The association between vitamin D deficiency and the risk of herpes zoster and COVID-19

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Declaration

I, Liang-Yu Lin, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: 

Date: 18 May 2022

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Abstract

BACKGROUND: Acute viral infections or reactivations, such as Coronavirus Disease 2019 (COVID-19) and herpes zoster (HZ), can cause high morbidity and mortality among older adults. Although vitamin D has immunomodulatory effects, the association between vitamin D status and COVID-19 and HZ is unclear. In this thesis, I investigated the association between vitamin D deficiency and HZ and COVID-19.

METHODS: This thesis comprises four parts: one systematic review and three analytic studies using UK Biobank. First, I systematically reviewed studies about vitamin D and human herpesviruses infection or reactivation. Second, I conducted a cross-sectional study which described the distribution of vitamin D status and identified demographic risk factors for vitamin D deficiency and insufficiency in UK Biobank. In the third part, I undertook a cohort study to explore the association between vitamin D status, supplementation, and prescriptions and the risk of incident HZ. Finally, I assessed the association between vitamin D status and COVID-19 diagnosis, hospitalisation, and mortality.

RESULTS: My systematic review and meta-analysis (Chapter 3) included ten studies, and the results demonstrated that vitamin D deficiency was not associated with cytomegalovirus (CMV) diseases in transplant patients, but vitamin D supplementation was associated with a lower risk of HZ in individuals receiving haemodialysis. All included studies were hospital-based and conducted among immunosuppressed people. In my cross-sectional study (Chapter 5) of 449,943 participants aged 40 to 69 years with vitamin D records, I found that the winter and spring seasons, northern regions, male sex, abnormal body mass index (BMI), non-white ethnic backgrounds, smoking, and socioeconomic deprivation were associated with vitamin D deficiency and insufficiency. My cohort study of vitamin D and HZ (Chapter 6) included 177,572 participants with linked clinical records. I found no association between vitamin D deficiency and incident HZ (deficient: adjusted hazard ratio (HR) = 0.99, 95% confidence interval (CI) = 0.90–1.10). Vitamin D supplementation or prescriptions were not associated with incident HZ. Finally, in the cohort study of vitamin D and COVID-19 (Chapter 7), including 307,512 people with linked COVID-19 clinical records, I found an inconsistent association between vitamin D deficiency and COVID-19 diagnosis during different follow-up periods (during British summertime (BST) months: HR=0.86, 95% CI=0.77–0.95; during non-BST months: HR=1.14, 95%CI=1.01–1.30). I found no evidence that vitamin D deficiency or insufficiency was associated with either hospitalisation or mortality due to COVID-19 in any time stratum.

CONCLUSION: In summary, I found no association between vitamin D status, supplementation, or prescriptions and the risk of HZ or COVID-19. According to currently available evidence, extra vitamin D supplementation should not be recommended to prevent HZ or COVID-19.

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Abbreviations

ACIP	Advisory Committee on Immunisation Practices
BMI	Body mass index
BST	British summertime
Camp	cathelicidin antimicrobial peptide gene in mouse
CAMP	Human cathelicidin antimicrobial peptide gene
CKD	Chronic kidney disease
CTV 3	Clinical Term Version 3
CMV	Cytomegalovirus
CI	Confidence intervals
COPI	Control of Patient Information
COVID-19	Coronavirus disease 2019
DM+D	Dictionary of medicines and devices
EHR	Electronic health record
EBV	Epstein–Barr virus
GP	General practitioner
HR	Hazard ratio
HZ	Herpes zoster
HES	Hospital Episode Statistics
ICD	International Classification of Diseases
IMD	Index of Multiple Deprivation
NAAT	Nucleic acid amplification test
OR	Odds ratio
OPCS	OPCS Classification of Interventions and Procedures
PCR	Polymerase-chain-reaction
PPV	Positive predictive value
PHN	Post-herpetic neuralgia
RNI	Reference nutrient intake
RT-PCR	Reverse transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SNOMED-CT	Systematized Nomenclature of Medicine – Clinical Terms
BNF	the British National Formulary

TPP	The Phoenix Partnership
SACN	the Scientific Advisory Committee on Nutrition
US-CDC	US Centers for Disease Control and Prevention
VZV	Varicella-Zoster virus
VDR	Vitamin D receptors

Chapter 1. Introduction

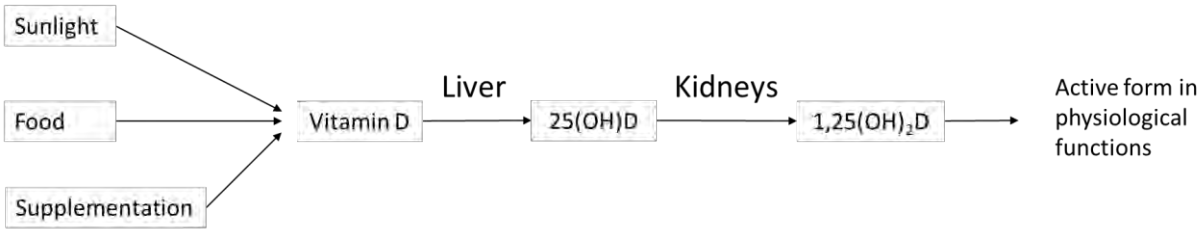
Chapter overview

In this chapter, I present an overview of vitamin D, including its metabolism, deficiency, supplementations, and non-musculoskeletal effects. I also introduce the clinical characteristics, disease burden, treatment, and prevention of herpes zoster (HZ) and COVID-19. This chapter ends by describing the uncertainty about vitamin D as a public health intervention for viral infections.

1.1. Vitamin D

1.1.1. Vitamin D metabolism

Vitamin D is an essential element in bone formation. It was discovered in cod liver oil, which treats rickets (1). **Figure 1** briefly illustrates the mechanism of vitamin D metabolism. The skin can produce vitamin D after sun exposure, and vitamin D can also be obtained from food and supplementation. After production or ingestion, vitamin D is metabolised in the liver into 25-hydroxyvitamin D (25(OH)D), which can be measured in the blood. Later, in the kidneys, 25(OH)D is transformed into 1,25-dihydroxy vitamin D (1,25 $(OH)_2D$), the active form of vitamin D. This active vitamin D metabolite can significantly increase calcium and phosphate absorption and facilitate bone mineralisation. Vitamin D deficiency impairs bone mineralisation, leading to osteopenia or osteoporosis (2).



Chapter 1. Figure 1 A brief diagram of vitamin D metabolism.

1.1.2. Vitamin D deficiency

Currently, vitamin D status is evaluated by measuring serum 25(OH)D because its half-life is much longer than that of the active 1,25 $(OH)_2D$ (3). The 25(OH)D analytic methods are chemiluminescence immunoassay and tandem mass spectrometry, both of which are used and recognised by the Royal Osteoporosis Society and Public Health England (3, 4). However, currently, no global consensus has been reached regarding normal serum vitamin D levels. Each study may use different standards and definitions of vitamin D deficiency. **Table 1** summarises different countries' definitions of vitamin D status. In the UK, Public Health England defined vitamin D deficiency as a 25(OH)D level of less than 25 nmol/L. In the US, the Endocrine Society regards vitamin D deficiency as a 25(OH)D level of less than 50 nmol/L (20 ng/ml) (3, 5). In this thesis, I used Public Health England's approach to define vitamin D deficiency among the study population.

Vitamin D levels also vary in different seasons, ages, and countries. Using the criteria of a serum vitamin D level lower than 25 nmol/L, a national cohort in the UK indicated that during the winter and spring, the average prevalence of vitamin D deficiency was 18.6% for people older than 19 years old (6). In Taiwan, a nationwide cross-sectional survey on nutrition and health revealed

that among people over 45 years old, the average prevalence of vitamin D deficiency was over 30% (7).

Chapter 1. Table 1 The normal range of serum vitamin D levels adapted in different countries and studies

Country	**Reference research**	**Deficiency (nmol/L)***	**Insufficiency (nmol/L)**	**Adequate (nmol/L)**
UK-Public Health England	Pearce and Cheetham, 2010 (8)	<25	25–50	≥ 50
US- NIH	Institute of Medicine, 2011 (9)	<30	30–50	≥ 50
US-Endocrine Society	Holick et al., 2011 (5)	<50	50–75	≥ 75
Taiwan	Hanley et al., 2010 (10)	<50	50–75	≥ 75

* 1 nmol/L = 0.4 ng/mL

1.1.3. Vitamin D supplementation

The official standards in different countries vary regarding the necessary intake levels of nutrients to ensure the health of individuals or populations. In the UK, reference nutrient intake (RNI) is the reference for protein, vitamin, and mineral intake, defined as adequate nutrition intake for 97% of the population to minimise the risk of deficiency (11). During the seasons with less sun exposure, the recommended intake aims to maintain serum

vitamin D levels above 25 nmol/L. In 2016, the Scientific Advisory Committee on Nutrition (SACN) modified the RNI for vitamin D, recommending 10 µg/day (400 IU/day) intake for anyone older than four years old (3). Based on this revision of RNI, Public Health England recommends daily vitamin D supplementation of 10 µg in the autumn and winter (12)

Regarding vitamin D supplementation, two forms of vitamin D exist, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 can be found in mushrooms, and vitamin D3 is more abundant in oily fish and meat. Commercially fortified products use both forms of vitamin D (5). Vitamin D supplements include single supplements, multivitamins/minerals, fish oil, fish liver oil, and calcium (13). In addition to food and over-the-counter supplementation, vitamin D is also widely prescribed in clinical practice in the UK, especially for people over 65 years old (14, 15).

1.1.4. Vitamin D and bone health

Adequate vitamin D and calcium levels are crucial for maintaining calcium homeostasis and bone mineralisation (16). A systematic review and meta-analysis among people over 65 years found some evidence that increasing serum vitamin D levels by 10 ng/mL and taking daily vitamin D supplements with calcium were associated with a lower risk of fracture (serum vitamin D levels: hip fracture: pooled rate ratio (RR): 0.80, 95% CI: 0.75–0.86, 5 studies; daily supplements: hip fracture: RR= 0.84; 95% CI:0.72–0.97). (17). Another systematic review and meta-analysis summarising results from 53 trials among post-menopausal women or men over 65 years old indicated some evidence that taking vitamin D plus calcium supplements reduced the

risk of hip fracture (pooled risk ratio: 0.84, 95% CI: 0.74 – 0.96). Among high-risk populations, such as individuals living in institutions, taking vitamin D and calcium supplements prevented nine hip fractures for every 1000 person-years (18).

