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Weekly cholecalciferol supplementation results in significant reductions in infection risk among the vitamin D deficient: results from the CIPRIS pilot RCT

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Abstract

Background: Observational studies suggest vitamin D deficiency may contribute to the risk of acute infections. We undertook a randomised controlled trial (RCT) of cholecalciferol supplementation as an intervention against acute infections.

Methods: A cohort of 34 healthy adults was randomised to 20,000 IU/week cholecalciferol or identical placebo and followed for 17 weeks during winter 2012. Acute infections, defined as the occurrence of sustained (at least an hour) infection symptoms, either of severity 2/5 or greater or sustained over 24 h, were monitored by daily online symptom reporting, with potential infections assessed in clinic. No microbiological verification of symptoms was available, however. Primary endpoint was the occurrence of acute infection; secondary endpoints were infection duration and infection severity; and tertiary endpoints were change in serum 25-hydroxyvitamin D (25(OH)D) and adverse events.

Results: No treatment effect was observed for infection risk (HR: 0.83, 95% CI: 0.53, 1.31), nor duration or severity. However, on stratification by baseline serum 25(OH)D (levels chosen on the basis of average levels in our cohort and known minimums needed for bone health), a significant treatment effect on infection risk was evident among those who were vitamin D deficient at the start of the study, such that those of baseline 25(OH)D < 40 nmol/L ($n = 4$) realised a 44% reduction in infection risk (HR: 0.56; 95% CI: 0.32, 0.96; $P = 0.007$), this increasing to 73% on restriction to clinically verified infections (HR: 0.27; 95% CI: 0.07, 1.00; $P = 0.050$). A similar but less consistent and nonsignificant effect was seen for infection severity. Treatment was associated with significantly higher 25(OH)D compared to placebo; however, the maximum 25(OH)D was 154 nmol/L and no adverse events occurred.

Conclusions: The results of this study suggest a protective effect of vitamin D supplementation against acute infection risk among persons who are vitamin D deficient. Larger studies are needed to validate these findings.

Keywords: Vitamin D, Acute infection, Randomised controlled trial

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Background

Acute infections, particularly respiratory tract infections (RTIs), are a major cause of mortality, particularly among those at the extremes of age and those requiring significant support [1,2]. Moreover, RTIs are among the most frequent sources of absenteeism from work and school, accounting for millions of lost work and school days and billions of lost wages each year [3]. Thus, an intervention to reduce the frequency of acute infections is indicated.

A significant modulator of the immune response is vitamin D's active metabolite, 1,25-dihydroxyvitamin D, which has been shown to regulate antimicrobial peptides that comprise a significant part of the innate immune response and is known to modulate the expression of nearly 1,000 genes [4,5]. In persons who are vitamin D deficient, as occurs in winter at high latitudes and year-round for long-term medical inpatients [6,7], there is a potential for significant dysfunction in the innate immune response, leading to a greater frequency of infections.

This has been supported by observational studies, which found a potent relationship between vitamin D deficiency and acute infection [8-13]. Randomised controlled trials (RCTs) of cholecalciferol supplementation have had mixed results, however, with some finding protective treatment effects [14-17] and others not [18-20], reflecting in part varied methodologies. Recently, Murdoch and colleagues ran a 2-year trial of monthly cholecalciferol supplementation for 18 months in 322 healthy adults, with comprehensive monitoring of infections and clinical assessment, but finding no difference in infection occurrence, duration or severity [20]. Despite some null findings, systematic reviews [21,22] have indicated a potential protective effect against infections from vitamin D supplementation.

We hypothesised that by improving the vitamin D sufficiency of participants, their innate immune response would more effectively clear pathogens prior to their establishing a solid colonisation, reducing the occurrence of symptomatic infections. Therefore, we undertook a pilot RCT of 20,000 IU/week cholecalciferol vs. placebo in a cohort of 34 adults to evaluate its efficacy at reducing the frequency, duration and severity of acute infections.

Methods

Recruitment and follow-up

CIPRIS (Cholecalciferol Intervention to Prevent Respiratory Infections Study) was a double-blind randomised placebo-controlled trial of 20,000 IU/week cholecalciferol supplementation, undertaken in Hobart, Australia (latitude 42.9°S). This dose was chosen with a goal to give the equivalent of roughly 3,000 IU per day to realise a replete 25(OH)D status. The study was registered at www.clinicaltrials.gov (NCT01549938) and the Australia-New Zealand Clinical Trials Register (ACTRN12612000054819). The study was approved by the Southern Tasmania Human Research Ethics Committee, conforming to the principles embodied in the Declaration of Helsinki. All participants provided written informed consent. The study protocol was developed and finalised prior to the start of recruitment. No alterations to protocol were made during the study recruitment or follow-up periods.

