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COMMENTARY

Vitamin D to prevent COVID-19: recommendations for the design of clinical trials

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The coronavirus disease 2019 (COVID-19) pandemic has focused attention on the potential role of vitamin D supplementation to prevent COVID-19. In this issue, Merzon and colleagues report epidemiologic data on the vitamin D status of 7807 individuals and their risk of developing COVID-19. In multivariable analyses, low vitamin D status was associated with increased risk of both COVID-19 infection and hospitalization. The authors call for clinical trials of vitamin D supplementation. In this Commentary, we discuss some of the challenges of vitamin D research and provide recommendations for the design of randomized controlled trials of vitamin D supplementation to prevent COVID-19.

Vitamin D and acute respiratory infection

The health effects of vitamin D have garnered attention for years. Beyond the established connection between vitamin D deficiency and rickets, many observational studies conducted in a diverse array of participants have shown that individuals with lower levels of 25-hydroxyvitamin D (25OHD), the best available marker of vitamin D status, are at higher risk of acute respiratory infection (ARI) [1]. These observational studies have led to randomized controlled trials (RCTs) of vitamin D supplementation, which have yielded 'mixed' results but have tended to support benefit among specific populations using specific dosing regimens. In 2017, we published an individual participant data meta-analysis involving 10 933 participants from 25 RCTs [2]. We reported an overall decrease in ARI (adjusted odds ratio, 0.88; 95% confidence interval, 0.81–0.96). In a recent update, using aggregate data from 29 841 participants from 39 RCTs [3], we observed similar results (adjusted odds ratio, 0.89; 95% confidence interval, 0.81–0.98). While the overall results indicate that vitamin D supplementation protects against ARI, we also found strong evidence

Abbreviations
25OHD, 25-hydroxyvitamin D; ARI, acute respiratory infection; COVID-19, coronavirus disease 2019; RCT, randomized controlled trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

of heterogeneity across the 39 trials, suggesting that the situation is more complicated than was initially appreciated.

The role of vitamin D in the COVID-19 pandemic

The ongoing COVID-19 pandemic has focused attention on the potential for vitamin D supplementation to prevent COVID-19 infection [4,5]. In this issue of *The FEBS Journal*, Merzon *et al*. [6] studied 7807 individuals who had at least one prior blood test for 25OHD and who were later tested for COVID-19. Overall, the mean plasma 25OHD was ~ 20 ng·mL^{-1} (or ~ 50 nM; multiply by 2.496 for conversion), and 782 (10.1%) cohort participants were COVID-19-positive. In multivariable analyses that adjusted for many potential confounders, low plasma 25OHD level was associated with increased risk of both COVID-19 infection and hospitalization. The authors of this large population-based observational study conclude that their findings 'could guide healthcare systems in identifying populations at risk and contribute to interventions aimed to reduce the risk of COVID-19 infection'.

The biological plausibility for vitamin D affecting risk of viral ARI, including COVID-19, is strong. Vitamin D is known to have modulatory and regulatory roles in many relevant processes, including host defense, inflammation, immunity, and epithelial repair [7]. Recently, Mok *et al*. [8] extended this line of research to SARS-CoV-2, the cause of COVID-19. They demonstrated for the first time that calcitriol, the active form of vitamin D, exhibits potent activity against SARS-CoV-2.

Designing randomized controlled trials

As we look ahead to clinical trials of vitamin D supplementation against COVID-19, it is important to first appreciate the distinction between prevention of COVID-19 (including severe COVID-19) and treatment of COVID-19. We focus on prevention here and, based on our experience with vitamin D supplementation to prevent ARI, we offer guidance for the design of trials to prevent COVID-19. Consideration of these design issues will greatly enhance the inferences that can be drawn from future trials, and may explain why some trials show benefit, while others do not.

Firstly, we encourage adherence to the CONSORT guidelines in the design of all RCTs [9]. Key features are concisely summarized in the Cochrane Collaboration Risk of Bias tool [10]. Vitamin D trialists also should consider the Heaney criteria for clinical studies of nutrient effects [11]. With regard to COVID-19, the relative infrequency of COVID-19 outcomes—even during pandemic conditions, with major health system challenges in the face of only 5–10% of population infected—suggests the importance of enrolling and retaining a large sample. We encourage all COVID-19 trialists to register their intent to study the effects of vitamin D supplementation beyond COVID-19 *per se*—that is, to also examine the prevention of ARIs in general.

Box 1. Considerations for RCTs on vitamin D supplementation for the prevention of ARI, including COVID-19.