Taking vitamin D supplements is an economic public health intervention for preventing osteoporotic fracture. A Belgian study which modelled the cost-effectiveness of vitamin D and calcium supplements and fracture among people over 60 years with osteoporosis, indicated that the cost for gaining one quality-adjusted life-year (QALY) decreased as people aged. The cost even became negative among people in their eighties, meaning that vitamin D and calcium supplements were cost-saving (cost per QALY: among aged 60: women: €40,578, and men: €23,477; among aged 80: women: - €12,815, and men: - €6,723) (19). Another study indicated that universal vitamin D supplements for individuals with osteoporosis would save annual hospital expenses for fractures by €5.71 billion in Europe and $3.31 billion in America (20). In addition to supplementation, food fortification could further reduce the spending on vitamin D testing and prescription (21).

1.1.5. Vitamin D and the human immune system

In addition to bone health, recent studies have indicated that vitamin D may have potential immunomodulatory effects. Previous cell studies have shown that immune cells, such as monocytes and macrophages, upregulate their expression of vitamin D receptors (VDRs) and the enzymes that catalyse vitamin D activation in response to pathogen exposures. With the increase of active 1, $25(OH)_2D$, these immune cells synthesise an antimicrobial peptide

cathelicidin to protect against pathogens (22, 23). Despite the evidence from in vitro studies, the effect of vitamin D supplementation is inconsistent across epidemiological studies. A systematic review and meta-analysis of 37 randomised clinical trials indicated that taking vitamin D supplements may decrease 8% of the risk of acute respiratory tract infections (odds ratio (OR) = 0.92, 95% confidence interval (CI) = 0.86–0.99) (24). However, it is unclear whether vitamin D supplements are associated with protection against other viral infections, such as herpesviruses.

1.1.6. Animal studies about vitamin D and immune function

Previous evidence about vitamin D's effects on immune function was mainly from cell studies, and only a few were from animal models. In humans, activated vitamin D regulates the expression of the antimicrobial peptide cathelicidin. Similarly, in mice, cathelicidin is also important for protecting against bacterial infections. Transgenic mice without a gene producing cathelicidin, cathelicidin antimicrobial peptide (Camp) gene, were susceptible to Group A streptococcus infection of the skin and E. coli O157:H7 colonization in the gut (25, 26). However, unlike the human cathelicidin antimicrobial peptide (CAMP) gene, the Camp gene among non-primate mammals is not regulated by vitamin D because the vitamin D receptors (VDR) elements are not encoded in the promoter of Camp gene. Therefore, in mice or other animal models, vitamin D cannot induce the expression of cathelicidin (27).

In 2020, a new transgenic mouse model was introduced to study in vivo antimicrobial effects of human cathelicidin protecting against gastrointestinal

and skin infections (28). Lowry et al. substituted mice's Camp gene with the humans' CAMP gene with vitamin D receptors sequence, so these mice can produce vitamin D-induced human cathelicidin. These transgenic mice were reported to be more resistant to Salmonella typhimurium infection in their guts. In addition, these transgenic mice were infected with Staphylococcus aureus on their skin, and later treated with topical activated vitamin D ($1,25(OH)_2D$) or with comparators with control vehicles, 50% glycerine / ethanol solution. In the following biopsy of the infected wounds, the topical vitamin D treatment group had a lower bacterial load (74,400 mean CFU) than the control group receiving the control vehicle (1,840,000 mean CFU) (28).

1.2. Herpes zoster (HZ) in humans

1.2.1. Pathogenesis, clinical symptoms, diagnosis, and treatment

Herpes zoster (HZ), commonly called shingles, is caused by the reactivation of clinically latent varicella-zoster virus (VZV). VZV belongs to the family Herpesviridae, a group of double-stranded DNA viruses widely prevalent in nature (29). VZV infection typically results in chickenpox in young children and less commonly in adolescents and adults, characterised as a pruritic vesicular rash that spreads over the body and becomes crusted within days (30). After recovering from varicella, instead of being eradicated, VZV leads to lifelong latent infection. This virus remains in the cranial nerves or dorsal root ganglia, and its reactivation from latency may lead to HZ, which increases in incidence with age. HZ is characterised by a rash consisting of painful erythematous vesicles, which usually progress to pustules before

forming scabs and typically occur in a unilateral dermatomal distribution. It is generally self-limited, and the rash resolves after 10–20 days (31). However, people with compromised immunity may suffer from severe systemic infections (32). In people with trigeminal nerve involvement, VZV may cause zoster ophthalmicus, resulting in ocular complications such as keratopathy and even blindness without proper treatment in some patients (33).

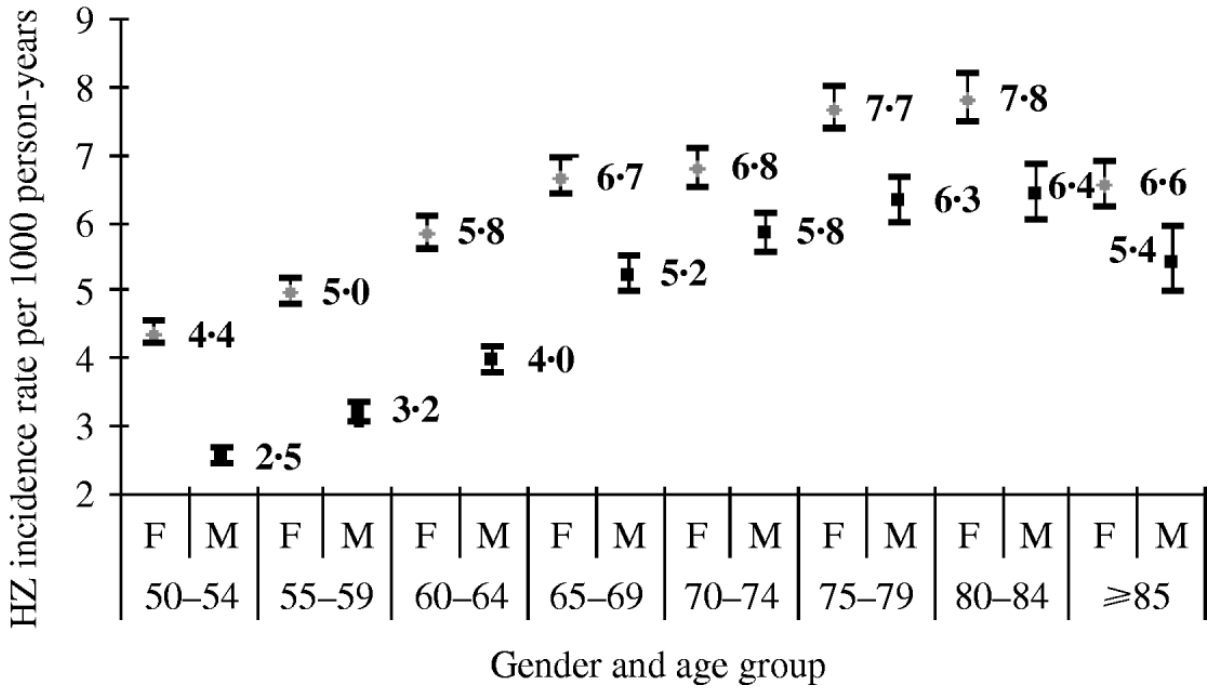
After an acute episode of HZ, some people may experience persisting neuropathic pain called post-herpetic neuralgia (PHN), which is traditionally defined as any persisting pain at least 90 days after the rash appears (34). HZ diagnosis is mainly based on clinical symptoms and signs, whereas laboratory tools such as polymerase-chain-reaction (PCR) can provide further evidence of infection (35).

Oral antiviral agents such as aciclovir, valaciclovir, and famciclovir are indicated for people with HZ (36). Although antiviral agents cannot eradicate VZV, these medications can reduce the severity of pain, hasten rash healing and reduce the period of viral shedding (35). It is recommended that people initiate antiviral treatment within 72 hours after the onset of the rash if they are immunocompromised, older than 50 years, or at any age if they have moderate or severe symptoms (37).

1.2.2. Epidemiology and risk factors for herpes zoster

According to a historical analysis of electronic health records (EHRs), the incidence of HZ is between 2 to 5 cases per 1000 person-years in many countries (34). In 2009, the annual average incidence of HZ was 5.23/1000

person-years in the UK. HZ incidence increased with ageing, and the incidence was higher in females than in males (**Figure 2**) (38).



Chapter 1. Figure 2 The HZ incidence rate in the UK by age group and gender (38)

Ageing is the most important risk factor of HZ, especially for those older than 50 years, because the immune system declines with age; this decline is also associated with decreased T-cell immunity against VZV (35). Another major risk factor for HZ is being severely immunocompromised, such as having certain diseases, including HIV infection, lymphoma, leukaemia or myeloma, or undergoing certain treatments, such as chemotherapy or immunosuppressants after organ transplantation (35). Furthermore, some diseases or their treatments are also associated with higher HZ risk, such as rheumatoid arthritis, inflammatory bowel disease, chronic obstructive

pulmonary disease, asthma, chronic kidney disease (CKD), depression, and diabetes (39).

1.2.3. Herpes zoster vaccination policies in the UK

Vaccination against VZV can prevent HZ and post-herpetic neuralgia. Two vaccines are available for HZ, a live attenuated vaccine, and a recombinant vaccine. For live attenuated vaccines, the vaccine efficacy is 61.1% against HZ and 66.5% against post-herpetic neuralgia among people over 60 years old (40); for the recombinant vaccines, the vaccine efficacy is 91.3% against HZ and 88.8% against post-herpetic neuralgia among people over 70 years old (41). Compared with the live attenuated vaccine, the recombinant vaccine has higher efficacy and is more cost-effective. In 2018 the Advisory Committee on Immunisation Practices (ACIP) recommended recombinant vaccine use in adults over 50 years old (42). Currently, both live attenuated and recombinant vaccines are approved in the UK.

