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Vitamin D and COVID-19—Revisited

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Vitamin D, when activated to 1,25-dihydroxyvitamin D, is a steroid hormone that induces responses in several hundred genes, including many involved in immune responses to infection. Without supplementation, people living in temperate zones commonly become deficient in the precursor form of vitamin D, 25-hydroxyvitamin D, during winter, as do people who receive less sunlight exposure or those with darker skin pigmentation. Studies performed pre-COVID-19 have shown significant but modest reduction in upper respiratory infections in people receiving regular daily vitamin D supplementation. Vitamin D deficiency, like the risk of severe COVID-19, is linked with darker skin colour and also with obesity. Greater risk from COVID-19 has been associated with reduced ultraviolet exposure. Various studies have examined serum 25-hydroxyvitamin D levels, either historical or current, in patients with COVID-19. The results of these studies have varied but the majority have shown an association between vitamin D deficiency and increased risk of COVID-19 illness or severity. Interventional studies of vitamin D supplementation have so far been inconclusive. Trial protocols commonly allow control groups to receive low-dose supplementation that may be adequate for many. The effects of vitamin D supplementation on disease severity in patients with existing COVID-19 are further complicated by the frequent use of large bolus dose vitamin D to achieve rapid effects, even though this approach has been shown to be ineffective in other settings. As the pandemic passes into its third year, a substantial role of vitamin D deficiency in determining the risk from COVID-19 remains possible but unproven.

Keywords: COVID-19, vitamin D

Introduction

It was recognised early during the COVID-19 pandemic that many of the phenotypic markers of poor prognosis were also known correlates of vitamin D deficiency [1]. Obesity and darker skin colour both associate with increased risk of vitamin D deficiency. Old age is the strongest risk factor for poor COVID-19 prognosis, to a much greater extent than seen with other viral pandemics such as influenza. Although old age is not itself consistently associated with increased risk of vitamin D deficiency, living in a care home certainly is, unless the residents get out in the summer sunshine—which is almost never the case in UK care settings—or receive regular vitamin D supplements. Similarly, incarceration in prison is also associated with increased risk for vitamin D deficiency, although the England & Wales Prison Service did begin offering daily vitamin D 25 micrograms to its inmates from September 2020. It is almost impossible to obtain sufficient vitamin D from dietary sources alone, and most vitamin D is derived from synthesis in the skin by the action of ultraviolet B (UVB; wavelength 315–280 nm), which breaks a carbon-to-carbon bond in the 7-hydroxycholesterol precursor. UVB is largely removed during passage through the earth's ozone layer, and also by particulate air pollution, so vitamin D can only be synthesised when the sun is high in the sky. In the Northern Hemisphere at more than 35 degrees

latitude, sufficient UVB for vitamin D synthesis can only be obtained under cloudless skies during the late morning and early afternoon from March to September, and deficiency becomes increasingly common as winter progresses.

After synthesis in the skin, vitamin D undergoes two metabolic conversions. The first, which occurs mainly in the liver, produces 25-hydroxyvitamin D (25[OH]D), the main circulating form of vitamin D and the metabolite of vitamin D that is most commonly measured in laboratories around the world. However, 25(OH)D is an inactive form of vitamin D that requires further metabolism to produce 1,25-dihydroxyvitamin D (1,25[OH]2D), a secosteroid hormone that acts by binding to ubiquitous nuclear vitamin D receptors (VDR) that regulate gene transcription. Gene promoter vitamin D response elements for the 1,25(OH)2D-VDR complex are found in around 5% of genes in the human genome. There is extensive in vitro and in vivo evidence for a role of 1,25(OH)2D in the regulation of both innate and adaptive immune responses to bacterial and viral infection [2]. Circulating levels of 1,25(OH)2D are dependent on the metabolism of 25(OH)D by the kidney, but within the immune system 1,25(OH)2D can be synthesised by cells such as macrophages and dendritic cells. Localised vitamin D metabolism is then able to drive antimicrobial innate immunity in an intracrine fashion depending on the availability of 25(OH)D for conversion to 1,25(OH)2D and may thus be compromised in the setting of vitamin D deficiency [3]. Beyond antimicrobial actions, 1,25(OH)2D also promotes potent anti-inflammatory effects on T lymphocytes (T cells) that protect against possible tissue damage following infection. This was initially thought to involve a paracrine effect via 1,25(OH)2D synthesised by macrophages or dendritic cells but recent studies of T cells from patients with COVID-19 have shown that T cells themselves are able to convert 25(OH)D to 1,25(OH)2D [4]. As a result, it now appears that anti-inflammatory adaptive immunity is also dependent on 25(OH)D availability and will therefore be compromised under conditions of vitamin D deficiency.

A causal association between vitamin D deficiency and the risk or severity of COVID-19 is therefore entirely plausible. It has, however, been pointed out by government advisory bodies such as the UK National Institute for Health and Care Excellence (NICE) that the evidence supporting a role of vitamin D is mainly circumstantial and lacks the high-quality randomised control trial evidence nowadays regarded as essential to justify therapeutic interventions. NICE has concluded that clinicians should not 'offer a vitamin D supplement to people solely to prevent COVID-19, except as part of a clinical trial' and that 'Randomised controlled trials in all care settings with a minimum 8-week follow up are recommended' [5]. Consequently, governmental messaging advocating avoidance of vitamin D deficiency in winter has been muted. However, at the time of writing, we are well over 24 months into the pandemic and large, high-quality randomised trials of vitamin D supplementation have yet to be published. There are several reasons for this. Randomised controlled trials (RCTs) of vitamins or hormones are very hard to perform, partly because supplementation is unlikely to have any benefit in people who are already replete. The 'ideal' study would recruit people with known vitamin deficiency and then ask them to be randomised to vitamin supplement or placebo, but this is unethical as anyone with vitamin D deficiency should routinely be supplemented. Studies are therefore comparing high-dose vitamin D with a low dose as a control, but there is good evidence that a regular daily low-dose supplement, up to 1000 IU or 25 micrograms per day, may be at least as effective and possibly more effective than a higher dose at reducing risk for respiratory infection [6]. Perhaps because nutrition research is currently less 'fashionable' than other research areas such as genetic engineering or systems biology, it has also proven difficult to obtain funding for trials of vitamins. The Wellcome Trust/Bill Gates/Mastercard COVID-19 Therapeutics Accelerator fund, which has generated around 125 million US dollars towards COVID research, expressly excluded vitamin research as 'out of scope' from funding [7]. Consequently, it is quite likely that we will never obtain high-quality RCT evidence to support or refute the role of vitamin D in determining COVID-19 outcomes. This does not mean though that there is no evidence worth considering, particularly given that vitamin D deficiency is very common and easily preventable and that vitamin D supplementation is extremely cheap, very safe unless taken in great excess and anyway likely to have beneficial effects on bone health in many individuals.

The associations between vitamin D status and COVID-19 prognostic factors were reviewed previously in this journal [1] and the interactions of vitamin D with the immune system have also been

thoroughly reviewed elsewhere [8]. The present review, therefore, focuses on evidence published since the start of the pandemic for/against an effect of vitamin D status in determining the infection risk and severity of COVID-19.

Methods

This is a narrative review but informed by a PubMed literature search that included as search terms 'COVID-19 and vitamin D' and with emphasis on reports published since January 2021.

Seasonality, associations between latitude, UV exposure and COVID-19 outcomes

SARS-CoV-2, the causative organism of COVID-19, is a coronavirus and coronaviruses, like other respiratory viruses such as influenza and respiratory syncytial virus, tend to be seasonal [9]. Although COVID-19 infections have been prevalent throughout the year, there is good evidence for an impact of seasonality on the risk for infection and severity [10]. The mechanisms underlying seasonality are not clearly understood but one possible factor is UV light exposure, acting either via vitamin D synthesis or by some other effect such as direct viral killing on sun-exposed surfaces. In contrast, changes in temperature and humidity do not appear to have an impact consistently.

It was noticed early in the pandemic that there was an association between latitude and COVID-19 mortality. Although much of this could be accounted for by the relatively low average age of populations living close to the equator, a significant association between latitude and COVID-19 mortality per head of population remained after adjustment for this [11]. Further studies have looked in more detail at the impact of UV exposure on COVID-19 risk. Most, but not all, studies have shown a significant negative association [12].