1 Population (trial participants)
- Age-group (e.g., newborns *vs* elderly adults)
- Baseline vitamin D status (e.g., 25OHD level < 25 *vs* 75+ nM)
- Race/ethnicity (e.g., European white *vs* African black)
- Body mass index (e.g., adults < 25 *vs* 30+)
- Comorbidities (e.g., chronic diseases and immunodeficiencies)

2 Vitamin D intervention (dosing regimen)
- Frequency (daily *vs* less often)
- Initial bolus dose (yes/no)
- Regular dose (e.g., standard *vs* high; with amounts dependent on participant)
- Trial duration (e.g., 3 *vs* > 12 months)

3 Comparison intervention
- Placebo (true) = no vitamin D supplement nor change in sunlight exposure
- Placebo + 'allowance' of vitamin D usage (e.g., up to 800 IU/day)
- Different doses of vitamin D supplement (e.g., low *vs* high dose)
- None assigned (i.e., vitamin D intervention is open label)

4 COVID-19 outcomes
- SARS-CoV-2 positivity (e.g., nasal swab *vs* blood antibody)
- ARI outcomes (multiple), including specific focus on COVID-19
- Quality of outcome (e.g., clinical diagnosis/laboratory testing *vs* self-report)

Less obvious perhaps are the issues summarized in Box 1 and discussed briefly here.

1 Population (trial participants)

- Age-group (e.g., newborns *vs* elderly adults). While prevention of COVID-19 is important in all age-groups, the emphasis might differ by age. For example, RCTs in young children might focus on prevention of SARS-CoV-2 positivity, while RCTs in older adults might focus on the prevention of severe COVID-19. Investigators should always prespecify their subgroups of interest.
- Baseline vitamin D status (e.g., 25OHD level <25 *vs* 75+ nM). For all types of ARI, including COVID-19, we believe it is helpful to focus on individuals with low baseline vitamin D status. There are, however, ethical challenges of enrolling vitamin D-deficient individuals into a placebo-controlled trial of longer duration; presumably, these individuals should receive vitamin D treatment for their newly diagnosed deficiency state (disease). For this reason, baseline 25OHD measurements are often deferred until trial completion—a practice that also permits enrollment of individuals with adequate vitamin D status. Algorithms to identify individuals at risk of low vitamin D status may help. Unfortunately, there is no easy solution for this conundrum.
- Race/ethnicity (e.g., European white *vs* African black). Given the strong links between race/ethnicity and vitamin D status (and COVID-19), it is critical to include individuals of diverse racial/ethnic backgrounds.
- Body mass index (e.g., < 25 *vs* 30+). Likewise, trials should strive to enroll individuals across the spectrum of body mass index.
- Comorbidities (e.g., chronic diseases and immunodeficiencies).

2 Vitamin D intervention (dosing regimen)

- Frequency (daily *vs* less often). Based on our recent meta-analysis [3], we strongly encourage daily dosing. While this may lower protocol fidelity (as compared to less frequent dosing), daily dosing appears critical for the anti-infective effects of vitamin D supplementation.
- Initial bolus dose (yes/no). While an initial bolus can accelerate the intervention-induced increase in vitamin D status, at least per blood 25OHD levels, we discourage this practice based on the ARI prevention trials [2].
- Regular dose (e.g., standard *vs* high; with amounts dependent on participant). Based on our recent meta-analysis [3], we encourage 'standard' dosing (i.e., 400–1000 IU daily). We speculate that obese adults may require higher doses (e.g., up to 2000 IU/day) but caution that 'more' is not always better!
- Trial duration (e.g., 3 *vs* > 12 months). We encourage a focus between 4 and 12 months. Shorter trials risk assessing impact of the vitamin D intervention before blood 25OHD stabilizes (at ~ 2 months). Longer trials (12+ months) capture seasonal variation in the outcome but may suffer from lower protocol fidelity (e.g., from participants missing assigned doses, or placebo group starting vitamin D and thereby contaminating the comparison).

3 Comparison intervention

- Placebo (true) = no vitamin D supplement nor change in sunlight exposure. While such trials were permissible years ago, the likely health benefits of treating vitamin D deficiency make this challenging today. For ethical reasons, true placebo-controlled trials need to be shorter (e.g., 4 months), with vitamin D supplementation given to everyone who is identified as deficient during end-of-trial testing of frozen baseline blood samples.
- Placebo + 'allowance' of vitamin D usage (e.g., up to 800 IU/day). If most participants take the allowed amount, the RCT effectively turns into a comparison of lower-dose *vs* higher-dose vitamin D. The advent of mega trials in the late 20th century was a major research advance, but those participants usually could not access the intervention (e.g., thrombolytic agents for acute myocardial infarction). In vitamin D supplement trials, participants can simply take a multivitamin to 'hedge their bet'—or just spend more time outside. Ideally, vitamin D trials would record baseline intake of vitamin D (all sources, including sunlight exposure) and monitor this potential co-intervention during the trial.
- Different doses of vitamin D supplement (e.g., low *vs* high dose). While these trials address important dose–response questions, they may yield a 'null' result because vitamin D is ineffective against ARI—or because both doses work!
- None assigned (i.e., vitamin D intervention is open label). This practical design forgoes blinding. The aforementioned 'allowance' issue also may apply.