A routine shingles vaccination program was initiated in the UK in 2013. From 2013 to 2014, the vaccine was only provided to people aged 70 and 79 years, but from 2014 to 2015, it became available to people aged 78 years (43, 44). However, in 2017, the eligibility criteria were revised. Previously eligible but unvaccinated people can receive the vaccination until their 80th birthday (45). Therefore, according to the latest regulations, people aged 70 to 74 years and 78 to 80 years are eligible for vaccination (**Table 2**) (46).

Chapter 1. Table 2 The eligibility criteria for the HZ vaccine and vaccine coverage in the UK

Year	Eligible age (years)	Vaccine Coverage[1] (%)
2013 – 2014 (43)	Routine: 70	61.8
	Catch-up: 79	59.6
2014 – 2015 (44)	Routine: 70	59
	Catch-up: 78, 79	57.8[2] - 58.5[3]
2015 – 2016 (47)	Routine: 70	54.9
	Catch-up: 78, 79	55.5
2016 – 2017 (45)	Routine: 70 – 73	48.3
	Catch-up: 78, 79	49.4
2017 – 2018 (48)	Routine: 70 – 74	44.4
	Catch-up: 78 – 80	46.2
2018 – 2019 (49)	Routine: 71 – 76	31.9
	Catch-up: 79 – 80	32.8
2019 – 2020 (50)	Routine: 70 – 77	26.5
	Catch-up: 78 – 80	25.8

1. Live-attenuated vaccine; 2. among aged 78 years; 3. Among aged 79 years

1.3. COVID-19

1.3.1. Epidemiology and impact of COVID-19

Coronavirus Disease 2019 (COVID-19) was first reported in Wuhan, China, in December 2019 and later induced a global pandemic (51). As of October 2021, more than 240 million cases had been reported globally, and nearly five million deaths had been recorded (52). More than 10,000,000 people have tested positive in the UK, and over 145,000 deaths have been reported (53). In addition to the casualties directly caused by COVID-19, pandemic-related interruption and collateral damage had tremendous economic and social impacts. In the UK, the utilisation of primary care for other diseases

has significantly decreased, and people's mental health has worsened (54, 55).

1.3.2. Pathogenesis, transmissions, and clinical symptoms

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus. The primary mode of transmission of SARS-CoV-2 is respiratory transmission, including large droplets or small aerosols; direct contact and fomite transmission are also possible transmission routes (56). After infection, the median incubation period is approximately five to six days, although people can be infectious one day before symptoms appear (56, 57). However, not every infected person becomes symptomatic. Approximately one-third of people who tested positive did not report any symptoms (58). Among symptomatic people, 81% have only mild symptoms, 14% need hospital care, and 5% require intensive care treatment (59). Common symptoms include fever, cough, dyspnoea, loss of smell/taste, and some people may also have fatigue, anorexia, myalgia, sore throat, diarrhoea, and nausea/vomiting (60). Acute COVID-19 symptoms usually resolve within four weeks, whereas some patients may experience ongoing symptoms longer than four weeks or even over 12 weeks (61).

1.3.3. Risk factors and diagnoses

SARS-CoV-2 can infect anyone who has close contact with confirmed cases, but some people have a higher risk of developing severe disease and mortality. Older adults, males, people of non-white ethnicity, those with low socioeconomic status, and pregnant women are at higher risk of severe

COVID-19. In addition, the hazards of adverse COVID-19 outcomes are increased for people with comorbidities such as diabetes, asthma, cardiovascular diseases, CKD, severe chronic respiratory diseases, and immunosuppressive status (60, 62).

COVID-19 diagnosis relies on a laboratory approach. Reverse transcription-polymerase chain reaction (RT-PCR), a nucleic acid amplification test (NAAT), is the standard for diagnosing COVID-19. Other standard diagnostic methods are rapid antigen tests, such as lateral flow tests used in the UK, which are cheaper and quicker than RT-PCR. A systematic review reported that among people with active COVID-19 symptoms, the overall sensitivity of rapid antigen test is approximately 70%, and the specificity is over 99% (sensitivity: 72%, 95%CI: 63.7–79.0%; specificity: 99.5%, 95%CI:98.5–99.8%) (63). However, the testing accuracy is influenced by the timing of testing and the quality of clinical specimens (64). A study indicated 89% of cases were identified from nasopharyngeal samples within four days after symptom onset, which decreased to 54% after ten days (65). The SARS-CoV-2 virus is more likely to be detected in clinical specimens obtained from the lower respiratory tract, such as bronchoalveolar lavage fluid (93%), compared with nasal (63%) or pharyngeal swabs (32%) (66). In addition, testing techniques also affect the accuracy of the COVID-19 tests (67).

1.3.4. Prevention and treatment of COVID-19

In addition to non-pharmacological interventions, vaccinations are effective against symptomatic infections, decreasing the chance of hospitalisation and death. The efficacies of the COVID-19 vaccines currently approved in the UK

are approximately 70% to 95% (68-70). Despite the emergence of more transmissible variants, the COVID-19 vaccines remain more than 70% effective in protecting against symptomatic disease (71).

The primary treatment for COVID-19 is supportive for people with mild or moderate symptoms (72). Molnupiravir, a new oral antiviral drug recently approved in the UK, was reported to reduce the risk of hospitalisation and death by 50% among people with mild to moderate COVID-19 symptoms (73, 74). For those who need inpatient care for oxygen supplementation, corticosteroids such as dexamethasone can reduce mortality (75). Monoclonal antibodies, such as casirivimab, imdevimab, and tocilizumab, are also recommended (76).

1.4. Is vitamin D a possible public health intervention for preventing viral diseases?

The previous section introduced the reported antimicrobial effects of vitamin D, which may be attributable to its immunomodulatory properties. However, epidemiological evidence on the association between vitamin D deficiency and an increased risk of viral infections is still lacking. If vitamin D deficiency is associated with viral infections or reactivation, such as COVID-19 or HZ, vitamin D supplementation may be a potential public health intervention for preventing these viral diseases. Although routine vitamin D supplementation is recommended for general health for adults in the winter, the evidence for immune support in preventing infections is still limited.

1.5. Chapter summary

- Vitamin D is essential to bone health. Studies have suggested that vitamin D may be associated with protection against some respiratory infections, perhaps through its immunomodulatory effects.
- It is unclear whether vitamin D is associated with protection against other viral infections.
- HZ, caused by VZV reactivation, leads to a significant disease burden, especially among older people and those with compromised immunity
- Vaccination against the VZV can effectively reduce the risk of HZ and post-herpetic neuralgia. This vaccine is available for people aged 70 to 80 years in the UK.
- SARS-CoV-2 is responsible for the COVID-19 pandemic, leading to five million deaths and a significant societal impact worldwide.
- Age, male sex, ethnicity, and severe immunosuppression are risk factors for severe diseases of COVID-19.
- Vaccines are effective at preventing severe health consequences of COVID-19.
- If vitamin D deficiency is associated with an increased risk of HZ and COVID-19, and vitamin D supplementation may be a cost-effective public health intervention.

Chapter 2. PhD Aim, Objectives, and Research Questions

Chapter overview

Following the brief overview of vitamin D, HZ, and COVID-19 in the previous chapter, this chapter frames the aim and research questions of this thesis.

2.1. PhD Aim

This PhD project aims to explore the association between vitamin D deficiency and the risk of HZ and COVID-19.

2.2. Objective 1: To systematically review the literature about serum vitamin D deficiency and the risk of infection with or reactivation of human herpesviruses.

2.2.1. Research Questions:

1. Is serum vitamin D deficiency/insufficiency associated with increased risk of infection with or reactivation of human herpesviruses?
2. Does oral vitamin D supplementation protect against infection with or reactivation of human herpesviruses?

2.3. Objective 2: To investigate the demographic, seasonal and regional factors associated with vitamin D deficiency in the UK Biobank.

2.3.1. Research Questions:

3. What is the distribution of serum vitamin D levels among UK Biobank participants?

4. What are the factors associated with vitamin D deficiency and insufficiency among UK Biobank participants?

2.4. Objective 3: To explore the association between serum vitamin D levels and the risk of HZ among people of middle and older ages using UK Biobank with linked EHRs.

2.4.1. Research Questions:

5. Does vitamin D deficiency increase the risk of HZ?

2.5. Objective 4: To explore the association between serum vitamin D levels and the risk of COVID-19 among people of middle and older ages using UK Biobank and linked EHRs.

2.5.1. Research Questions:

6. Does vitamin D deficiency increase the risk of SARS-CoV-2 infections, hospitalisation, or mortality due to COVID-19?

Chapter 3. Vitamin D deficiency or supplementation and the risk of human herpesvirus infections or reactivation: a systematic review and meta-analysis

Chapter overview

In the previous chapter, I elaborated on the aims and objectives of this thesis, namely, investigating the association between vitamin D, HZ and COVID-19. In this chapter, I present a review of the existing epidemiological evidence regarding the association between vitamin D and herpesviruses, which comprises two published papers, a systematic review, and a related study protocol.

In this chapter, I aim to answer two research questions:

1. Is serum vitamin D deficiency/insufficiency associated with increased risk of infection with or reactivation of human herpesviruses?
2. Does oral vitamin D supplementation protect against infection with or reactivation of human herpesviruses?

In this systematic review, I searched for the literature up to 31 August 2019. I further summarised papers published between September 2019 and November 2021 subsequently.

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SECTION A – Student Details

Student ID Number	1803144	**Title**	Mr
First Name(s)	Liang-Yu		
Surname/Family Name	Lin		
Thesis Title	The association between vitamin D deficiency and the risk of herpes zoster and COVID-19		
Primary Supervisor	Sinéad Langan		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMJ Open		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Liang-Yu Lin contributed to the design of the study and the manuscript writing, and I revised the paper according to other authors' comments. Charlotte Warren-Gash contributed to the design of the study, made critical comments on the protocol and revised the paper critically; Sinéad Langan contributed to the design of the study, made critical comments on the protocol and revised the paper critically. Ketaki Bhate, Harriet Forbes and Liam Smeeth contributed to the design of the study and revised the paper critically. All authors approved the final version of the protocol.

