease groups. • **Did authors review all outcomes?** Yes. • **Cost/benefit effectiveness** Not assessed. • **Findings supported by previous literature?** Previous findings discussed. **Strengths:** Large sample size, long duration, sufficient power. Daily dosing regimen. High rates of follow-up and adherence to the trial regimen. Rigorously adjudicated endpoints. BL and follow-up blood samples from many participants and achieved mean 25-hydroxyvitamin D levels in the targeted range. **Weaknesses:** Median duration of follow-up varies. Only tested one dose of vitamin D. No control for multiple hypothesis testing, and no formal adj made to the P values or CIs. **Plausible explanations for the results?** Yes.
Conclusion *Supplementation with vitamin D did not result in a lower incidence of invasive cancer or cardiovascular events than placebo*			
Country UK			
Data collection period 2011-2017			