A study of 417,342 participants from the UK Biobank cohort found that ambient UVB, measured over the previous 135 days, although not associated with COVID-19 infection risk, was strongly and inversely associated with hospitalisation ($p < 2 \times 10^{-16}$) and death ($p < 2 \times 10^{-16}$) in a multivariable analysis that adjusted for various factors including age, gender and BMI. Median UVB (kJ/m^2) in those who died (43.09, interquartile range [IQR] 31.89–74.10) was less than half that in those with COVID-19 not requiring hospital admission (90.89, IQR 67.43–98.95) [13].

A study based on the large US Nurses Health cohort had similar findings. Data from 39,315 participants in periodic studies within Nurses Health II from May 2020 to March 2021 showed that participants in the highest quartile for average annual UVB exposure based on the state of residency had a lower risk of SARS-CoV-2 infection compared with the lowest quartile (multivariable-adjusted odds ratio [OR] 0.76, 95% confidence intervals [CI] 0.66, 0.87; *p*-trend 0.002) [14] (Fig. 1). Winter-time UVA (400–315 nm) exposure, which would not induce vitamin D synthesis, also showed a similar size effect (OR 0.76, 95% CI 0.66, 0.88; *p*-trend <0.001), but this was not adjusted for annual UVB exposure, with which it was likely associated. A separate study, also performed in the United States, has, however, also shown an association between winter UVA exposure and reduced COVID-19 mortality, thought possibly to be mediated via cutaneous release of nitric oxide [15].

Nationwide and global studies have also investigated associations between UV exposure and COVID-19 infection rates. A study of SARS-CoV-2 transmission from March to December 2020 across 2669 US counties calculated that the fractions of the SARS-CoV-2 reproduction number (R_t) attributable to cold temperature, reduced specific humidity and lower UV radiation were 3.73%, 9.35% and 4.44%, respectively. UVB and UVA were not separately measured [16].

A global analysis conducted up until late April 2020 estimated the daily reproduction number at 3739 global locations and found a significant negative association with higher temperature (>27.5°C) and a U-shaped relationship with outdoor UV exposure [17]. Another study performed over approximately the same time period included data from 3235 regions across 173 countries [18]. No association was found between COVID-19 growth rates and either temperature or humidity but there was again a significant negative association with UV radiation. A one standard deviation (SD) increase in ambient UV was accompanied by approximately a one percentage point reduction in daily growth rate over the subsequent 2.5 weeks compared with an average growth rate of 13.2%. A direct effect of UV on the virus in the environment is likely but the time scale of the response, peaking in magnitude after 9–11 days, is also compatible with vitamin D synthesis and subsequent activation in response to dermal UV exposure

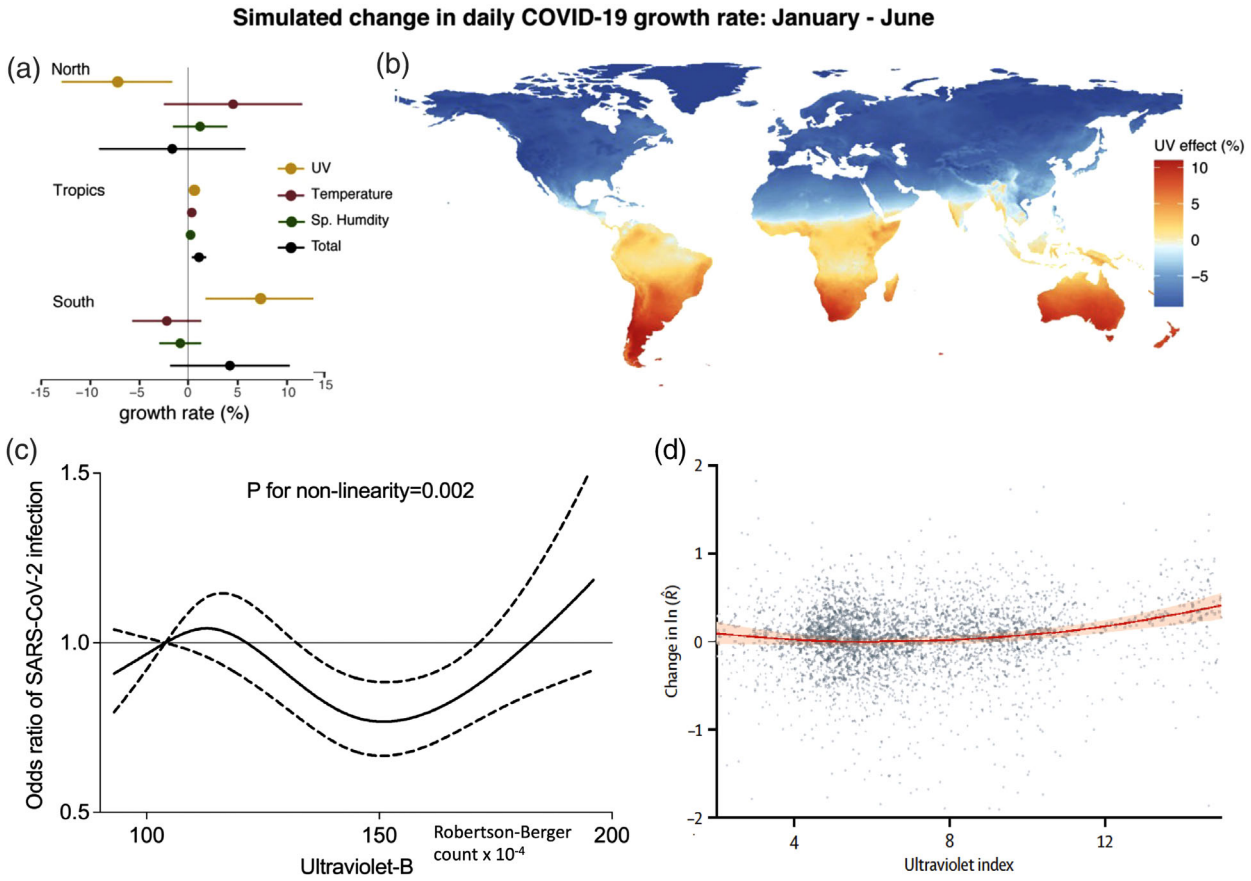

Fig. 1 *Impact of ultraviolet (UV) exposure on COVID-19 growth rates. (a) Modelling based on a global dataset of daily COVID-19 cases and local environmental conditions found that increased daily UV radiation lowers the cumulative daily growth rate of COVID-19 cases over the subsequent 2.5 weeks whereas impacts of temperature and humidity were not significant. (b) Map of the influence of expected seasonal changes in UV alone on the COVID-19 growth rate from January to June. (from Carleton et al. [18] with permission). (c) Modelling using data from periodic sampling of 39,315 participants (1768 Sars-CoV2 positive) within the US Nurses Health Study II. A significant (U-shaped) relationship is shown between predicted UVB exposure and SARS-CoV-2 infection rates. Average annual UVB exposure was based on the state of residence. The figure shows restricted cubic spline smoothing for the relationship between regional UVB and risk of SARS-CoV-2 infection compared with the lowest quartile median UVB (Robertson–Berger count × 10^{-4}) as the reference and adjusted for age; White race; smoking pack-years; the Alternate Healthy Eating Index (quintiles); body mass index; physical activity, alcohol intake; being a frontline healthcare worker; chronic comorbidities including hypertension, hypercholesterolaemia, diabetes, heart disease, cancer and asthma; and 2010 census tract median income (from Suppl Fig. S3, Ma et al. [16] with permission). (d) Daily reproduction number (R) for SARS-CoV-2 infection at 3739 global locations between December 2019 and April 2020 showing a U-shaped relationship with outdoor UV exposure (from Fig. 2B, Xu et al. [17] with permission).*

Vitamin D status as a possible explanation for differing national mortalities from COVID-19

There are marked differences in COVID-19 mortality between countries and many of these cannot be accounted for by latitude or the age of the population. There are of course many other possible determinants of COVID-19 outcome including vaccine availability and uptake, government responses affecting social distancing and mask wearing, ethnicity, deprivation and population density. Moreover, latitude is not the only determinant of vitamin D status. Cloud cover, atmospheric pollution, supplementation, fortification, clothing and social customs and occupations impacting on sunlight exposure will all have an impact. It was noted early in the pandemic that there was some correlation between COVID-19 mortality by country and

historical vitamin D status [19]. A more recent study across 47 European and Asian countries has confirmed this association (r = 0.35; p = 0.016) [20].