SECTION E

Student Signature	[redacted]
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Vitamin D deficiency or supplementation and the risk of human herpesvirus infections or reactivation: a systematic review protocol

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ABSTRACT

Introduction Human herpesviruses induce lifelong latent infections and may reactivate as the immune system deteriorates. Recent studies have suggested that vitamin D, an essential element of bone health, may have some effect of protecting against infections, but investigations of its potential to prevent herpesvirus infection or reactivation are limited. We will review the current literature examining vitamin D and the risk of herpesvirus infections or reactivation.

Methods and analysis Our systematic review will address two research questions: (1) Do deficient/insufficient serum vitamin D levels increase the risk of herpesvirus infections and (2) Does vitamin D supplementation protect against herpesvirus infections? We will include only intervention studies with control groups, cohort studies and case-control studies. We will use subject headings and keywords to search for synonyms of 'vitamin D' and 'herpesviruses' (including herpes simplex virus type 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus and human herpesviruses type 6, 7 and 8) in Medline, Embase, Global Health, Web of Science, Scopus and Cochrane Central Register of Controlled Trials, and the grey literature databases Open Grey, EThOS and BASE from inception to 31 August 2019. References to the included articles and relevant systematic reviews will also be examined. Two reviewers will independently screen the study titles and abstracts, and examine the full texts to decide the final eligibility. They will independently extract data from the studies and assess bias using the Cochrane Collaboration approach. A third researcher will solve any discrepancies. The results will be narratively synthesised; if an adequate number of studies is included and the homogeneity between studies is acceptable, a meta-analysis will be performed. We will assess the quality of evidence using the Grading of Recommendations, Assessment, Development and Evaluation framework, and display the results in a summary of findings table.

Ethics and dissemination Ethical review is not required for a systematic review. We will publish the results in a peer-review journal. Any amendments to the protocol will be recorded in the supplementary section.

PROSPERO registration number CRD42019130153.

Strengths and limitation of this study

- This systematic review will be undertaken following the predefined population, exposure, comparator and outcome framework.
- All available databases, including six major databases and threegrey literature databases, will be searched to obtain all eligible studies.
- The summarised results will improve our understanding of the current evidence of the possible association between vitamin D and herpesvirus infection/reactivation.
- The number of sufficient eligible studies may be inadequate, especially for some viruses that are harder to diagnose; also, the included studies may not have an adequate quality of evidence to answer the research questions.

INTRODUCTION

Rationale

Herpesviruses are a group of double-stranded DNA viruses that infect humans and some animals. After infecting their hosts, these viruses cannot be eradicated; instead, they establish latency and persist for life. As the host's immunity declines, these viruses can reactivate to induce various symptoms. There are eight human herpesviruses (table 1).[1] Reactivation of herpesviruses may induce serious complications. For instance, herpes simplex virus 1 (HSV-1) can lead to herpetic keratitis, which is the major cause of blindness in high-income countries[2]; Epstein-Barr virus may induce nasopharyngeal cancer[3] and varicella-zoster virus would cause herpes zoster and postherpetic neuralgia, which increases financial burdens, especially for people older than aged 65 years.[4] Consequently, investigating immunomodulatory factors associated with infection or reactivation of this virus family is important.

Table 1 List of human herpesviruses

Common name	Abbreviation
Herpes simplex virus type 1	HSV-1
Herpes simplex virus type 2	HSV-2
Varicella-zoster virus	VZV
Epstein-Barr virus	EBV
Cytomegalovirus	CMV
HHV-6 variant A	HHV-6A
HHV-6 variant B	HHV-6B
HHV-7	HHV-7
Kaposi's sarcoma-associated HV	KSHV

Vitamin D is mainly endogenously synthesised by the skin after sun exposure and can be supplied through dietary intake and supplementation. It plays an important role in absorbing calcium and phosphate, which are essential for bone health.[5] Recently, some studies have indicated that vitamin D may have potential immunomodulatory effects associated with the regulation of antimicrobial peptides (AMPs).[6] In previous cell studies, vitamin D induced gene expression of an AMP named cathelicidin. In response to pathogen exposure, immune cells such as monocytes or macrophages, upregulate vitamin D receptors and enzymes to increase the production of cathelicidin.[7–9] In addition, evidence suggests that vitamin D has some effects on the adaptive immune system. Vitamin D suppresses CD4+ T helper (Th)1 lymphocytes and increases Th2 lymphocytes, and it also intensifies the effect of regulatory T lymphocyte responses.[10 11] Regarding the effects of vitamin D-associated AMPs on herpesviruses, a cell study indicated that cathelicidin decreased HSV-1 viral titres isolated from patients with keratoconjunctivitis[12]; furthermore, another cell study also showed that vitamin D supplementation reduced HSV-1 viral load and mRNA expression in HSV-1-infected cells.[13]

Vitamin D also shows some anti-infective potential in epidemiological studies. A meta-analysis using original patient data from 25 randomised controlled trials showed that among the general population, vitamin D supplementation reduced the risk of acute respiratory infections.[14] Furthermore, there is some evidence to suggest an anti-infective effect of vitamin D in specific patient groups, such as patients with chronic kidney disease (CKD), human immunodeficiency virus (HIV) or hepatitis C virus (HCV) infection. Among patients with CKD receiving dialysis, a case-control study indicated that the risk of herpes zoster reactivation was significantly lower in those who received vitamin D supplementation[15]; another meta-analysis also showed that patients with CKD with higher or normal serum vitamin D levels had a lower risk of infection.[16] Among HIV-infected patients, lower serum vitamin D levels were also associated with a higher risk of clinical progression to AIDS and all-cause mortality in a cohort study,[17] while vitamin D supplementation did not affect mortality, CD4 cell count or viral load.[18] For HCV-infected patients, serum vitamin D levels were inversely associated with the grade of liver inflammation and the stage of fibrosis,[19] while no protective effect of vitamin D supplementation was seen in a meta-analysis of clinical trials.[20] However, the effect of vitamin D on herpesvirus infection or reactivation in the general population is unclear.

In this study, we will comprehensively review studies of the effect of serum vitamin D levels or the use of oral vitamin D supplementation on infection with or reactivation of any of the eight human herpesviruses.

OBJECTIVE

This review aims to explore the association between vitamin D and herpesviruses. The proposed systematic review will address two primary research questions:

1. Is serum vitamin D deficiency/insufficiency associated with an increased risk of infection with or reactivation of human herpesviruses?
2. Does oral vitamin D supplementation protect against infection with or reactivation of human herpesviruses?

METHODS

This study protocol will be reported according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols.[21]

Patient and public involvement

Patients and/or the public were not involved in this systematic review protocol.

Eligibility criteria

Study design and characteristics

To identify the possible causal association between vitamin D and herpesvirus infections, we will review observational studies, including cohort and case-control studies, and intervention studies with any type of control group, either placebo, active comparator or no treatment, including randomised or non-randomised controlled trials. Descriptive studies, ecological studies, cross-sectional studies, case reports and case series will not be included. If a systematic review relevant to our topic is found, we will review its references.

Participants

Only human studies will be included in our review, and animal or cell studies will be excluded. Studies from all age groups or involving patients with any immune status are eligible.

Exposure

We have two exposures of interest for each research question in our review. For the first research question, the exposures are deficient or insufficient serum vitamin D levels in the participants. We will include articles in which the serum vitamin D levels are identified as deficient or

Table 2 The normal range of serum vitamin D levels adapted in different studies

Study	Deficiency (nmol/L)	Insufficiency (nmol/L)	Adequate (nmol/L)
Pearce and Cheetham[26]	<25	25–50	50–75
Institute of Medicine[27]	<30	30–50	≥50
Holick *et al*[28]	<50	52.5–72.5	
Hanley *et al*[29]	<25	25–75	

1 nmol/L=0.4 ng/mL.

insufficient. Notably, no consensus exists about serum vitamin D levels, and different studies may use different definitions (table 2). If eligible studies provide original values for the serum 25(OH)D levels, we will define vitamin D deficiency as 25(OH)D<25 nmol/L and insufficiency as 25(OH)D in the range from 25 to 50 nmol/L in accordance with the standards of Public Health England.[22]

For the second research question, the intervention is oral vitamin D supplementation or oral vitamin D analogue treatment used for secondary hyperparathyroidism or osteoporosis (online supplementary table 1). We will exclude studies using topical vitamin D analogues, because their effects on systemic serum vitamin D levels are unknown.

Comparators

The comparator groups for vitamin D insufficiency and deficiency are those with sufficient serum vitamin D levels. For those receiving oral vitamin D supplementation or treatment, the comparators are those without vitamin D supplementation or treatment or receiving placebo or another active comparator, respectively.

Outcomes

The outcomes will be infection with or reactivation of all eight human herpesviruses listed in table 1. Infection with and reactivation of herpesviruses are defined using clinical or laboratory criteria. The clinical criteria include patients presenting with classical symptoms, for instance, the painful rash of herpes zoster, or a diagnosis recorded by physicians. Laboratory criteria include using laboratory techniques to confirm the diagnosis, such as evaluation of the serum viral load by PCR. Studies reporting only serum antibodies against herpesviruses will not be included.

Information sources

We will search the following database from inception to 31 August 2019: Medline (Ovid), EMBASE (Ovid), Web of Science, Scopus, Cochrane Library and Global Health (Ovid). To enhance the sensitivity of our search and reduce the risk of publication bias, we will also search grey literature databases such as Open Grey, BASE, EThOS and the clinical trials register at ClinicalTrials.gov.