Study methods, results and interpretations thereof are presented as per the CONSORT guidelines (Additional files 1 and 2).

Participants were recruited from the student and staff and their friends and families of the Menzies Research Institute Tasmania and the University of Tasmania School of Medicine, the Royal Hobart Hospital and the TasTAFE Campbell Street Campus, all essentially healthy controls representative of the general population. All clinics were conducted at the Menzies Research Institute Tasmania, however. Recruitment was in March-June 2012 conducted via flyers, email invitation and word-of-mouth communication. Exclusion criteria were 1) age <18 or >60 years; 2) using immunomodulatory medication; 3) diagnosed with an autoimmune, immune-deficiency or chronic respiratory condition; 4) diagnosis of hypercalcemia or malabsorption syndrome, or any parathyroid, liver or kidney disorder; 5) using vitamin D supplements (>1,000 IU/day) in the preceding 3 months; 6) being pregnant or planning to so within the study period; 7) inability to swallow capsules; or 8) inability to provide informed consent. Interested persons were invited to complete an online assessment questionnaire querying the exclusion criteria, with acceptable participants asked to provide contact information for the study investigators to contact them on. Of the 173 persons who accessed the assessment questionnaire, 52 were ineligible and 89 either did not provide contact information or declined to participate on discussing the study protocol, leaving 32 participants.

Follow-up was during the winter months, from May to October 2012, much of which is when ambient UV is too low to generate vitamin D [7].

At baseline assessment, participants were queried for demographic information and diet and behaviour affecting infection or vitamin D. At weekly updates, participants were queried about changes in health status, medication, supplements, health status, or time outside or physical activity.

Participants were also queried whether they had travel out of the state in the interval since the last weekly update. Excluding these people did not materially affect any analyses (data not shown).

Sample size for this pilot was set at 32 to allow a balance between treatment arms, with the number selected to test methods and principals in anticipation of a larger study. Participants were a convenience sample of healthy

adults and did not represent any groups at risk of vitamin D deficiency or infection susceptibility.

Randomisation and treatment

Participants were randomised simply 1:1 to parallel treatment using a computerised randomisation program (www.randomization.com), using the first generator. A person outside the study was asked to run the randomisation and to affix the treatment labels with study IDs to the respective bottles of treatment and placebo.

Cholecalciferol and placebo capsules were obtained from Dartnells Pharmacy in Victoria, Australia. Both cholecalciferol and placebo were identical white capsules. Treatment allocations were dispensed at weekly clinics.

Blinding

As a double-blind RCT, all CIPRIS staff (including nurses and database entry personnel), investigators and participants were blinded to treatment allocation until the conclusion of follow-up.

Biological specimens

At each monthly review, a blood sample was taken from participants for measuring 25(OH)D. The first two monthly specimens were also tested for corrected calcium and phosphate. Blood samples were centrifuged and the serum aliquoted and stored at −80°C until analysis.

Biological assays

Serum corrected calcium and phosphate were assessed using standard methods by the Pathology Department of the Royal Hobart Hospital each week.

After the conclusion of the study, serum 25(OH)D was assessed by tandem mass spectroscopy by Canterbury Health Laboratories in New Zealand.

Infection assessment

Participants completed daily online questionnaires querying the occurrence and magnitude (0–5, where 0 is no presence of that symptom and 5 is most severe) of acute infection symptoms, including respiratory, gastrointestinal, urinary tract, eye, ear, skin and cold sore infections, as well as nonspecific symptoms. Online questionnaire responses were monitored each day by the chief investigator. When a participant reported symptoms of magnitude greater than 2/5 (thought to be of sufficient magnitude for a single day's occurrence to warrant assessment in clinic), or two successive days of symptoms of any magnitude, the participant was invited to come into clinic for objective assessment by our study nurse.

After the conclusion of the study, infection reports from daily online surveys, and from clinic assessments, were reviewed by the chief investigator and the study nurse, classifying infections and infection type. In the event of disagreement between reviewers, infection status was agreed upon by discussion. Respiratory, gastrointestinal, urinary tract, eye, skin and cold sore infections were defined by symptoms reflective of these infection types. Ear infections were defined as earache symptoms in the absence of RTI symptoms. Systemic/nonspecific infections were defined as nonspecific symptoms like fever, malaise, fatigue, headache, and/or arthromyalgia, in the absence of other infection symptom types.