Several of the Nordic countries have impressively low COVID-19 mortalities and a recent estimation of overall excess mortality rates during the pandemic supports this [21]—in Iceland, excess mortality per 100,000 is (minus)47.8 (95% uncertainty intervals −107.1 to 1.6), Norway 7.2 (−0.0 to 15.9) and Finland 80.8 (66.2–94.0) in comparison with the much higher excess mortalities seen in the UK, 126.8 (122.3–130.9), and across Western Europe as a whole, 140.0 (133.5–146.3). Even before the pandemic, Norwegians maintained healthy vitamin D levels through a high intake of vitamin D, either through regular daily consumption of a teaspoonful of cod liver oil, other vitamin D supplements, fortification of dairy products or frequent consumption of oily fish. Blood levels of vitamin D in Norwegians consequently have been shown to vary relatively little from the end of winter (average 58 nmol/L) to the end of summer (average 69 nmol/L) [22] whereas in the UK, average blood levels in White men fall by about 50% from their peak (average 70 nmol/L) in September to a low point in February at an average of around 35 nmol/L—well below the 50 nmol/L 'sufficiency' level and dangerously close to the 25 nmol/L 'severe deficiency' level [23]. Icelanders, like Norwegians, have a strong tradition of supplementing vitamin D to prevent deficiency in winter [24] and have an even lower COVID-19 mortality. In Finland, an active policy of food fortification with vitamin D has led to a massive improvement in vitamin D status in recent years [25].

Some countries close to the Equator have suffered high COVID-19 mortalities. These include, particularly, countries in central and southern America such as Peru, with an estimated excess mortality of 528.6 (497.5–556.4), Brazil 186.9 (172.2–199.8), and Ecuador 333.4 (315.1–348.0) [21]. Perhaps surprisingly, these countries do, however, have quite high rates of vitamin D deficiency (defined here as <50 nmol/L). A study of teenagers in Peru found that 28% were vitamin D deficient and a smaller study in Peruvian adults performed in June 2016 reported deficiency in 46% of 144 adults from an impoverished community [26]. A study of 2374 older adults living in Ecuador showed that 22% were vitamin D deficient, particularly those living in mountainous regions [27]. Similarly, a study of 39,004 Brazilians of all ages found deficiency in 34% with marked seasonal variation [28].

Relevance of vitamin D status to immune function—Evidence from COVID-19

As outlined earlier in this review, studies published before the pandemic have extensively documented interactions between vitamin D and the immune system. These are reviewed at length elsewhere [1, 8]. VDR are ubiquitously expressed by immune cells and their effects include downregulation of inflammatory cytokines, induction in macrophages and epithelial cells of the antimicrobial peptide cathelicidin and promotion of differentiation of regulatory T cells. Vitamin D also induces expression of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 receptor. This is particularly relevant since ACE2, by hydrolysing angiotensin II, has a protective effect against the development of acute respiratory distress syndrome. In experimental mouse models of lung damage, vitamin D deficiency or VDR knockout both result in greatly increased lung damage in response to intratracheal bacterial lipopolysaccharide [1].

A set of key experiments performed on bronchoalveolar lavage T cells from patients with severe COVID-19 has provided direct evidence for the relevance of vitamin D to immune defence against SARS-CoV-2 [4]. In these studies, single-cell RNA sequencing of COVID-19 pulmonary T-helper T cells showed upregulation of genes that regulate type 1 T helper (T_H1) cells that are likely to be pro-inflammatory and also showed derepression of genes normally downregulated by vitamin D. Addition of either 1,25(OH)2D or 25(OH)D repressed interferon gamma production and induced the anti-inflammatory cytokine IL-10. The efficacy of 25(OH)D, as well as 1,25(OH)2D, in achieving this confirmed the ability of the activated T cells to synthesise their own 1,25(OH)2D from 25(OH)D and suggested that this effect is likely to be impaired in subjects with vitamin D (25(OH)D) deficiency. The authors also noted that corticosteroids such as dexamethasone could induce expression of VDR and speculated that there could be a beneficial synergistic interaction between dexamethasone and vitamin D.

Recent studies have also examined the relationship between the immune response to SARS-CoV-2 vaccination and serum 25(OH)D levels with contradictory results. In a cohort of healthy German adults,

SARS-CoV-2 IgG antibody responses and neutralisation potency along with 25(OH)D concentrations were analysed for 24 weeks from the time of vaccination [29]. No significant differences were found in the dynamic increase or decrease of SARS-CoV-2 IgG as a function of 25(OH)D status. In contrast, a study of UK healthcare workers reported that antibody response to immunisation was significantly affected by vitamin D status with a 29.3% greater peak antibody response in individuals with 25(OH)D >50 nmol/L [30].

Identifying the optimal blood concentrations and supplement dosing strategy for vitamin D

Before considering the evidence linking vitamin D status with COVID-19 outcomes, it is necessary to understand what constitutes a healthy vitamin D status and what is likely to be an effective form of supplementation to prevent the consequences of deficiency. Regrettably, these questions do not have unequivocal answers.

Vitamin D status is usually assessed by measuring blood levels of 25(OH)D. This is because blood levels of the fully activated 1,25(OH)2D are too low to be easily measured; moreover, many cells, including most immune cells, express the 25(OH)D-1α-hydroxylase (1α-hydroxylase) enzyme that produces 1,25(OH)2D and are therefore able to complete activation of vitamin D independent of the circulating levels of 1,25(OH)2D. It is important to remember that 1,25(OH)2D is a steroid hormone. It follows that too much vitamin D, as well as too little, is likely to be harmful and, whilst hypercalcaemia is an easily diagnosed and well-recognised consequence of extreme vitamin D toxicity, there might be other much subtler consequences of too much vitamin D. It is frustrating that a 100 years on from the discovery of vitamin D, there is still disagreement about both the lower and higher limits of a healthy blood 25(OH)D concentration. There is a reasonably strong consensus that 50 nmol/L is an appropriate lower limit. This is supported by the US Institute of Medicine and by the European Union Food Safety Authority [31]. The UK Scientific Advisory Committee on Nutrition has set a lower limit of 25 nmol/L, albeit without clearly documented evidence to support this and there are many published cases of the bone disease rickets with blood 25(OH)D levels between 25 and 50 nmol/L [32]. The US Endocrine Society has set a higher threshold of 75 nmol/L for sufficiency, by which standard a majority of the world's population would be judged insufficient. This higher level is based partly on the relationship between 25(OH)D levels and parathyroid hormone concentration as well as calcium absorption, and also on post-mortem studies of relationships between 25(OH)D and the presence of uncalcified osteoid suggesting that plateauing of its effect may not occur until 25(OH)D concentration reaches at least >75 nmol/L [33, 34]. The US Institute of Medicine concluded that there was insufficient clinical evidence of benefit above 75 nmol/L. It also set an upper level of 125 nmol/L, above which there was 'reason for concern' [31], although levels in excess of this are regularly achieved in populations having high sunlight exposure to bare skin such as beach lifeguards and traditional herders.

There is also controversial literature (pre-COVID-19) suggesting a possible 'U-shaped' (or 'reverse-J') curve for the relationship between blood 25(OH)D levels and clinical outcomes, most importantly all-cause mortality. A Danish community-based study of 247,574 subjects found a reverse J-shaped association between blood 25(OH)D and all-cause mortality, with the lowest mortality for those at 50–60 nmol/L, although arguably their data could be interpreted as showing low mortality across a broader optimal range of 50–75 nmol/L [35] (Fig. 2). A study of 24,094 hospital in-patients from Boston, United States, showed a U-shaped relationship between prehospitalization 25(OH)D and 90-day all-cause mortality but inferred a much broader optimal range of 25(OH)D 50–150 nmol/L [36]. An individual participant data meta-analysis across 26,916 individuals from eight European prospective studies also showed increased all-cause mortality below 50 nmol/L but no significant increase at high levels up to 125 nmol/L [37]. Similarly, a study of 365,530 participants in the UK Biobank cohort showed no evidence of a U-shaped curve for serum 25(OH)D and all-cause mortality [38].