Search strategy

We will search for synonyms of 'human herpesviruses' and 'vitamin D' using both controlled vocabularies and keywords in each database. A search strategy in Medline (Ovid) is listed in online supplementary table 2. The subject headings will be modified for different databases. The results will be combined using the Boolean logic operator 'AND'. For some database with limited search functions, such as single line search, keywords will be split into small sections to fulfil the requirement (online supplementary table 3). In addition to searching electronic databases, we will manually search for the reference lists of the included articles and relevant systematic reviews.

Study records

Data management

One researcher will import the search results from different databases into the citation management software EndNote X9 (Clarivate Analytics, V.9.1/2019). Duplicated results will be identified and deleted.

Selection process

Two researchers will independently screen the titles and abstracts of all identified studies. We will obtain full texts of all studies fulfilling the review criteria, and the two researchers will screen all articles to establish their eligibility for inclusion. Any discrepancy in the reviewing process will be adjudicated by the third researcher.

Data collection process

Data extraction for the first three studies will be performed by two independent researchers to ensure the integrity of the process; then, one researcher will extract data from the remaining studies. If any data are missing or unclear, we will contact the authors for further clarification and information.

Data items

We will summarise and extract data from the included studies using a Population, Exposure/Intervention, Comparator, Outcomes and Study characteristics framework as follows:

1. Population: sample sizes of each study, inclusion or exclusion criteria for the participants and demographic characteristics, such as sex, age or immune status

2. Exposure 1: insufficient or deficit serum vitamin D levels of the study participants. Exposure 2: oral vitamin D supplementation or vitamin D analogue
3. Comparator 1: sufficient serum vitamin D levels among the participants. Comparator 2: without vitamin D supplementation or vitamin D analogue use.
4. Outcomes: definition of herpesvirus infection/reactivation, that is, clinically diagnosed or laboratory-confirmed herpesvirus infections; the number of study subjects with the outcomes
5. Study characteristics: publication details (authors, publication year, country and journal), study designs, confounders measured, confounders adjusted and study results

Outcomes and prioritisation

The outcomes of the studies are herpesvirus infections or reactivation. The disease can be diagnosed either clinically or through laboratory confirmation. Some herpesvirus infections, such as herpes zoster, may lead to characteristic clinical symptoms, and some laboratory approaches, such as PCR, can detect the viral load, which can also be proof of herpesvirus infection/reactivation. Studies measuring serum antiherpesvirus antibodies will not be included, because antibodies detected in the absence of clinical symptoms cannot ascertain the timing of infection. Regarding the study results, our focus is on the incidence of herpesviruses infection or reactivation. We will report the outcome definition used for each study, and will extract data on the appropriate effect measure. The effect measures will include ORs for case-control studies and risk, rate or HRs for cohort studies or clinical trials. If studies report only continuous outcomes such as viral load, then we will summarise these using means or medians as appropriate.

Risk of bias in individual studies

We will assess the risk of bias from the included studies using a template form based on the Cochrane approach for trials and observational studies, and all relevant domains of bias will be assessed for different study types. For randomised controlled trials, we will evaluate bias due to the following sources: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants, personnel or assessment; (4) incomplete outcome data and (5) selective reporting. For observational studies, we will consider bias due to the following sources: (1) confounding factors; (2) selection of participants or controls; (3) differential and non-differential misclassification of the exposure or outcome; (4) reverse causation and (5) missing data (online supplementary table 4). A piloted form will be tested by applying it to extract data from the first three studies. To ensure the quality and consistency of the risk of bias assessment, the first three studies will be evaluated by two independent researchers. Then, one researcher will complete the evaluation of the remaining included studies. Any discrepancies will be examined by the third researcher.

Data synthesis and meta-bias (es)

We will comprehensively search different databases including grey literature databases to minimise bias in our search. We will use a narrative synthesis to summarise the data and results from the eligible studies included in our review. Since each herpesvirus subtypes have different pathogenic pathways, we will display the results separately by virus. If an adequate number of studies with acceptable homogeneity are included, a meta-analysis will be performed to integrate the study results. We will decide whether to use fixed-effects or random-effects models by considering the I^2 value for heterogeneity; values >50% will be considered to represent substantial heterogeneity.[23] If the number of included studies is sufficient, we will carry out subgroup analyses of subjects with baseline vitamin D status, intervention trials, study durations, different ages, immune statuses or latitude to explore sources of heterogeneity. A funnel plot will be used to present the distribution of studies and examine any possible publication bias.[24] All statistical analyses will be performed by using STATA V.15.

Confidence in cumulative evidence

We will evaluate the quality of evidence using the Grading of Recommendations, Assessment, Development and Evaluations framework.[25] We will evaluate the design and risk of bias across all included studies. Studies with significant effects, strong dose responses or that are well adjusted for plausible confounders will receive higher grades, whereas those with a higher risk of bias, inconsistency, indirectness or imprecision will be downgraded. We will conclude the quality of evidence using a 'Summary of Findings' table and assign each outcome a quality rank of 'high', 'moderate', 'low' or 'very low' for the level of confidence.

Contributors L-YL contributed to the design of the study, drafted the introduction, methods and analysis and revised the protocol according to other authors' comments; CW-G contributed to the design of the study, made critical comments on the protocol and revised the paper critically; SML contributed to the design of the study, made critical comments on the protocol and revised the paper critically; KB, HF and LS contributed to the design of the study and revised the paper critically. All authors approved the final version of the protocol.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical review is not required for a systematic review. We will publish the results in a peer-review journal. Any changes in the study protocol will be recorded and reported. Any revision of the protocol will be recorded in the supplementation of the final published review.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES

1. Knipe DM, Howley P. Fields virology: Wolters Kluwer health 2013:1806; 2013.
2. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. *Cornea* 2001;20:1–13.
3. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. *Nat Rev Cancer* 2016;16:789–802.
4. Scott FT, Johnson RW, Leedham-Green M, *et al*. The burden of herpes zoster: a prospective population based study. *Vaccine* 2006;24:1308–14.
5. Holick MF, deficiency VD. Vitamin D deficiency. *N Engl J Med Overseas Ed* 2007;357:266–81.
6. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;415:389–95.
7. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (cAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *Faseb J* 2005;19:1067–77.
8. Wang T-T, Nestel FP, Bourdeau V, *et al*. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004;173:2909–12.
9. Weber G, Heilborn JD, Chamorro Jimenez CI, *et al*. Vitamin D induces the antimicrobial protein hCAP18 in human skin. *J Invest Dermatol* 2005;124:1080–2.
10. Cantorna MT, Yu S, Bruce D. The paradoxical effects of vitamin D on type 1 mediated immunity. *Mol Aspects Med* 2008;29:369–75.
11. Mann EH, Pfeffer PE, Hawrylowicz CM. *Vitamin D and adaptive immunology in health and disease*. Fourth ed. Elsevier, 2018: 937–49.
12. Gordon YJ, Huang LC, Romanowski EG, *et al*. Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. *Curr Eye Res* 2005;30:385–94.
13. Kumar A, Singh MP, Kumar RS, *et al*. 25-Hydroxyvitamin D3 and 1,25 dihydroxyvitamin D3 as an antiviral and immunomodulator against herpes simplex virus-1 infection in HeLa cells. *Viral Immunol* 2018;31:589–93.
14. Martineau AR, Jolliffe DA, Hooper RL, *et al*. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;356.
15. Chao C-T, Lai C-F, Huang J-W. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. *Eur J Intern Med* 2012;23:711–5.
16. Su G, Liu Z, Qin X, *et al*. Vitamin D deficiency and treatment versus risk of infection in end-stage renal disease patients under dialysis: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2019;34:146–56.
17. Viard J-P, Souberbielle J-C, Kirk O, *et al*. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. *AIDS* 2011;25:1305–15.
18. Visser ME, Durao S, Sinclair D, *et al*. Micronutrient supplementation in adults with HIV infection. *Cochrane Database Syst Rev* 2017;5:CD003650.
19. Petta S, Cammà C, Scazzone C, *et al*. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010;51:1158–67.
20. Bjelakovic G, Nikolova D, Bjelakovic M, *et al*. Vitamin D supplementation for chronic liver diseases in adults. *Cochrane Database Syst Rev* 2017;11:CD011564.
21. Shamseer L, Moher D, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;349:g7647.
22. The Scientific Advisory Committee on Nutrition. Vitamin D and health: public health England; 2016.
23. Deeks JJ, Higgins JPT, Altman DG. 9.5.2 Identifying and measuring heterogeneity. In: Douglas G, Altman D, eds. *Cochrane Handbook for systematic reviews of intervention version 510*. The Cochrane Collaboration, 2011.
24. Sterne JAC, Egger M, Moher D. 10.4.1 Funnel plots. In: Moher D, ed. *Cochrane Handbook for systematic reviews of intervention version 510*. The Cochrane Collaboration, 2011.
25. Guyatt GH, Oxman AD, Vist GE, *et al*. Grade: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924–6.
26. Pearce SHS, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;340:b5664.
27. Institute of Medicine. *Dietary reference intakes for calcium and vitamin D*. Washington (DC: National Academies Press (US), 2011.
28. Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al*. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911–30.
29. Hanley DA, Cranney A, Jones G, *et al*. Vitamin D in adult health and disease: a review and guideline statement from osteoporosis Canada (summary). *Can Med Assoc J* 2010;182:1315–9.

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Student ID Number	1803144	**Title**	Mr
First Name(s)	Liang-Yu		
Surname/Family Name	Lin		
Thesis Title	The association between vitamin D deficiency and the risk of herpes zoster and COVID-19		
Primary Supervisor	Sinéad Langan		

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Liang-Yu Lin contributed to the design of the study, searched for databases, extracted data, assessed the risk of bias, underwent statistical analysis, and drafted and revised the manuscript according to other authors' comments. Ketaki Bhate contributed to the design of the study, extracted data, assessed the risk of bias, and revised the paper critically. Harriet Forbes contributed to the design of the study and revised the paper critically; Charlotte Warren-Gash contributed to the design of the study protocol, determined study eligibility, made critical comments on the manuscript, and revised the paper critically; Sinéad Langan contributed to the design of the study, determined study eligibility, made critical comments on the manuscript, and revised the paper critically; Liam Smeeth contributed to the design of the study and revised the paper critically. All authors approved the final version of the manuscript.