Infection duration was defined from the first day of reported symptoms and concluded at the resolution of symptoms. Where infections were confluent, a judgement was made on the basis of symptoms and symptom severities as to the end of one infection and start of another.

Infection severity for each infection was evaluated in three fashions. One was to take the maximum reported infection symptom severity for that infection. We also summated the infection severity scores for all infection symptoms for each day of the infection and summated this for a total infection severity score. Finally, we averaged the daily total infection symptom severities. For RTIs, only RTI-specific symptoms were used.

While the majority of infections were seen in clinic, a subset (11.8%) was only reported on online daily questionnaire but not seen in clinic. Sub-analyses excluding these are reported.

Adverse events

Serum collected at the first 2 months was evaluated for the levels of corrected calcium and phosphate. These reports were reviewed by an external monitor, who could terminate a participant's participation if levels exceeded safe levels.

In addition, on daily questionnaire and at weekly and infection clinics, participants were queried about the occurrence and severity (0–5) of symptoms potentially indicative of hypercalcemia or hyperphosphatemia, including excessive thirst, abnormally high urine output, change in urine colour, bone pain, groin-area pain, confusion, irritability or other neurological symptoms. Any sustained occurrence of these could have resulted in that subject's participation being stopped.

Statistical analyses

Analysis was by intention-to-treat.

Primary outcomes were time to infection. Secondary outcomes were infection severity and duration. Tertiary outcomes were change in serum 25(OH)D and the occurrence of adverse events.

Predictors of time-to-acute infection were assessed by survival analysis, using Cox proportional hazard models for repeated events [23]. All covariates satisfied the proportional hazard assumption.

Significance of differences in study follow-up duration and inter-review interval duration were evaluated by Kruskal-Wallis rank test.

Predictors of having an infection during the study were evaluated by logistic regression.

Predictors of infection duration, infection severity and longitudinally measured 25(OH)D were assessed by multilevel mixed effects linear regression. Predictors of infection count during the study were assessed by linear regression. Predictors of baseline-measured 25(OH)D were assessed by linear regression. As the distribution of all these variables was skewed, transformation was applied as required to satisfy homoscedasticity; however, all coefficients are reported on the scale of the original value.

Where interaction was assessed, a product term containing the primary predictor and the interaction covariate was generated and included in the model.

For all instances where data was missing, analyses were restricted to persons with complete data.

All analyses were performed using STATA/SE for Windows.

Results

Cohort characteristics

We initially recruited 32 participants, the majority (23/32) attending their first clinic in the 2 weeks of the study. Two participants (both on treatment) dropped out during the study due to not being able to meet the study's schedule rigour (one after week 1, the other after week 4), and these were replaced with an additional two participants who ran out their period of follow-up. Analyses excluding these additional two participants did not materially affect the results (data not shown).

Participants were followed for an average of 16.4 weeks. Follow-up time did not differ significantly between treatment groups (treatment: 15.7 weeks; placebo: 17.2 weeks; $P = 0.10$). While the interval between reviews (not including infection clinics) was 7 days, there was some variation in this, with a mean interval of 7.07 days; this interval did not significantly differ between treatment groups ($P = 0.77$).

Over the course of an average follow-up, 34 participants experienced 98 infections. Of these, the majority (69.4%) were RTIs. Virtually everyone in the cohort had at least one infection (31/34, 91.2%)—six people had one infection, eight had two infections, seven had three infections and ten had four or more infections.

As in Table 1, the cohort was majority female, majority Australian-born and of mean age 32 years. None of the cohort characteristics were significantly different between treatment arms. A small number of participants (two on treatment, one on placebo) reported taking multivitamins, but otherwise no one was taking any supplements which might contain vitamin D.

Predictors of infection, infection duration and severity

Having any infection during the study was less frequent among those on treatment (OR: 0.53; 95% CI: 0.04, 6.51), though this was not statistically significant. Infection count was lower among those on treatment (mean: 2.01; 95% CI: 1.12, 2.89) than those on placebo (mean: 3.01; 95% CI: 1.87, 4.15), though this did not reach statistical significance ($P = 0.17$). On restriction to infections seen in clinic, this difference was increased but still did not reach statistical significance ($P = 0.13$). These analyses could not be stratified by baseline-measured 25(OH)D. Overall, there was no evidence of statistically significant treatment effects on infection overall.