Optimum dosing regimens for vitamin D supplementation will depend on the target 25(OH)D blood level. The UK SACN recommendation of 400 IU/day for adults in the winter months aims to ensure that at least 97.5% of the population receiving this will attain a blood level of at least 25 nmol/L [39]. If, however, the more widely accepted target level of at least 50 nmol/L is chosen, then a higher daily dose, for example, 600–800 (for the elderly) IU/day as recommended by the US Institute of Medicine [31], is needed and some data

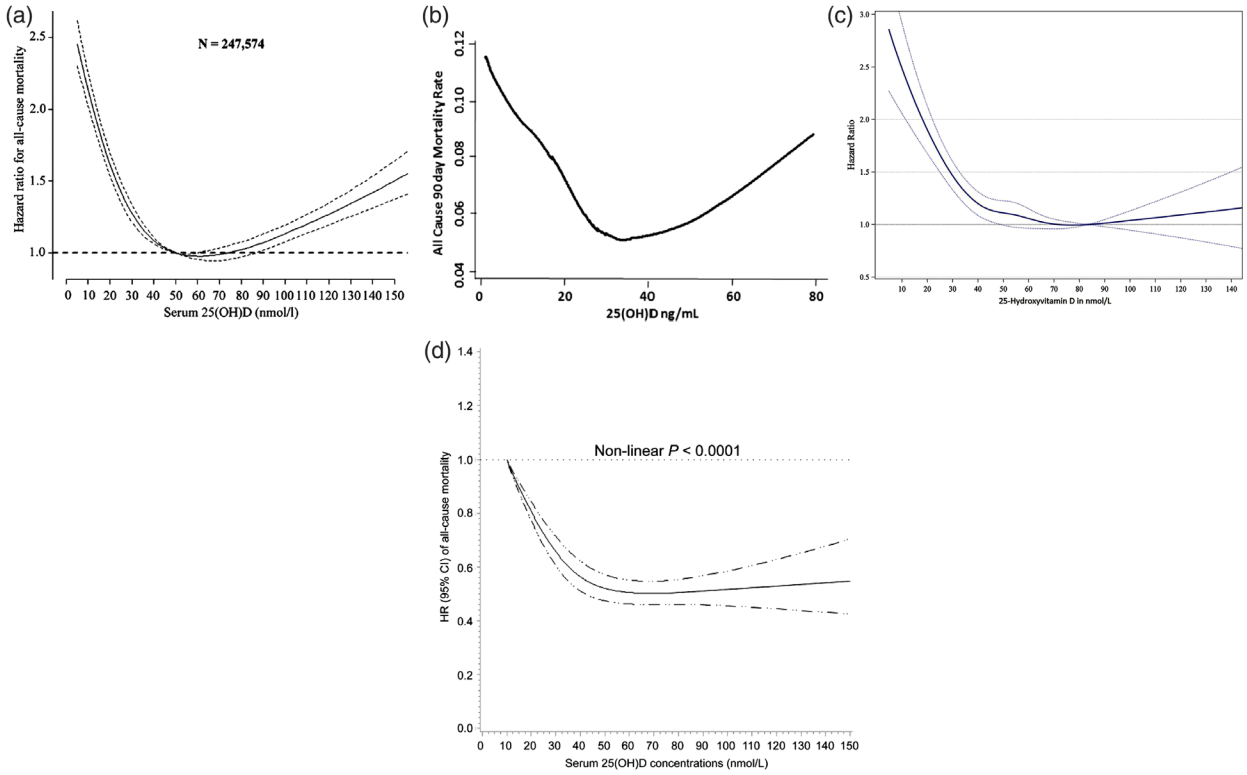

Fig. 2 *Relationships between serum 25(OH)D concentration and all-cause mortality pre-COVID-19. (a) Relationship between serum 25(OH)D and all-cause mortality in 247,574 subjects, mean age 51, from the Copenhagen general practice sector with median follow-up 3.07 years during which 15,198 (6.1%) died (from Durup et al. [35] with permission). (b) Relationship between pre-hospital serum 25(OH)D and risk of 90-day mortality after hospital admission in a retrospective cohort of 24,094 adult inpatients admitted to two Boston, United States, teaching hospitals (20 ng/ml is equivalent to 50 nmol/L) (from Amrein et al. [36] with permission). (c) Relationship between the hazard ratio for all-cause mortality and standardised 25(OH)D concentration in 26,916 participants (median age 61.6 years) pooled from eight prospective cohort studies with a median follow-up of 10.5 years during which 6802 died. Data were adjusted for age, sex, body mass index and season of blood drawing concentrations. No significant U-shaped relationship is shown (from Gaksch et al. [37] with permission). (d) Relationship between the hazard ratio for all-cause mortality and serum 25(OH)D concentration in 365,530 participants from the UK Biobank with a median follow-up of 8.7 years during which 10,175 died. Multivariate Cox regression model based on restricted cubic splines. Again, no U-shaped relationship is shown (from Fan et al. [38] with permission).*

would suggest a slightly higher dose still, around 1000 IU/day [40]. This assumes a 'one dose fits all' policy, which would be much cheaper than tailored dosing according to blood 25(OH)D measurements, although it has been pointed out that it might imply reaching an average 25(OH)D level of 90 nmol/L to ensure that almost all supplemented people achieve >50 nmol/L [31]. Obesity is a major factor determining the need for a higher regular dose to achieve sufficiency. The mechanisms behind obesity negatively affecting serum 25(OH)D are not well defined but both sequestration of vitamin D in fat stores and reduced hepatic 25-hydroxylation have been suggested as possible explanations [1].

An even more important issue than the size of dose is that of daily dosing versus intermittent 'bolus' dosing. In recent years, intermittent high-dose bolus supplementation of vitamin D, without intervening maintenance dosing, has gained traction, both in routine clinical practice and in RCTs. Bolus replacement achieves satisfactory blood levels of 25(OH)D without obvious toxicity [41]. There is, however, growing evidence, now substantial, that this strategy is probably ineffective or even

harmful [42]. Recent evidence comes from a negative RCT of bolus vitamin D (100,000 IU every 3 months) to prevent rickets in children [43]. A recent RCT of 6-weekly bolus vitamin D also showed no benefit in healing radius fractures in post-menopausal women and some evidence of a detrimental effect on bone stiffness in those receiving the higher bolus dose of 75,000 IU every 6 weeks [44]. This adds to negative trials of bolus vitamin D supplementation in tuberculosis [45], and, most relevantly, in acute respiratory infections [46]. The most recent meta-analysis of vitamin D supplementation in the prevention of acute respiratory infections has not only shown that intermittent bolus, whether weekly or less frequently, is ineffective but that daily doses of >1000 IU/day are also ineffective [6]. There is a plausible biological explanation for why bolus and/or high dose vitamin D may not be effective: not only will transiently high levels of 25(OH)D induce the inhibitory, catabolic, enzyme vitamin D-24-hydroxylase CYP24A1, which may persist for several weeks after the 25(OH)D has fallen, but they are also likely to induce fibroblast growth factor 23 (FGF23), which can then suppress the activating enzyme 1α-hydroxylase in both renal [46] and extrarenal tissues [47]. Blood 25(OH)D levels of >100 nmol/L are likely to result in significant increase in FGF23 [48].

Definitions of vitamin D deficiency and the validity of supplementation dosing regimens therefore need to be taken carefully into account when assessing the results of studies investigating a role of vitamin D in determining COVID-19 outcomes. The case can be made for excluding from meta-analysis all studies reporting a pure intermittent bolus regimen (rather than single bolus for rapid normalisation of serum levels followed by daily maintenance).