SECTION E

Student Signature	[redacted]
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REVIEW ARTICLE

Vitamin D Deficiency or Supplementation and the Risk of Human Herpesvirus Infections or Reactivation: A Systematic Review and Meta-analysis

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Background. Vitamin D may protect against respiratory virus infections, but any association with herpesviruses is unclear.

Methods. We undertook a systematic review of vitamin D deficiency or supplementation and the risk of 8 human herpesviruses. Six databases and 4 gray literature databases were searched for relevant cohort studies, case–control studies, and clinical trials.

Results. Ten studies were included, all conducted among immunosuppressed patients. There was no evidence that vitamin D deficiency is associated with cytomegalovirus (CMV) disease (pooled risk ratio, 1.06; 95% CI, 0.66–1.7), herpes zoster after transplantation (1 study), or HHV-8 among HIV patients (1 study). Vitamin D supplementation may decrease herpes zoster among hemodialysis patients (1 study) or CMV disease after renal transplantation (1 study), but supplementation was not associated with reduced EBV viral load among multiple sclerosis patients (1 study).

Conclusions. Any association between vitamin D and herpesviruses remains inconclusive. Further studies in the general population are needed.

Keywords. cytomegalovirus; Epstein-Barr virus; herpes zoster; herpesviridae; systematic review; vitamin D deficiency; vitamin D supplementation.

Herpesviruses are a family of 8 DNA viruses that induce lifelong latency after infecting humans; they include herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), HHV-6, HHV-7, and Kaposi's sarcoma–associated herpesvirus (KSHV). These viruses can reactivate and lead to clinical symptoms when the immunity of the host declines [1]. Although many primary herpesvirus infections are mild or self-limited, both infection and reactivation may lead to rare but serious complications that affect quality of life and lead to a substantial burden on health care services. For example, VZV causes chickenpox among children and herpes zoster in adults. Especially among people older than age 65 years, zoster may lead to post-herpetic neuralgia, which is associated with an increased risk of cardiovascular outcomes and financial burden [2, 3]. CMV infection is usually asymptomatic in healthy adults, but infection of immunocompromised hosts can lead to graft loss or death [4]. Consequently, it is important to explore the immunomodulatory factors associated with infection or reactivation of herpesviruses.

Vitamin D is synthesized by the skin after sunlight exposure, or it can also be consumed through food or supplements. Its concentration in the blood is greatly affected by season and latitude as well as nutritional intake. This vitamin plays an essential role in absorbing calcium and phosphate, which are important to bone health. Vitamin D deficiency may lead to rickets in children or osteomalacia in adults [5]. Currently, no consensus exists about the threshold serum levels for defining vitamin D deficiency. Some studies have defined vitamin D deficiency as serum 25(OH)D levels <50 nmol/L[6], while other studies and Public Health England recommendations have used 25(OH)D levels <25 nmol/L as their cutoff [7, 8]. To protect bone health, Public Health England recommends people taking 10 μg (400 IU) of vitamin D every day in the winter [9].

In addition to bone health, some studies have shown that this vitamin may have some immunomodulatory effects and anti-infective potential. At a cellular level, some studies have shown that vitamin D regulates the production of the antimicrobial peptide cathelicidin [10–13], and 1 study indicated that vitamin D supplementation was associated with a decrease in HSV-1 viral load and mRNA expression in HSV-1-infected cells [14]. In addition, among epidemiological studies, a meta-analysis showed that the risk of infection was lower in chronic kidney disease patients with

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normal or higher serum vitamin D levels [15], and another meta-analysis using original patient data from 25 randomized controlled trials showed that people receiving vitamin D supplementation had a lower risk of respiratory tract infection [16].

However, whether vitamin D is associated with protection against herpesviruses is still unclear, and no comprehensive review exists of this possible association. As vitamin D supplementation is an inexpensive public health intervention, studying its possible association with herpesviruses may help us find a novel approach to mitigate the health impact of these infectious diseases. We therefore undertook a systematic review to examine the relationship between serum vitamin D levels or oral vitamin D supplementation and the risk of infection with or reactivation of any of the 8 human herpesviruses.

METHODS

Protocol and Registration

The protocol of this study has been previously registered on PROSPERO (registration number: CRD42019130153) and published [17].

Eligibility Criteria

As previously described, we included only human studies in our review [17]. The exposures were serum vitamin D deficiency or oral vitamin D supplementation, including vitamin D analog treatment. Vitamin D deficiency was defined as serum 25(OH) D <25 nmol/L, to be consistent with the Public Health England approach. The comparator for vitamin D–deficient participants was people with sufficient serum vitamin D levels, and the comparator for vitamin D supplementation was participants without vitamin D supplementation or a placebo group. The outcomes were infection or reactivation of any human herpesvirus, confirmed based on physicians' clinical diagnoses or by laboratory-based techniques such as polymerase chain reaction. For more rigorous causal estimation, only cohort, case–control, and intervention studies were eligible to be included.

Information Sources and Search

One researcher (L.Y.L.) searched 6 main databases and 4 gray literature databases. The main databases were Medline (Ovid), EMBASE (Ovid), Web of Science, Scopus, the Cochrane Library, and Global Health (Ovid). The gray literature databases included Open Grey, BASE, EThOS, and the clinical trials register at ClinicalTrials.gov. The search was updated to August 31, 2019.

The search strategy was reviewed and revised by a librarian. Synonyms of "human herpesviruses" and "vitamin D" were searched using both controlled vocabularies and keywords in different databases, in which subject headings were modified. The search results were combined using the Boolean logic operator "AND." One author (L.Y.L.) also hand-searched the final included studies to identify potential eligible articles. Duplicated search results were removed using EndNote X9 [18].

Study Selection, Data Collection, and Data Items

The titles and abstracts of the included articles were independently screened by 2 researchers (L.Y.L. and K.B.), and the full texts of the eligible studies were further examined. Any discrepancy in these processes was resolved by a third researcher (S.M.L.). Two researchers (L.Y.L. and K.B.) independently extracted data from the first 3 studies using a predefined form, and 1 researcher (L.Y.L.) extracted the other included articles. The complete data extraction form is available in the Supplementary Data. Briefly, data were extracted following the framework of population, exposure/intervention, comparator, and outcomes. In addition, study characteristics such as study design, study population, results, statistical analysis methods, and confounders were extracted. The numbers of subjects with outcomes were obtained for each exposure group, and the reported crude results and adjusted results were recorded. If a study reported >1 vitamin D outcome, such as deficiency, insufficiency, and sufficiency, only the outcomes of vitamin D deficiency and sufficiency were extracted. For studies reporting continuous outcomes, their mean or median change of outcome and standard deviation were recorded. The authors of the included studies were contacted for unclear or missing data.

Risk of Bias in Individual Studies

Predefined templates based on the Cochrane approach were used to assess the risk of bias in the included studies. For observational studies, 5 domains were assessed: confounding factors, selection of participants, misclassification of variables, bias due to missing data, and reverse causation. For intervention studies, bias was assessed using version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2) to assess random sequence generation; allocation concealment; blinding of participants, personnel, and assessment; incomplete outcome data; and selective reporting [19]. The assessment tools were piloted and tested, and the risk of bias of the first 3 included studies was assessed by 2 independent researchers (L.Y.L. and K.B.) to ensure consistency and quality. One researcher (L.Y.L.) evaluated the risk of bias of the other studies. Discrepancies in the assessment were resolved by a third researcher (S.M.L. or C.W.G.).

Summary Measures

The results of the included studies were synthesized by vitamin D exposure and by different herpesviruses using a narrative approach. The main adjusted risk ratio (RR), hazard ratio (HR), or odds ratio (OR) was presented for each study. If the numbers of outcomes among exposure and comparator groups were reported without an estimate of relative risk, the unadjusted risk ratio and 95% confidence interval were calculated. For the studies with similar study designs,

exposures, and outcomes, we further assessed their heterogeneity and synthesized them using a meta-analysis. The heterogeneity between studies was assessed using I^2, and a random-effects meta-analysis was used for substantial heterogeneity ($I^2 > 50\%$). A funnel plot was used to evaluate publication bias [20]. All analysis was conducted using Stata, version 16/IC (StataCorp, College Station, TX, USA).

Risk of Bias Across Studies

For studies reporting similar exposures and outcomes, we further analyzed the risk of bias. The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) framework was used to assess the overall quality of evidence [21]. The levels of confidence were established through the design of included studies, which were further downgraded or upgraded according to the domains of risk of bias, inconsistency, indirectness, imprecision, publication bias, strong association, dose response, and confounding. A rank of "high," "moderate," "low," or "very low" was given to the quality of evidence.

Patient Consent Statement

Patient consent is not required for publication. Ethical review is not required for a systematic review.

RESULTS

Study Characteristics

Figure 1 displays the steps to study selection. In our search, 4537 articles were initially identified. After removing duplicated studies, we scanned 2548 titles and abstracts. We excluded articles that did not meet our inclusion and exclusion criteria, and we reviewed the full text of 62 articles for eligibility.