Hazard of infection was not significantly predicted by treatment (Table 2) nor was infection duration or infection severity by any of the measures used.

While there was no significant effect on infection hazard by treatment overall, on stratification by baseline-measured serum 25(OH)D, a protective effect from treatment became evident among those who were deficient at the start of the study (Table 3). This effect was strongest on setting the threshold at 40 nmol/L ($p_{\text{interaction}} = 0.042$), attenuating at a threshold of 50 nmol/L ($p_{\text{interaction}} = 0.68$) and disappearing altogether at a threshold of 60 nmol/L ($p_{\text{interaction}} = 0.40$). On restriction to infections which were seen in clinic, these trends were increased in magnitude, though of reduced significance, likely reflecting the decreased number of infections. Associations persisted on adjustment. On restriction to RTIs, trends were similar (data not shown). Importantly, any protective effect was abrogated on adjustment for extant levels of serum 25(OH)D, indicating a true effect of treatment via increasing 25(OH)D (data not shown).

On stratification by baseline-measured 25(OH)D, no effect of treatment on infection duration was apparent. For infection severity, on stratification by level of baseline 25(OH)D, there were inconsistent trends for the maximum infection severity score and the average daily infection severity score, but no trend was apparent for the total infection severity score (Additional file 3). Similar results were found for RTIs seen in clinic (data not shown). No associations were found for infections not seen in clinic (data not shown).

Distribution and predictors of serum vitamin D

The mean 25(OH)D at study entry was 67.9 nmol/L (SD: 23.0), with those randomised to treatment having significantly lower baseline 25(OH)D than those allocated to placebo (60.5 vs. 76.4, $P = 0.040$). Thereafter, the mean 25(OH)D for those on treatment was 100.7 nmol/L (SD: 23.9), while that of placebo was 56.0 nmol/L (SD: 24.2) ($P < 0.001$). Trends in 25(OH)D over time are shown in Figure 1.

There was a negative interaction between baseline-measured BMI and treatment in predicting 25(OH)D,

Table 1 Distribution of cohort characteristics, overall and between treatment allocations

	Placebo *n* = 16 (47.1%)	Treatment *n* = 18 (52.9%)
Total infections during the study	54	44
Infection type		
Respiratory tract infection	32 (59.3)	36 (81.8)
Gastrointestinal infection	11 (20.4)	5 (11.4)
Urinary tract infection	0	0
Skin infection	2 (3.7)	0
Ear infection	1 (1.9)	0
Eye infection	0	1 (2.3)
Cold sore	5 (9.3)	2 (4.6)
Systemic/nonspecific	3 (5.6)	0
Sex		
Male	5 (31.3)	9 (50.0)
Female	11 (68.8)	9 (50.0)
Birthplace		
Australia	15 (88.2)	14 (82.4)
New Zealand	1 (5.9)	1 (5.9)
United Kingdom	0	2 (11.8)
Singapore	1 (5.9)	0
BMI at baseline		
< 18.5	1 (6.3)	0
18.5–24.9	8 (50.0)	10 (55.6)
25.0–29.9	6 (37.5)	7 (38.9)
30 or greater	1 (6.3)	1 (5.6)
Taking any medications at baseline?		
No	9 (56.3)	9 (50.0)
Yes	7 (43.8)	9 (50.0)
Taking any supplements at baseline?		
No	12 (75.0)	15 (83.3)
Yes	4 (25.0)	3 (16.7)
Ever smoked?		
No	12 (75.0)	15 (83.3)
Yes	4 (25.0)	3 (16.7)
How much time do you usually spend in the sun on weekends and holidays in summer?		
< 30 min/day	1 (6.3)	1 (5.6)
30–60 min/day	2 (12.5)	3 (16.7)
1–2 h/day	6 (37.5)	4 (22.2)
2–3 h/day	5 (31.3)	4 (22.2)
3–4 h/day	2 (12.5)	4 (22.2)
4 or more hours/day	0	2 (11.1)
Age	35.0 (12.5; 19–51)	30.3 (11.8; 18–52)
Baseline 25(OH)D (nmol/L)	76.4 (27.3; 36–132)	60.5 (13.9; 32–78)

Results for categorical and dichotomous variables are presented at *n* (%). Results for age and baseline 25(OH)D are presented as mean (SD; range). Significance of difference in dichotomous or categorical variables between treatment arms is assessed by chi-square test. Significance of difference in continuous variables between treatment arms is assessed by Kruskal-Wallis test.
25(OH)D 25-hydroxyvitamin D, *nmol/L* nanomoles per litre.