Vitamin D status and COVID-19 outcomes—Hospital studies

There has been much interest in the possible benefits of vitamin D supplementation and normalisation of serum 25(OH)D levels with respect to its antimicrobial effects and risk of COVID-19 infection. In this context, vitamin D can be viewed as a nutritional factor for improving 'immune health' in the general population. However, vitamin D supplementation may also have therapeutic applications that are more consistent with its use as a drug rather than a nutrient. Several studies have examined the association between serum 25(OH)D concentrations and COVID-19 outcomes in hospitalised patients. There is wide heterogeneity among these studies in various aspects, including study design, time of sample draw, definition of end points and sample size. Among studies with ≥200 subjects and with in-hospital mortality as the endpoint, a majority show an association between low serum 25(OH)D and increased mortality from COVID-19 (Table 1). This is in keeping with the result of a recent systematic review that included 13 observational studies [49].

Serum 25(OH)D has been reported to be a negative acute phase reactant and thus a low 25(OH)D might be more likely in a severe disease of any etiology. Controlled studies in calves infected with bovine diarrhoea virus showed that serum 25(OH)D levels fell by 57% during the acute phase response to illness [81] and similar falls, albeit of lower magnitude, have been documented in humans although generally in the context of invasive procedures, for example, following orthopaedic surgery and acute pancreatitis [82]. There is a biological explanation as the majority of 25(OH)D is bound to serum vitamin D binding protein (DBP) and albumin, both of which fall in acute illnesses. Measurement of unbound or free 25(OH)D has been suggested as a more accurate marker of 25(OH)D status, analogous to free thyroid hormones (although it remains contentious whether free testosterone is physiologically more relevant than total concentration). Two recent studies from the UK investigated the correlation between free 25(OH)D and mortality. In a multicentre study of 295 hospitalised patients from the UK, a correlation was noted between both total and directly measured free 25(OH)D and receipt of in-hospital mechanical ventilation [72]. However, in a larger study from two acute hospitals, performed by some of the authors of the present review, we did not find a correlation between computed free 25(OH)D and in-hospital mortality [80]. However, a total serum 25(OH)D of <25 nmol/L was associated with in-hospital mortality when assessed by quartiles. An increase in mortality was also seen in those with high levels (>100 nmol/L), suggesting a possible U-shaped relationship; however, this was not significant when 25(OH)D was analysed as a continuous variable (Fig. 3). In this study, the negative acute phase effect seemed modest as mean serum 25(OH)D concentration was approximately 50nmol/L at CRP <5 mg/L compared with approximately 40 nmol/L at a median CRP of 200 mg/L.

Table 1. *Correlation between serum 25(OH)D and in-hospital mortality among hospitalised COVID-19 patients*

Author and year	Location	Study design	*N*	Direction of association	Key findings
Maghbooli et al., 2020 [50]	Iran	Cross-sectional study	235	↑	Serum 25(OH)D (>30 ng/ml or 75 nmol/L) associated with decreased severity and mortality from COVID-19
Luo et al., 2021 [51]	China	Retrospective cohort study	335	↑	Low serum 25(OH)D (<30 nmol/L) associated with increased severity of COVID-19
Alguwaihes et al., 2020 [52]	Saudi Arabia	Cross-sectional study	439	↑↑	Low serum 25(OH)D (<12.5 nmol/L) associated with increased mortality
Hutchings et al., 2021 [53]	Armenia	Cross-sectional study	330	↔	No association between serum 25(OH)D and COVID-19 severity or mortality
Gavioli et al., 2021 [54]	USA	Retrospective cohort study	437	↑	Low serum 25(OH)D (<20 ng/ml or 50 nmol/L) associated with increased need for oxygen support but not mortality from COVID-19
Basaran et al., 2021 [55]	Turkey	Retrospective cohort study	204	↑	Low serum 25(OH)D associated (<20 ng/ml) with increased severity of COVID-19
Mazziotti et al., 2021 [56]	Italy	Retrospective cohort study	348	↔	Low serum 25(OH)D (<12 ng/ml or 30 nmol/L) associated with increased hypoxic respiratory failure, but not mortality
Charoenngam et al., 2021 [57]	USA	Retrospective cohort study	287	↑↑	Serum 25(OH)D ≥30 ng/ml or 75 nmol/L associated with decreased mortality in patients >65 or those with Body Mass Index (BMI) <30 kg/m^2
Jevalikar et al., 2021 [58]	India	Cross-sectional study	410	↔	No association between serum 25(OH)D and COVID-19 severity or mortality
Tehrani et al., 2021 [59]	Iran	Cross-sectional study	205	↑	No association between serum 25(OH)D and COVID-19 mortality except in severe disease
Osman et al., 2021 [60]	Oman	Cross-sectional study	329	↔	No association between serum 25(OH)D and COVID-19 mortality
Nasiri et al., 2021 [61]	Iran	Retrospective cohort study	329	↑	Insufficient serum 25(OH)D (20–30 ng/ml) associated with increased length of stay, but not mortality

(*Continued*)

Table 1. *Continued*

Author and year	Location	Study design	*N*	Direction of association	Key findings
Reis et al., 2021 [62]	Brazil	Retrospective cohort study	220	↔	No association between serum 25(OH)D and length of stay, COVID-19 severity or mortality
AlSafar et al., 2021 [63]	UAE	Cross-sectional study	464	↑↑	Low serum 25(OH)D (<12 ng/ml) associated with increased COVID-19 severity, Intensive Therapy Unit (ITU) admission and mortality
Diaz-Curiel et al., 2021 [64]	Spain	Cross-sectional study	1549	↑	Serum 25(OH)D as a continuous variable was independently associated with ITU admission but not mortality
Al-Jarallah et al., 2021 [65]	Kuwait	Cross-sectional study	231	↔	No association between serum 25(OH)D and increased COVID-19 mortality
Guven and Gultekin, 2021 [66]	Turkey	Retrospective cohort study	520	↔	No association between serum 25(OH)D and COVID-19 mortality
Bianconi et al., 2021 [67]	Italy	Cross-sectional study	200	↔	No association between serum 25(OH)D COVID-19 severity or mortality
Shakeri et al., 2022 [68]	Iran	Cross-sectional study	293	↔	No association between serum 25(OH)D and mortality
Vasheghani et al., 2021 [69]	Iran	Retrospective cohort study	508	↑↑	Low serum 25(OH)D associated with increased COVID-19 severity, ITU admission and mortality
Afaghi et al., 2021 [70]	Iran	Retrospective cohort study	646	↑↑	Low serum 25(OH)D associated with increased mortality
Freitas et al., 2021 [71]	Portugal	Cross-sectional study	491	↑↑	Low serum 25(OH)D (<20 ng/ml) associated with increased COVID-19 severity and mortality
Hurst et al., 2021 [72]	UK	Cross-sectional study	295	↑↑	Low serum 25(OH)D associated with invasive mechanical ventilation (19.6 vs. 31.9 nmol/L) and increased mortality (23.2 vs. 29.5 nmol/L)
Ramirez-Sandoval et al., 2021 [73]	Mexico	Retrospective cohort study	290	↑↑	Low serum 25(OH)D (<12.5 ng/ml) associated with increased in-hospital mortality
Derakhshanian et al., 2021 [74]	Iran	Retrospective cohort study	290	↑↑	Serum 25(OH)D levels (<20 ng/ml) associated with increased death and ITU admission rates but not mechanical ventilation

(*Continued*)

Table 1. *Continued*

Author and year	Location	Study design	*N*	Direction of association	Key findings
Seven et al., 2021 [75]	Turkey	Retrospective cohort study	403	↑	Serum 25(OH)D levels (<14.5 ng/ml) independently associated with increased severity in pregnant COVID-19 patients
Apaydin et al., 2021 [76]	Turkey	Retrospective cohort study	219	↔	No association between serum 25(OH)D levels and COVID-19 severity, Intensive Care Unit (ICU) admission or mortality
Hernandez et al., 2021 [77]	Spain	Case-control study	216	↔	No association between categorical or continuous 25(OH)D levels and COVID-19 mortality
Jenei et al., 2022 [78]	Hungary	Retrospective cohort study	257	↑↑	Serum 25(OH)D levels independently associated with increased mortality in >60-year olds (30 ± 12 in the deceased compared to 21 ± 13 nmol/L in the recovered group)
Juraj et al., 2022 [79]	Slovakia	Retrospective cohort study	357	↑↑	Serum 25(OH)D levels (<12 ng/ml or 30 nmol/L) independently associated with increased mortality
Subramanian et al., 2022 [80]	UK	Retrospective cohort study	472	↑↑	Serum 25(OH)D <25 nmol/L and >100 nmol/L associated with increased mortality (when assessed by quartiles but not significant as continuous variable)

Note: We included studies with a sample size of more than 200 subjects and an outcome of in-hospital mortality. Studies with a sample size lesser than 200 or not including mortality as endpoint were excluded. ↑ represents increased severity of COVID-19 in patients with low serum 25(OH)D, ↑↑ represents increased mortality from COVID-19 in patients with low serum 25(OH)D and ↔ represents no association between serum 25(OH)D and COVID-19 mortality. Conversion of 25(OH)D ng/ml to nmol/ml is approximately ×2.5, that is, 20 ng/ml = 50 nmol/L.