Ten studies were finally included in our review, and their characteristics are summarized in Table 1. They were 8 cohort studies, 1 case–control study, and 1 randomized controlled trial. All cohort and case–control studies were undertaken in single-hospital settings, while the only randomized controlled trial used data from 5 hospitals in the Netherlands. All studies were conducted in different regions: 4 studies from Asia, 3 from Europe, 2 from North America, and 1 from Africa. All studies were conducted with patients with the following underlying

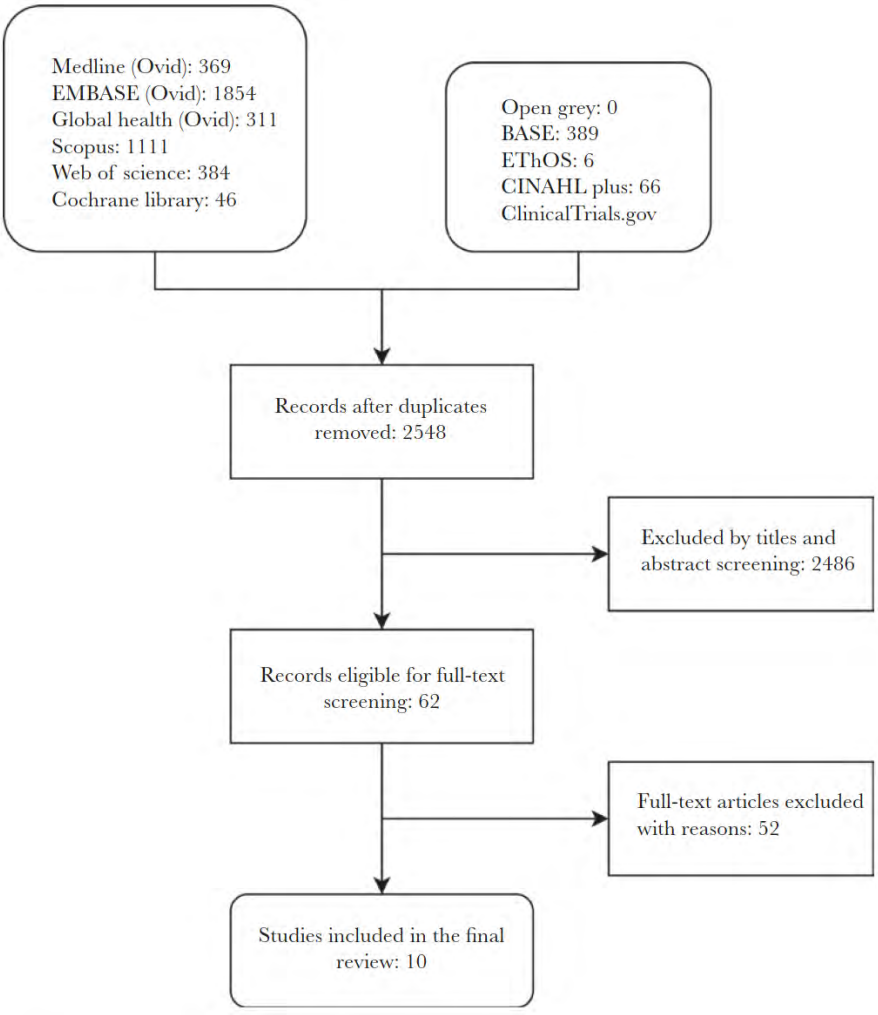

Figure 1. Flow diagram of study selection.

Table 1. Characteristics of the Included Studies

Author, Year	Design	Study Period	Setting	Study Population at Recruitment	Intervention/Exposure Definition and Ascertainment	Comparator Definition and Ascertainment	Outcome Type	Outcome Definition and Ascertainment
Chao et al. (2012) [24]	Case–control study	January 2000 to December 2009	Taiwan, single-hospital study	Patients received dialysis	Vitamin D supplementation (1α-hydroxylated vitamin D)	No vitamin D supplementation	Primary	Clinically diagnosed herpes zoster
Erlandson et al. (2014) [23]	Historical cohort	June 2003 to May 2005	Zimbabwe, single-hospital study	Adult patients with HIV-1 and Kaposi's sarcoma	Serum 25(OH)D levels (nmol/L): • Deficiency: ≤50	Serum vitamin D levels (nmol/L): • Adequate ≥75 nmol/L	Secondary	HHV-8 viral load in plasma and peripheral blood mononuclear cells
Lee et al. (2014) [43]	Historical cohort	January 2005 to December 2010	USA, single-hospital study	Patients received kidney transplantation	Serum 25(OH)D levels (ng/mL): ≤ 20	Serum 25(OH)D levels (ng/mL): > 20	Secondary	Laboratory-confirmed CMV disease
Saber et al. (2015) [33]	Prospective cohort study	June 2013 to December 2013	Iran, single-hospital study	Potential kidney transplant patients	Serum 25(OH)D levels (ng/mL): • Deficiency: <15	Serum 25(OH)D levels (ng/mL): • Normal: >30	Primary	Laboratory-confirmed CMV infection
Moscarelli et al. (2016) [25]	Historical cohort	May 2005 to August 2014	Italy, single-hospital study	Patients received kidney transplantation	Vitamin D supplementation (calcitriol)	No vitamin D supplementation	Primary	Laboratory-confirmed CMV infection
Ban et al. (2017) [22]	Historical cohort	January 2011 to December 2013	Korea, single-hospital study	Patients received kidney transplantation	Serum 25(OH)D levels in low tertile groups (≤8.3 ng/mL)	25(OH)D levels in the high tertile group (>12.1 ng/mL)	Secondary	Laboratory-confirmed CMV infection and clinically diagnosed herpes zoster
Park et al. (2017) [32]	Historical cohort	January 2011 and December 2013	Korea, single-hospital study	Patients received kidney transplantation	Serum 25(OH)D levels (ng/mL): <20	Serum 25(OH)D levels (ng/mL): ≥20	Primary	Laboratory-confirmed CMV infection
Rolf et al. (2018) [26]	Randomized controlled trial	March 2011 to February 2014	The Netherlands, a substudy of a clinical trial	Patients with relapsing-remitting multiple sclerosis	Vitamin D supplementation (calcitriol)	Placebo	Secondary	EBV viral loads in peripheral blood mononuclear cells and B cells
Astor et al. (2019) [35]	Historical cohort	January 2004 to June 2014	USA, single-hospital study	Adult patients (age >18 y) received kidney transplantation	Serum 25(OH)D levels (ng/mL): Deficiency: <20	Serum 25(OH)D levels (ng/mL): ≥30	Primary	Laboratory-confirmed CMV infection
Fernandez-Ruiz et al. (2019) [34]	Prospective cohort	November 2014 to December 2016	Spain, single-hospital study	Patients received kidney transplantation	Serum 25(OH)D levels (ng/mL): <20	Serum 25(OH)D levels (ng/mL): ≥20	Primary	Laboratory-confirmed CMV disease

Abbreviations: CMV, Cytomegalovirus, HIV, human immunodeficiency virus.

[a]1 nmol/L = 0.4 ng/mL.

health conditions: end-stage renal disease requiring hemodialysis (n = 1), organ transplants (n = 7), multiple sclerosis (n = 1), and HIV (n = 1). Seven included studies analyzed the association between serum vitamin D levels and herpesvirus infections, and the other 3 studies assessed the correlation between vitamin D supplementation and herpesviruses.

Among studies assessing serum vitamin D status and the risk of different herpesvirus infections or reactivation, the definition of vitamin D deficiency varied. Five cohort studies defined vitamin D deficiency as serum 25(OH)D below 20 ng/mL or 50 nmol/L, 1 study used vitamin D levels <15 ng/mL (37.5 nmol/L), and the other used tertiles to classify its participants, with the mean vitamin D level in the lowest tertile being 20.78 nmol/L.

Among studies of vitamin D supplementation and the risk of herpesviruses infection or reactivation, 2 used activated vitamin D analog (calcitriol or 1α-hydroxylated vitamin D) supplementation, and the other used inactive vitamin D3 (cholecalciferol) supplementation (Table 1).

Meta-analysis of Vitamin D Deficiency and the Risk of CMV Infection or Reactivation Among Patients With Organ Transplants

A random-effects meta-analysis was conducted to pool 6 observational studies, which analyzed the association between serum vitamin D deficiency and the risk of CMV disease after transplantation therapy. No evidence was found that serum vitamin D deficiency increased the risk of CMV infection or reactivation in patients receiving transplants (RR, 1.06; 95% CI, 0.66–1.70) (Figure 2). The sample sizes of these studies were relatively small (Table 2), and the heterogeneity between studies was high (I^2 = 55.4%). Five of the included studies did not adjust for confounding factors, and bias due to missing data was unclear in 2 studies (Table 3). Due to the high risk of bias and imprecise estimates of these studies, the overall quality of evidence was low (Table 4). The funnel plot of the included studies showed a relatively symmetric pattern, which suggests that the risk of publication bias was low (Figure 3).

Vitamin D Deficiency and Herpesvirus Infection or Reactivation

One cohort study [22] also investigated whether vitamin D deficiency was associated with the risk of developing herpes zoster after kidney transplantation, showing no evidence of association (RR, 0.98; 95% CI, 0.337–2.87). Another study investigated the association between serum vitamin D levels and change in HHV-8 viral load among HIV-positive patients recruited from a trial [23]. It showed no evidence that inadequate vitamin D levels were associated with higher viral load in plasma (viral load decreased by 0.5 [0 to 1.5] log copies/mL in the inadequate vitamin D group and by 0.4 [0 to 1.5] log copies/mL in the adequate vitamin D group; P = .8) or in peripheral blood mononuclear cells (viral load decreased by 0.9 [−0.4 to 2.3] log copies/mL among the inadequate vitamin D group and by 1.0 [0 to 2.0] log copies/mL in the adequate vitamin D group; P = .9) (Table 2). Nevertheless, both studies had a high risk of bias in at least 1 domain (Table 3).

Vitamin D Supplementation and the Risk of Herpesvirus Infection or Reactivation

One case–control study (n = 126) indicated that hemodialysis patients receiving vitamin D supplementation had a lower risk of developing herpes zoster (OR, 0.06; 95% CI, 0.0–0.4) (Table 2) [24]. The assessed risk of bias in this study was moderate in 1 domain (Table 3).