Table 2 Treatment predicting infection hazard, infection duration and infection severity

		All infections	Infections seen in clinic	All URTIs	URTIs seen in clinic
		***n* = 98 (54 placebo; 44 treatment)**	***n* = 63 (34 placebo; 29 treatment)**	***n* = 68 (32 placebo; 36 treatment)**	***n* = 55 (28 placebo; 27 treatment)**
Infection hazard (HR (95% CI))	Placebo	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
	Treatment	0.83 (0.53, 1.31)	0.86 (0.50, 1.50)	1.11 (0.75, 1.65)	0.98 (0.59, 1.63)
		$P = 0.42$	$P = 0.60$	$P = 0.62$	$P = 0.94$
Infection duration (coefficient (95% CI))	Placebo	3.05 (2.35, 3.76)	4.15 (2.77, 5.53)	4.87 (3.29, 6.45)	5.28 (3.38, 7.18)
	Treatment	+1.04 (−0.25, 2.32)	+1.64 (−0.83, 4.11)	+0.51 (−1.80, 2.81)	+0.83 (−2.05, 3.70)
		$P = 0.11$	$P = 0.19$	$P = 0.67$	$P = 0.57$
Maximum infection symptom severity (coefficient (95% CI))	Placebo	2.19 (1.81, 2.57)	2.19 (1.69, 2.70)	2.41 (1.89, 2.93)	2.42 (1.85, 3.00)
	Treatment	+0.08 (−0.49, 0.65)	+0.19 (−0.58, 0.95)	+0.03 (−0.69, 0.76)	−0.05 (−0.85, 0.76)
		$P = 0.79$	$P = 0.63$	$P = 0.93$	$P = 0.91$
Total infection severity (coefficient (95% CI))	Placebo	12.26 (7.90, 16.62)	20.34 (9.01, 31.67)	25.47 (11.51, 39.43)	29.34 (12.28, 46.40)
	Treatment	+2.20 (−5.09, 9.49)	+5.95 (−13.15, 25.05)	−3.89 (−21.83, 14.06)	−2.87 (−25.70, 19.96)
		$P = 0.55$	$P = 0.54$	$P = 0.67$	$P = 0.81$
Average daily total infection symptom severity (coefficient (95% CI))	Placebo	4.00 (3.25, 4.75)	4.74 (3.52, 5.96)	5.28 (3.73, 6.82)	5.41 (3.81, 7.02)
	Treatment	−0.26 (−1.34, 0.82)	−0.22 (−1.97, 1.52)	−1.08 (−3.00, 0.85)	−0.91 (−2.98, 1.16)
		$P = 0.64$	$P = 0.80$	$P = 0.27$	$P = 0.39$

Infection hazard results are presented as HR (95% CI). Results for infection duration and severity are reported as geometric mean infection severity (95% CI) and then coefficient relative to the placebo group (95% CI). Note: a greater infection severity score or greater infection duration means a more severe infection or longer duration, respectively.

Table 3 Treatment predicting infections, for all persons and among those deficient in 25(OH)D at baseline at varying thresholds

			Number of participants with baseline 25(OH)D at level specified (%)	All infections		Infections seen in clinic	
				Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
All persons		Placebo	16 (47.1)	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
				0.83 (0.53, 1.31)	0.80 (0.50, 1.30)	0.86 (0.50, 1.50)	0.87 (0.52, 1.46)
		Treatment	18 (52.9)	$P = 0.42$	$P = 0.37$	$P = 0.60$	$P = 0.60$
25(OH)D threshold (nmol/L)	≤40	Placebo	2 (50.0)	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
		Treatment	2 (50.0)	0.56 (0.32, 0.96)[b]	0.41 (0.22, 0.78)[b]	0.27 (0.07, 1.00)[b]	0.26 (0.06, 1.17)
				$P = 0.036$[b]	$P = 0.007$[b]	$P = 0.050$[b]	$P = 0.079$
	≤50	Placebo	4 (50.0)	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
		Treatment	4 (50.0)	0.61 (0.38, 0.98)[b]	0.68 (0.34, 1.34)	0.48 (0.18, 1.30)	0.57 (0.18, 1.78)
				$P = 0.043$[b]	$P = 0.27$	$P = 0.15$	$P = 0.34$
	≤60	Placebo	4 (40.0)	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
		Treatment	6 (60.0)	0.86 (0.54, 1.35)	1.02 (0.60, 1.72)	0.66 (0.34, 1.30)	0.74 (0.36, 1.56)
				$P = 0.50$	$P = 0.95$	$P = 0.23$	$P = 0.43$
	≤70	Placebo	5 (29.4)	1.00 [reference]	1.00 [reference]	1.00 [reference]	1.00 [reference]
		Treatment	12 (70.6)	0.80 (0.51, 1.25)	0.82 (0.48, 1.41)	0.95 (0.46, 1.97)	0.85 (0.42, 1.70)
				$P = 0.33$	$P = 0.48$	$P = 0.89$	$P = 0.64$