In summary, several hospital studies show an association between low serum 25(OH)D levels and in-hospital mortality from COVID-19 and the putative negative acute phase effect seems insufficient to explain this association.

Vitamin D status and COVID-19 outcomes—Community studies

Many large studies have examined the association between serum 25(OH)D levels measured prior to illness in the community and subsequent SARS-CoV-2 infection risk as well as risk of severe COVD-19 and mortality (Table 2). Several of these studies are limited by a long delay, sometimes many years, between the measurement of serum levels and subsequent COVID-19 positivity. Notwithstanding this limitation, the majority of studies report an association between low pre-illness levels of 25(OH)D and subsequent increased risk of SARS-CoV-2 infection and hospitalisation from COVID-19. These include a large study from Israel [88], a US veterans affairs study [87] as well as the Nurses Health study [14]. Conversely, the association was lost after multivariate analysis in a UK Biobank study but this was significantly limited by the extremely long duration (median 11 years) between the measurement of 25(OH)D levels and the COVID-19

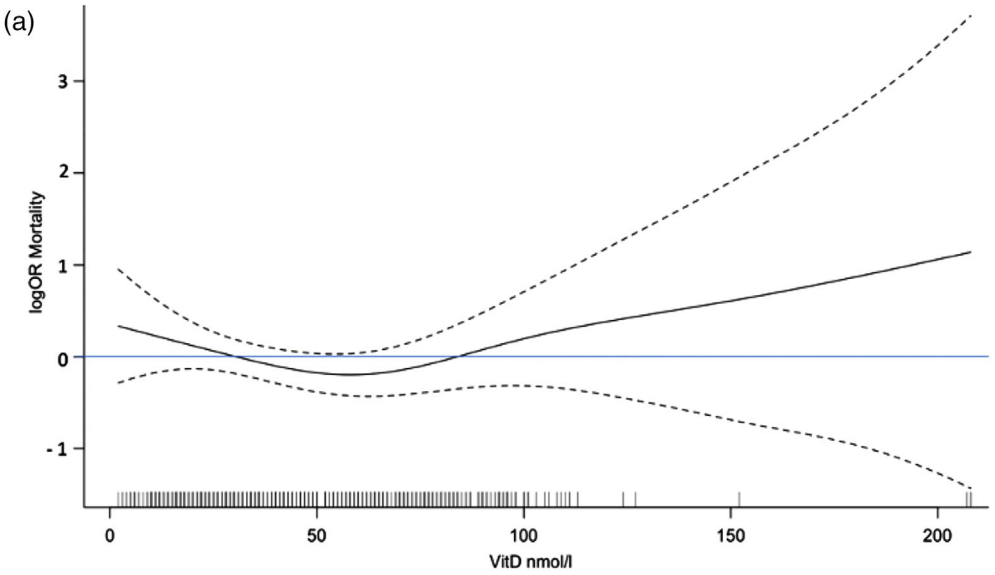

Fig. 3 *Relationships between 25(OH)D concentrations and COVID-19 mortality. Few published studies have included dose response data but two studies that have recorded it again reveal a mixed picture: (a) Relationship between 25(OH)D concentration on admission and 28-day mortality in 472 hospital patients admitted for COVID-19 in the UK. Log odds ratio for mortality compared with mean 25(OH)D (47.4 nmol/L) as reference with adjustment for age and sex and cubic spline smoothing. This analysis, with 25(OH)D as a continuous variable, was not significant, although a separate multivariable analysis with 25(OH)D by quartiles did show significant increased mortality if 25(OH)D <25 nmol/L or >100 nmol/L (from Subramanian et al. [80] with permission). (b) Relationships between serum 25(OH)D concentrations and COVID-19 severity in patients admitted to a single medical centre in Israel. Severity of illness was defined as per WHO/2019-nCoV/clinical/2020.5. Historical 25(OH)D concentrations measured 14–730 days prior to infection were available for 253 of 1176 admitted patients. No U-shaped curve was noted (from Dror et al. [117] with permission).*

pandemic [13]. Although there are inconsistencies among these studies, a recent systematic review including nearly 2 million adults concluded that vitamin D deficiency/insufficiency increased susceptibility to COVID-19 and its severity and mortality [89].

Interactions between race and vitamin D status also appear to modulate the risk of SARS-CoV-2 infection but findings are inconsistent. In a large cohort study from the United States, low serum 25(OH)D levels were associated with SARS-CoV-2 positivity among White but not Black individuals [86]. However, another study among US Black women reported an association between low serum 25(OHD) levels and infection risk [90] and similarly, a higher proportion of vitamin D deficient ethnic minority subjects had evidence of SARS-CoV-2 infection among UK healthcare workers [83]. This is further supported by another UK Biobank cohort study, which used structural equation modelling and reported that serum vitamin D levels mediate Asian and Black ethnic disparities in COVID-19 severity [91].

Mendelian randomisation studies

The perceived difficulty in interpreting serum 25(OH)D levels during illness and the lack of immediate pre-illness serum 25(OH)D levels have led investigators to consider alternative methods of association such as Mendelian randomisation. This uses gene polymorphisms that predict vitamin D status as a surrogate for vitamin D deficiency. Six studies to date have used this approach to test the association between genetically determined vitamin D levels and COVID-19 infection risk and/or severity and all of them report no association (Table 3).

However, there are obvious limitations to these studies: (i) all the studies used data from the UK Biobank, which limits the generalisability to a non-European population and (ii) serum

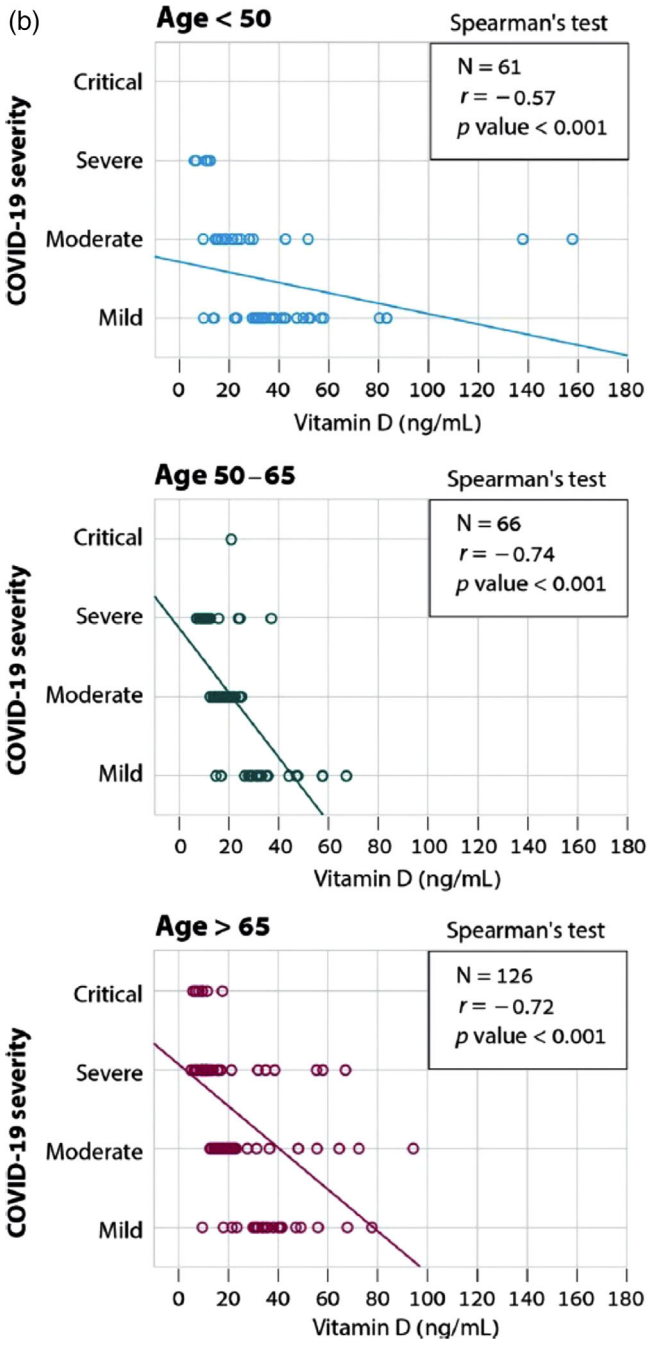

Fig. 3 *Continued*

25(OH)D levels are overwhelmingly an expression of environmental factors, with the heritability of serum 25(OH)D levels estimated to be less than 10%, thereby necessitating an extremely large sample size in order to detect any effect. Thus, whilst Mendelian randomization has the potential to interpret the impact of inherited variations in serum 25(OH)D concentration over the lifetime of an individual, these effects are very small and likely to be even less relevant for populations with