4 COVID-19 outcomes

- SARS-CoV-2 positivity (e.g., nasal swab *vs* blood antibody). If feasible, we recommend a focus on PCR-confirmed nasal swabs, regardless of symptoms. We also recommend batch analysis of end-of-trial blood samples using a high-quality antibody test [12].

- ARI outcomes (multiple), including specific focus on COVID-19. In addition to a simple yes/no outcome, we encourage a focus on severity of illness. Severe COVID-19 might include potentially overlapping outcomes—for example, hospitalization, intensive care, or death.
- Quality of outcome (e.g., clinical diagnosis/laboratory testing *vs* self-report). A weakness of most vitamin D trials on ARI (which includes the 'common cold') is their reliance on self-report. We strongly encourage a focus on validated outcomes. When self-report is required, more frequent questions are better for ascertainment than, for example, asking once at end of the trial; the latter approach will tend to drive results toward the null.

Conclusion

Randomized controlled trials are the optimal study design for causal interpretation, but they have their challenges. At best, RCTs provide answers to very precise questions—but clinical management and public health decisions often require extrapolation beyond the tight confines of any given trial. Accordingly, we believe that all types of research (basic, clinical/translational, and population) can provide valuable information. Taken together, they can provide evidence-based guidance for clinicians and public health leaders. As investigators design and implement RCTs of vitamin D supplementation to prevent COVID-19, we encourage consideration of the issues raised here. Likewise, we encourage a greater tolerance for 'mixed' results given the likely heterogeneity of future trial designs—and the inherent challenges of RCT research on vitamin D.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

CAC and ARM conceived, designed, and wrote the Commentary.

References

1 Camargo CA Jr (2018) Vitamin D, acute respiratory infection, and asthma/chronic obstructive pulmonary disease. In Vitamin D, 4th edn. (Feldman D, Pike JW, Bouillon R, Giovannucci E, Goltzman D & Hewison M, eds), pp. 1096–1120. Elsevier Academic Press, Cambridge, MA.

2 Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA *et al.* (2017) Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* **356**, i6583.

3 Jolliffe DA, Camargo CA Jr, Sluyter J, Aglipay M, Aloia J, Bergman P, Damsgaard C, Dubnov-Raz G, Esposito S, Ganmaa D *et al.* (2020) Vitamin D supplementation to prevent acute respiratory infections: systematic review and meta-analysis of aggregate data from randomised controlled trials. *medRxiv*. [preprint] version posted July 17, 2020. doi: https://doi.org/10.1101/2020.07.14.20152728.

4 Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL & Bhattoa HP (2020) Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients* **12**, 988.

5 Lanham-New SA, Webb AR, Cashman KD, Buttriss JL, Fallowfield JL, Masud T, Hewison M, Mathers JC, Kiely M, Welch AA *et al.* (2020) Vitamin D and SARS-CoV-2 virus/COVID-19 disease. *BMJ Nutr Prev Health* **3**, 106–110.

6 Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I & Frenkel Morgenstern M (2020) Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *FEBS J* **287**, 3693–3702.

7 Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL & Stanciu LA (2017) Vitamin D modulation of innate immune responses to respiratory viral infections. *Rev Med Virol* **27**, e1909.

8 Mok CK, Ng YL, Ahidjo BA, Hua Lee RC, Choy Loe MW, Liu J, Tan KS, Kaur P, Chng WJ, Eu-Li Wong J *et al.* (2020) Calcitriol, the active form of vitamin D, is a promising candidate for COVID-19 prophylaxis. *bioRxiv*, [preprint] version posted June 22, 2020. doi: 10.1101/2020.06.21.162396.

9 Schulz KF, Altman DG, Moher D & for the CONSORT Group. (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials. *BMJ* **2010**, c332.

10 Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L & Sterne JAC (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* **343**, d5928.

11 Heaney RP (2014) Guidelines for optimizing design and analysis of clinical studies of nutrient effects. *Nutr Rev* **72**, 48–54.

12 Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui L-P, Johnston JC, Lan Z, Law S, MacLean E, Trajman A *et al.* (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ* **370**, m2516.