Results are presented as HR (95% CI).
25(OH)D 25-hydroxyvitamin D, *nmol/L* nanomoles per litre.
[a]Analyses adjusted for age, sex, ever smoking and days per week engaging in vigorous physical activity.
[b]Statistically significant.

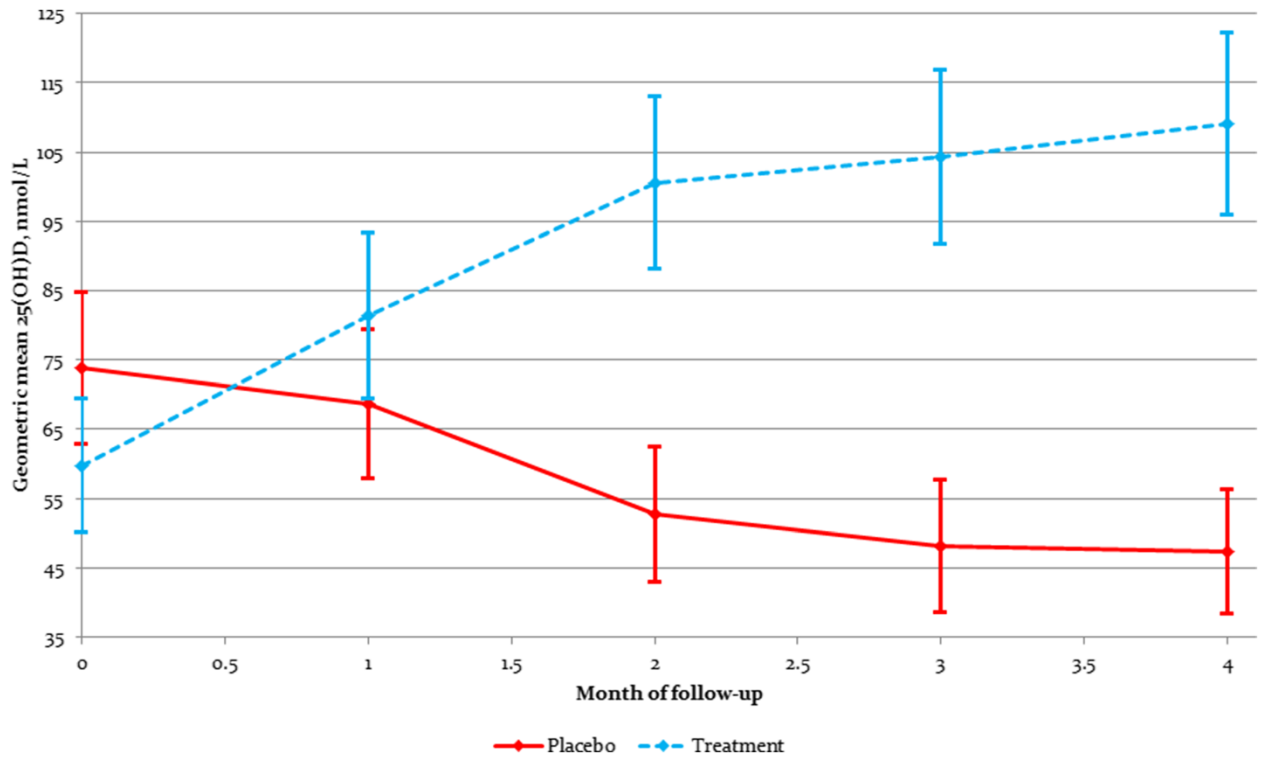

Figure 1 Trends in serum 25(OH)D by treatment group.

such that those of lower BMI (<24.9) realised a significant increase in 25(OH)D (mean increase: 30.0; 95% CI: 15.2, 44.8; $P = 0.001$), while those of higher BMI (25.0–29.9) realised a 21.2 increase (95% CI: 7.3, 35.0; $P = 0.003$), and those of the highest BMI (30 or greater) did not have a significant increase in their 25(OH)D from treatment (mean increase: 12.9; 95% CI: −14.6, 40.4; $P = 0.36$). While not statistically significant (test for difference: $P = 0.40$), the difference was appreciable and persisted on adjustment for age, sex and physical activity.