Table 2. *Association between vitamin D status and risk of SARS-CoV-2 infection*

Author and year	Location	Study design	*N*	Key findings
Faniyi et al., 2021 [83]	UK	Cross-sectional study	392	Healthcare workers with serum 25(OH)D levels <30 nmol/L independently associated with COVID-19 seroconversion
Li et al., 2021 [84]	USA	Cohort study	18,148	No association between serum 25(OH)D and SARS-CoV-2 infection risk
Jude et al., 2021 [85]	UK	Retrospective cohort study	80,670	Low serum 25(OH)D (<50 nmol/L) associated with increased risk of COVID-19 hospitalisation but not mortality
Cozier et al., 2021 [90]	USA	Retrospective cohort study	5081	Low serum 25(OH)D (<29 ng/ml) associated with increased SARS-CoV-2 infection risk among Black women
Crandell et al., 2021 [86]	USA	Retrospective cohort study	21,629	A 10 ng/ml increase in 25(OH)D lowered the odds of having a positive COVID-19 test overall and among White but not Black individuals
Li et al., 2021 [13]	UK	Retrospective cohort study	417,342	No association between 25(OH)D levels and SARS-CoV-2 infection risk[c]
Ma et al., 2021 [14]	USA	Retrospective cohort study	39,315	Higher predicted 25(OH)D levels associated with lower risk of SARS-CoV-2 infection (highest quintile median 34.7 ng/ml vs. lowest quintile 25.2 ng/ml) Vitamin D supplement intake >400 IU/d associated with lower hospitalisation risk
Seal et al., 2022 [87]	USA	Retrospective cohort study	4599	Independent inverse dose–response relationship between increasing continuous 25(OH)D concentrations (from 15 to 60 ng/ml) and decreasing the probability of COVID-19-related hospitalization and mortality
Israel et al., 2022 [88]	Israel	Retrospective cohort study	41,575[a] 2533[b]	Higher risk of infection among low serum 25(OH)D levels (<30 nmol/L) and SARS-CoV-2 positivity Low serum 25(OH)D associated with increased severity of COVID-19

[a]Number of patients with positive SARS-CoV-2 polymerase chain reaction (PCR) tests.
[b]Number of patients hospitalised for severe COVID-19.
[c]Median duration of 11 years between 25(OH)D measurement and COVID-19 pandemic.

inherently low vitamin D status, as is characteristic of countries such as the UK. Perhaps more importantly, much of the genetic effect on 25(OH)D concentration is mediated by polymorphisms in the DBP and although reduced DBP concentration or activity will reduce 25(OH)D concentration, it will simultaneously tend to increase free 25(OH)D and arguably has little or no impact on the biological effects of vitamin D [1]. Finally, Mendelian randomization is a useful tool for assessing the potential impact of factors such as vitamin D on diseases where long-term exposure may be important. A good example of this is the strong link between genetic determinants of vitamin D and the

Table 3. *Mendelian randomisation studies of genetically predicted vitamin D deficiency and COVD-19*

Author and year	Population	*N*	Key findings
Cui and Tian, 2021 [92]	European	1,683,768	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility or severity
Liu et al., 2021 [93]		1,079,768	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility or severity
Amin and Drenos, 2021 [94]	European	127,637	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility or severity
Patchen et al., 2021 [95]	European	1,388,512	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility
Li et al., 2021 [13]	European	417,343	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility
Butler-Laporte et al., 2021 [96]	European	443,774	No association between genetically determined 25(OH)D levels and COVID-19 susceptibility, severity or mortality

autoimmune disease multiple sclerosis [97]. However, it is unclear whether this approach is relevant to acute respiratory infections, where a transient, rather than sustained, rise in serum 25(OH)D may be sufficient to achieve a biological effect.

Community supplementation studies

More compelling evidence for a causal role of vitamin D status in determining SARS-CoV-2 infection risk and its severity could be inferred if pre-illness supplementation among deficient individuals were shown to attenuate the subsequent risk of infection or its severity compared with nonsupplemented subjects. However, this would require a very large sample size, especially if the study were performed on a population with a high vaccination rate since vaccines are now shown to be very effective in preventing serious illness. This is exemplified in an RCT from the UK recently reported as a non-peer-reviewed preprint [98]. This open-label study randomly assigned 6200 adults to 'test and treat' high dose vitamin D (3200 IU/day, $N = 1550$) or low dose (800 IU/day, $N = 1550$) to those with blood 25(OH)D concentration <75 nmol/L compared to a control group ($N = 3100$) who were not offered testing or additional supplementation but who were allowed to take the government recommended supplement of 400 IU vitamin D per day. Neither the primary outcome of the proportion of all participants developing at least one acute respiratory infection nor the secondary outcome of proportion of patients developing swab-confirmed COVID-19 were significantly different among the three groups. A number of other outcomes such as hospitalisation and mortality from COVID-19 were not significantly different between the groups. However, whereas on entry to the study, only 2.5% had received one or more vaccine doses, 89.1% had received at least one dose of the vaccine by the end of the study. Likely as a result of this, plus other public health measures, the proportion of people in the control group who became infected with SARS-CoV-2 was only 4.6% compared with 20% predicted in the sample size calculation. Consequently, the rate of COVID-19 infections makes even this relatively large study underpowered; moreover, it was not designed to study the severity, and mortality was 0% in all three groups. Furthermore, the control group had a mean serum 25(OH)D level of 66.6 nmol/L at the end of the study as roughly 50% of this group reported taking vitamin D supplements. There are further trials still in progress but most trial protocols continue to allow substantial albeit lower-dose supplementation in the control group, which makes it difficult to interpret the beneficial effect of supplementation.

Contrary to evidence from the single randomised trial, indirect evidence from nonrandomised community-based studies seems to suggest a protective effect of vitamin D supplementation on infection risk and adverse COVID-19 outcomes (Table 4).