Interestingly, there was also a significant positive interaction between sex and treatment in predicting 25(OH)D, such that females realised nearly three times greater 25(OH)D from treatment (mean increase: 36.7; 95% CI: 23.0, 50.5; $P < 0.001$) than males (mean increase: 12.5; 95% CI: −3.4, 28.5; $P = 0.12$) (test for difference: $P = 0.04$), despite virtually identical levels by sex among those on placebo. This effect persisted on adjustment for age, physical activity and BMI.

Adverse events

No participants suffered from hypercalcemia or hyperphosphatemia as determined by blood test. No participants reported any symptoms indicative of adverse events.

Treatment guess

There was no evidence of loss of blinding (data not shown).

Discussion

In this pilot RCT, we have shown evidence of a protective effect of 20,000 IU/week cholecalciferol supplementation against acute infection among persons who were vitamin D deficient at baseline. Treatment realised significantly higher 25(OH)D compared to placebo. While no significant effect of treatment on infection was seen in the aggregate, among those who were vitamin D deficient at the start of the study, a significant protective effect from treatment was evident. Importantly, the magnitude of effect was enhanced on restriction to infections which were assessed in clinic, and associations disappeared on adjustment for serum 25(OH)D. A similar but less consistent interaction by level of baseline 25(OH)D was seen for infection severity. No treatment effect on infection duration was found. None of our cohort members experienced adverse effects, and the highest 25(OH)D was 154 nmol/L. Since our cohort was comprised of healthy adults in a Western nation, its generalisability extends to similarly situated Western healthy adults.

While no effect of treatment is apparent in the aggregate, a significant protective effect was present among those who were vitamin D deficient at baseline. This effect among the deficient is similar to a trial of vitamin D supplementation as an intervention against exacerbations of COPD, which found efficacy only among those with 25(OH)D < 25 nmol/L [24]. Ours is the first trial of

cholecalciferol supplementation showing this effect for acute infections in vitamin D-deficient healthy adults. Murdoch and colleagues examined their infection analyses by level of baseline 25(OH)D of 50 nmol/L but reported no significant interactions [20]. Rees and colleagues also examined treatment effect by level of baseline 25(OH)D but found no protective effect among the deficient, though this may be due to the low dose used in that trial (1,000 IU/day) [19].

The protective effect on acute infection was clearest and most pronounced for infection risk. A similar but less consistent effect was seen for infection severity. The inconsistency of the trends of associations may indicate that no association exists with severity. However, severity was necessarily reported by participants, and the variability between participants as to what is severe no doubt affected the quality of the measure.

While no effect of treatment was evident for infection duration, this is not unexpected. Our hypothesis was that vitamin D enables the effective responsiveness of innate immune system pathways and thus improves the ability of the body to block the colonisation by pathogens [4,25-30], so an effect of vitamin D on absolute infection risk was expected. Vitamin D also has the capacity to depress inflammatory immune pathways like IP-10 and IL-8 [31,32], enabling an effective but less inflammatory response. Thus, an effect on infection severity is expected from vitamin D sufficiency. Once a pathogen has already secured a hold, however, the duration of infection is a function of the efficiency of the immune response and the characteristics of the aetiologic pathogen and thus more independent of vitamin D status.

On evaluating the impact of treatment on serum 25(OH)D, some interesting differences were seen by level of BMI and by sex, such that treatment was less impactful on 25(OH)D among those of higher BMI, while females had a greater increase in 25(OH)D from treatment. The difference by BMI is not surprising, given the vitamin D hoarding of adipose tissue would keep serum 25(OH)D lower than might occur in those of lower BMI [33,34], and others have demonstrated this as well [35]. However, the difference by sex was unexpected and not easily explained. Certainly, it is possible that females have a differential vitamin D metabolism than males, as has been demonstrated by others [36].