Table 4. *Community vitamin D supplementation and clinical outcomes*

Author and year	Location	Study design	*N*	Vitamin D dose	Key findings
Randomised controlled trial (RCT)					
Jolliffe, 2022 [98]	UK	Finger prick 25(OH)D measure at baseline followed by supplementation if levels <75 nmol/L versus no offer of supplementation	6200	3200 IU/d ($N = 1550$) 800 IU/d ($N = 1550$) No testing or supplementation ($N = 3200$)	No difference in acute respiratory infection or COVID-19 incidence rates across the three groups
Observational studies					
Ma et al., 2021 [99]	UK	Cohort study	8297	Not specified	Habitual use of vitamin D supplements associated with lower risk of COVID-19 infection (Odds ratio [OR], 0.66; 95% Confidence intervals [CI], 0.45–0.97; $p = 0.034$)
Ma et al., 2021 [14]	USA	Cohort study	39,315 (1768)[a]	Varying doses, 0 to ≥2000 IU/d	Intake of supplements ≥400 IU/d associated with a lower risk of COVID-19 hospitalisation
Oristrell et al., 2022 [100]	Spain	Population-based cohort study	4.6 m (30,557)[a]	Cholecalciferol or calcifediol-varying doses	Patients supplemented with cholecalciferol or calcifediol achieving serum 25OHD levels ≥30 ng/ml associated with better COVID-19 outcomes

(*Continued*)

Table 4. *Continued*

Author and year	Location	Study design	*N*	Vitamin D dose	Key findings
Louca et al., 2021 [101]	UK, USA and Sweden	Community survey	445,850 (30,746)[a]	Not specified but frequency >3 times/week for at least 3 months	Lower risk of SARS-CoV-2 infection among vitamin D supplement users
Annweiler et al., 2021 [102]	France	Quasi-experimental study	95	50,000 IU/ month, or 80,000 IU or 100,000 IU or 200,000 IU every 2–3 months or 800 IU/d	Lower adjusted mortality among the supplemented group
Arroyo-Diaz et al., 2021 [103]	Spain	Cross-sectional study	1267	Not specified	No association between vitamin D supplementation and death
Efird et al., 2021 [104]	USA	Retrospective cohort study	26,508	Not specified-daily 'low' dose	Use of vitamin D in conjunction with steroids reduced mortality
Nimer et al., 2022 [105]	Jordan	Cross-sectional survey	2148	Not specified	Use of vitamin D supplements was independently associated with low risk of hospitalisation and severe COVID-19

[a]Numbers in brackets represent number of COVID-19-positive individuals.

Amongst the larger studies, in the UK Biobank cohort, habitual use of vitamin D supplements was associated with a lower risk of COVID-19 infection [99]. In a US veterans cohort of 26,508 SARS-CoV-2-positive individuals, the benefit of vitamin D supplementation on mortality within 2 weeks of COVID-19 diagnosis was estimated using electronic prescription records [104]. Among hospitalised patients, a significantly decreased mortality rate was observed for the use of vitamin D in the absence of corticosteroids relative to patients who received steroids but not vitamin D. Among patients receiving systemic steroids such as dexamethasone, the use of vitamin D was associated with significantly fewer deaths in hospitalised patients compared with nonhospitalised patients. Similarly, in the Nurses Health study, subjects with a 'high' intake of vitamin D supplements (≥400 IU/day) had a lower risk of hospitalisation after adjusting for other factors [14]. In keeping with this, a recent systematic review concluded that vitamin D supplementation was associated with better clinical outcomes, although curiously this was only significant when vitamin D was administered after the diagnosis of COVID-19 [106].

Table 5. *Randomised trials of in-hospital vitamin D supplementation and COVID-19 outcomes*

Author and year	Location	Study design	*N*	Vitamin D dose	Key findings
Entrenas Castillo et al., 2020 [107]	Spain	Open-label RCT	Treatment arm (50), control (26)	Calcifediol (25[OH]D) 0.532 mg on day 1 followed by 0.266 mg on days 3 and 7 and weekly until discharge or ICU admission	Significant reduction in need for Intensive Care Unit (ICU) admission in the treatment arm
Murai et al., 2021 [108]	Brazil	Double-blind, placebo-controlled RCT	Treatment arm ($n = 120$) Placebo ($n = 120$)	Single oral dose of 200,000 IU of vitamin D3	No difference in length of hospitalisation, ICU admission or mortality
Cannata-Andia et al., 2022 [109]	International	Open-label RCT	Treatment arm (279), control (269)	Single oral bolus of 100,000 IU cholecalciferol	No difference in length of hospitalisation, ICU admission or mortality

Impact of vitamin D supplementation on COVID-19 outcomes—Hospital studies

It is also disappointing that there have been so few studies of vitamin D supplementation in hospitalised patients with COVID-19 (Table 5). A Cochrane systematic review published in June 2021 noted two RCTs that evaluated supplementation in hospitalised patients with moderate to severe disease [110]. The trials were too heterogenous to allow meta-analysis. One was a pilot study performed in Cordoba, Spain [107]. Seventy-six consecutive hospitalised patients were randomised in a ratio of 2:1 to receive oral calcifediol (25[OH]D) in a substantial dose (0.532 mg on admission followed by 0.266 mg on days 3 and 7, then weekly till discharge) compared with no added vitamin D, in addition to standard care, which, at that time, included hydroxychloroquine and azithromycin. The choice of calcifediol is interesting and likely to be relevant as a previous trial showed that calcifediol raised 25(OH)D levels more rapidly and in a greater proportion of patients than cholecalciferol [111]. The trial was, however, seriously underpowered to look at mortality—only two of the 76 patients died. Moreover, the treatment groups were not well matched for diabetes or hypertension and baseline 25(OH)D levels were not measured. Patients treated with calcifediol were less likely to be admitted to intensive care (OR after adjustment for diabetes and hypertension 0.03 [95% CI 0.003–0.25]). A larger study has been planned (NCT04366908) but has yet to report (estimated completion 31 December 2021).

The second RCT was performed in Sao Paulo, Brazil. This was conducted in 240 hospitalised patients with moderate to severe COVID-19 who were randomised 1:1 to receive a single oral dose of 200,000 IU cholecalciferol or placebo [108]. No significant difference was seen in vitamin D versus placebo groups for mortality (7.6% vs. 5.1%, p 0.43) or length of hospital stay. Patients in this study did not receive vitamin D until an average of 10 days from symptom onset but more importantly, as previously discussed, high dose bolus vitamin D supplementation is already known to be ineffective for various clinical conditions, including rickets. A further larger recent open-label RCT of moderate to severe COVID-19 patients randomised to a single oral bolus of cholecalciferol (100,000 IU) likewise showed no difference in the length of stay, Intensive Care Unit (ICU) admission or mortality between the treatment and control arms [109].

Several further systematic reviews have been published since the Cochrane review but although they have tended to set broader criteria for study inclusion, they have not noted any further prospective RCTs [89, 106, 112–115]. Several cohort observational studies have been reported but these can only provide relatively low quality evidence.

They include a large retrospective study looking at calcifediol (25[OH]D) and cholecalciferol use 15–30 days before hospital admission with COVID-19 across Andalucia, Spain [116]. This included all 15,968 patients hospitalised between January and November 2020, within which propensity score matching was conducted with adjustment for known variables associated with poor prognosis to yield 1269 individuals in each matched group. This showed reduced 30-day mortality in those receiving vitamin D within the previous 30 days with a larger effect for calcifediol (Hazard Ratio [HR] = 0.73, with 95% CI 0.57–0.95) than for cholecalciferol (HR = 0.88, with 95% CI 0.75, 1.03).

Conclusions

The impact of vitamin D deficiency on the risk of COVID-19 infection and perhaps particularly on the risk of its severity remains plausible but evidence to substantiate this is indirect, coming largely from association studies. There is growing evidence from studies performed prepandemic that intermittent high-dose bolus vitamin D supplementation is ineffective for various endpoints. Future studies should carefully consider the dose and formulation of vitamin D. For acute respiratory infection, calcifediol may be a better choice for both disease prevention and treatment due to its ability to raise serum 25(OH)D more rapidly compared to conventional vitamin D (cholecalciferol). Better quality evidence for an impact of vitamin D status on COVID-19 outcomes might still come from randomised trials of regular daily supplementation. Meanwhile, avoidance of vitamin D deficiency by regular low-dose daily supplementation, particularly in winter months, should be encouraged.

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Conflict of interest

M. H. has received speaking honoraria from DSM and Danone. R. Q. has participated in the Data Monitoring Committee for the Coronavit study of vitamin D in COVID-19 (NCT04579640). D. T. receives funding from the Health Technology Assessment/National Institute for Health Research for the 'Vitalize' study of vitamin D replacement in ITU patients. S. S. has received speaking honoraria and consultancy fees from several companies but none in relation to vitamin D. G. G., J. H., R. A. K., E. L. and J. M. R. have no conflict of interest to declare.

Author contributions

Sreedhar Subramanian: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. George Griffin: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. Martin Hewison: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. Julian Hopkin: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. Rose Anne Kenny: Conceptualization; Data curation; Writing - original draft; Writing - review and editing. Eamon Laird: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. Richard Quinton: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. David Thickett: Conceptualization; Data curation; Writing – original draft; Writing – review and editing. Jonathan M. Rhodes: Conceptualization; Data curation; Writing – original draft; Writing – review and editing.

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