Our study's methodology compares favourably with many of those undertaken previously. We designed our method to improve upon the designs reported in publications prior to when we undertook our study [14,18], which relied on passive detection. Our use of daily online follow-up to detect infections as close to the start of infection as possible, followed by objective assessment, was designed to allow an accurate and sensitive detection, while not affecting adherence. These methods are similar to that of Murdoch and colleagues [20] and to a lesser extent that of Rees and colleagues; however, these still relied on participant reporting. By placing the onus for detection on the study investigators, more infections may be detected closer to symptom onset. By relying on participants to report symptoms via daily questionnaire, the subjective nature of what is a reportable symptom is still a limitation.

Our hypothesis that vitamin D enhances the efficiency of the innate immune system to block colonisation by pathogens could not be evaluated due to the absence of pathogen sampling data. This is a limitation since it precluded our evaluation of pathogens present during infection or validation of infectious aetiology of symptoms. Further to this, our not obtaining valid nasal swab data to assess the aetiological pathogen and substantiate infection symptoms as a true infection is a limitation. The high frequency of infections during the study may indicate that some of the infections reported, particularly those not assessed in clinic, were not true infections. However, that we saw a potentiation in the treatment effect among the deficient on restricting to infections seen in clinic is supportive of a true effect, as this necessarily removes some symptoms which were not true infections. Another limitation is that our greatest impact of treatment was among a subgroup with only four people. While they comprised an appreciable proportion of our cohort (11.8%), this is nonetheless a small number. However, in addition to the statistical significance of this association, we think that the attenuation in association as the threshold of sufficiency is raised and that the treatment effect is enhanced on restriction to infections assessed in clinic are supportive of a true association, rather than artifact. Another measure that would have strengthened this study was to measure levels of activated vitamin D and antimicrobial peptides in the respiratory tract, which would have helped support the causality of a treatment effect. Finally, a small number ($n = 3$; two treatment, one placebo) reported taking multivitamins during the study, while four participants (all on placebo) started taking fish oil during the study (two at week 1, one at week 3, one at week 13), none of whom were also taking multivitamins. Consequently, there was some nontreatment supplementation of vitamin D, though of a relatively minor level (400 to 1,000 IU). Given the preponderance of fish oil use among controls, any impact of this would have been to reduce the difference in serum 25(OH)D over time, a reduction that if present was quite small indeed. Moreover, if there was a reduction in the spread of serum 25(OH)D between treatment arms, then potentially the observed reductions in infection risk may be an underestimate.

Conclusions

Our results from this pilot trial of weekly cholecalciferol supplementation vs. placebo among a cohort of 34 healthy

adults suggest a significant protective effect of treatment against acute infection risk among those which started the study vitamin D deficient. This effect persisted on adjustment for potential confounders, and the trends found on raising the threshold of deficiency/sufficiency and on restricting to infections seen in clinic are suggestive of a true effect. A larger study is needed to validate these results, and graded doses of supplementation are necessary to delineate the most optimal dose to realise a reduction in acute infection risk.

Additional files

Additional file 1: CONSORT 2010 checklist of information to include when reporting a randomised trial.

Additional file 2: CONSORT 2010 flow diagram.

Additional file 3: Treatment predicting symptom severity for infections seen in clinic, for all persons and among those deficient in 25(OH)D at baseline at varying thresholds. This supplemental table provides in detail the results for the three metrics of infection severity (maximum reported infection symptom severity for that infection, average daily total infection symptom severities, total sum of infection severity scores for all infection symptoms for each day of the infection and summated these for a total infection severity score) and stratified analyses by level of vitamin D sufficiency.

Abbreviations

25(OH)D: 25-hydroxyvitamin D; BMI: Body mass index; CIPRIS: Cholecalciferol Intervention to Prevent Respiratory Infections Study; IU: International unit; nmol/L: Nanomoles per litre; RCT: Randomised controlled trial; RTI: Respiratory tract infection.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The project was conceived and developed by SSJ, with the assistance of NS, BT, LB and IvdM. Funding was obtained by SSJ, NS, BT, LB and IvdM. The project was administered by SSJ. Statistical analysis was undertaken by SSJ, with the assistance of LB. Some additional analyses were undertaken by PT, including the development of participant correspondence letters reporting biological parameter tracking during the study follow-up. The initial manuscript draft was composed by SSJ, with the assistance of all authors in the revision process. All authors were involved in the critical revision of the manuscript and approved it for submission. SSJ had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis. Any persons interested in obtaining a copy of the trial protocol can do so by contacting SSJ.

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