A historical cohort study assessed the association between vitamin D supplementation before transplantation and the risk of CMV after transplantation [25]. The group unexposed to

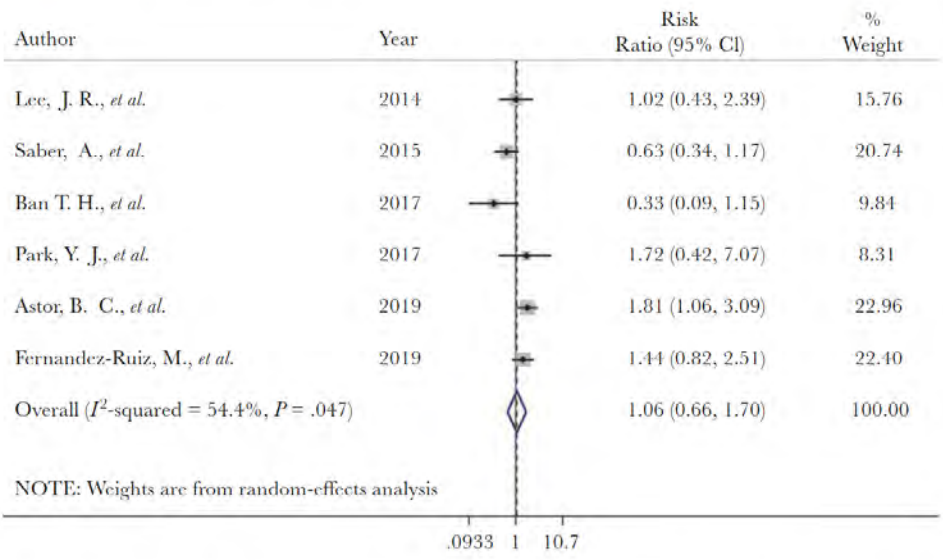

Figure 2. Forest plot of the summary of the effects of vitamin D deficiency on CMV risk after transplantation. Abbreviations: CMV, cytomegalovirus; RR, risk ratio.

Table 2. Summary of Results

Author, Year	Population Size, No.; Mean/Median Follow-up Time, mo	Subjects With Outcome [or Exposure for Case–Control Studies], No. (%)	Statistical Analysis Method Used	Main Reported Results	Covariates Adjusted for:
Vitamin D deficiency					
Serum vitamin D levels before transplantation and the risk of CMV infection after transplantation					
Lee et al. (2014)	n = 351; followed for 12 mo	Deficiency: n = 13/216 (6%) Sufficiency: n = 8/135 (5.9%)	Not reported	Risk ratio, 1.02 (95% CI, 0.43–2.39) [calculated by review authors]	Not adjusted
Saber et al. (2015)	n = 82; followed for 4 mo	Deficiency: n = 15/41 (37%) Sufficient: n = 7/12 (58%)	Not reported	Risk ratio, 0.6 (95% CI, 0.37–0.96) [calculated by review authors]	Not adjusted
Ban et al. (2017)	n = 174; median follow-up period 35.5 mo	CMV infection: Low tertile: n = 3/59 (5.1%) High tertile: n = 9/58 (15.8%)	Not reported	CMV: Risk ratio, 0.328 (95% CI, 0.09–1.15) [calculated by review authors]	Not adjusted
Park et al. (2017)	n = 164; followed for 24.8 mo	Deficiency: n = 16/135 (11.9%) Sufficiency: n = 2/29 (6.9%)	Not reported	CMV: Risk ratio, 1.72 (95% CI, 0.42–7.07) [calculated by review authors]	Not adjusted
Astor et al. (2019)	n = 1976; followed for 12 mo	Not reported	Cox proportional hazard regression models	Serum 25(OH)D ≥30 ng/mL: reference Serum 25(OH)D <20 ng/mL: 1.81 (95% CI, 1.06–3.09)	Age, sex, ethnicity, cause of ESKD, BMI, donor status, prior transplant, delayed graft function, induction immunosuppression, smoking status, HLA mismatch category, CMV serostatus, time from transplant to 25(OH)D measurement, history of acute rejection, estimated glomerular filtration rate category, season, maintenance immunosuppression, and quartile of calcineurin inhibitor level
Fernandez-Ruiz et al. (2019)	n = 215; followed up for at least 12 mo	Vitamin D deficiency: n = 34/135 (25.2%) No deficiency: 14/80 (17.5%)	Not reported	CMV: Risk ratio, 1.44 (95% CI, 0.82–2.51) [calculated by review authors]	Not adjusted
Serum vitamin D levels before transplantation and the risk of herpes zoster after transplantation					
Ban et al. (2017)	n = 174; median follow-up period 35.5 mo	Herpes zoster: Low tertile: n = 6/59 (10.2%) High tertile: n = 6/58 (10.3%)	Not reported	Herpes zoster: Risk ratio, 0.98 (95% CI, 0.337–2.87) [calculated by review authors]	Not adjusted
Serum vitamin D levels and the change of serum HHV-8 viral load					
Erlandson et al. (2014)	n = 85; followed for 24 mo	Not applicable	Mann-Whitney test	A decrease in HHV-8 viral load (log), median (IQR): In plasma: • Inadequate: 0.5 (0–1.5) • Adequate: 0.4 (0–1.5) In PBMC: • Inadequate: 0.4 (0–1.5) • Adequate: 1.0 (0–2.0)	Not adjusted
Vitamin D supplementation					
Vitamin D supplementation and the risk of herpes zoster among dialysis patients					
Chao et al. (2012)	n = 126; followed at least 1 mo before the event	Exposure among HZ cases: 3/63 (5.4%); exposure among controls: 29/63 (46%)	Conditional logistic regression model	OR, 0.06 (95% CI, 0.0–0.4)	Hepatitis or cirrhosis, cerebrovascular accident, use of iron therapy, use of corticosteroids, use of statins, CRP, intact PTH, ferritin
Vitamin D supplementation and risk of CMV infection after transplantation					

Table 2. Continued

Author, Year	Population Size, No.; Mean/Median Follow-up Time, mo	Subjects With Outcome [or Exposure for Case–Control Studies], No. (%)	Statistical Analysis Method Used	Main Reported Results	Covariates Adjusted for:
Moscarelli et al. (2016)	n = 360; followed for 12 mo	Nonuser group: n = 21 (9%); user group: n = 4 (3%)	Cox proportional hazards regression	HR, 2.31 (95% CI, 1.44–3.71)	Serum 1,25(OH)$_2$D3 deficiency, biopsy-proven acute rejection, BKV infection, CMV serostatus, steroid boluses, BMI
Vitamin D supplementation and change of EBV viral load in blood cells					
Rolf et al. (2018)	n = 53; followed for 7.3 mo	Not applicable	Mann-Whitney *U* test	Fold change relative to T0: Treatment: 1.38 (0.36–3.11) Placebo: 1.31 (0.16–3.17)	Not adjusted

Abbreviations: CMV, Cytomegalovirus; CI, confidence interval; HR, hazard ratio; HHV-8, Human herpesvirus-8; OR, odds ratio; PBMC, peripheral blood mononuclear cell.

[a]1 nmol/L = 0.4 ng/mL.

vitamin D supplementation had a 2.3-fold increased hazard of developing CMV disease after transplantation (HR, 2.31; 95% CI, 1.44–3.71) (Table 2). However, the risk of misclassification in this study was moderate, and the risk of bias due to missing data was unclear (Table 3).

One randomized controlled trial assessed the effect of oral vitamin D supplementation on EBV viral load in blood [26], showing that high-dose vitamin D supplementation did not decrease the viral load in peripheral blood mononuclear cells or B cells (Table 2). However, the overall risk of bias of this interventional study was high (Table 3).

DISCUSSION

We reviewed 10 studies examining serum vitamin D deficiency or vitamin D supplementation and the risk of herpesvirus infection or reactivation among patients with comorbidities. The results showed no consistent association between serum vitamin D deficiency and the risk of herpesvirus infection or reactivation, but some evidence that vitamin D supplementation may be associated with a reduced risk of herpes zoster or CMV disease. However, the risk of bias of most included studies was high. Therefore, the evidence for establishing an association between vitamin D deficiency or oral vitamin D supplementation and the risk of herpesvirus infection or reactivation is still inconclusive.

Ours is the first systematic review of vitamin D and herpesvirus infection or reactivation. Some previous studies have also explored the various association between vitamin D deficiency or vitamin D supplementation and similar chronic viral infections. Among patients with chronic hepatitis B virus infection, a meta-analysis showed that serum vitamin D levels were negatively associated with hepatitis B viral load [27]. For hepatitis C virus patients, 1 meta-analysis reported that baseline serum vitamin D was associated with a sustained virologic response to antiviral and interferon treatment [28], while another recent meta-analysis reported no association [29]. A previous systematic review found no clear evidence of an effect of vitamin D supplementation on HIV viral load [30]. The immunomodulatory mechanism of vitamin D is still unclear, and its potential for preventing clinical viral infections is also inconclusive. Due to the paucity of current literature, further research is needed.

Our meta-analysis found no evidence that vitamin D status affected the risk of CMV infection or reactivation among patients receiving transplantation. CMV and other herpesviruses are the most common viral infections among transplant recipients [31]. However, the detected incidence of CMV will vary according to the use of prophylactic measures, differences in testing frequency, and definitions of CMV disease. These factors may have contributed to heterogeneity in our analysis. There were 2 studies that did not mention prophylactic measures [32, 33], and 1 study provided prophylactic antiviral treatment only for people with a high risk of CMV disease [34]. Two studies regularly checked participants' CMV viral loads [22, 25], and 3 studies only examined CMV antigen or viral load when patients were symptomatic [33–35]. These inconsistencies may lead to different estimations of the risk of CMV infections. We suggest that future studies about vitamin D and CMV diseases consider using a standardized definition of CMV and a consistent follow-up approach [36], so that researchers can accurately estimate the association between vitamin D and CMV infections among transplant patients.

While our study did not focus on the outcome of transplant rejection, there is a close relationship between CMV infection and graft injuries, which increase the risk of rejection [37, 38]. A recent systematic review also indicated that there was weak evidence showing an association between vitamin D deficiency and acute or chronic graft-vs-host disease (GVHD) [39]. While viral infections such as CMV may act as mediators of any relationship between vitamin D and graft loss, further research into these complex relationships is needed.

The effects of vitamin D supplementation on the risk of EBV remain inconclusive. The only included study did not find a

Table 3. Assessment of Bias for Individual Studies

Observational Studies						
Included Studies		Confounding	Selection of Participants	Misclassification of Variables	Bias due to Missing Data	Reverse Causation
Serum vitamin D levels before transplantation and the risk of CMV infection after transplantation						
Lee et al. (2014)		High	Low	Low	Low	Low
Saber et al. (2015)		High	High	Low	Unclear	Low
Ban et al. (2017)		High	Low	Moderate	Low	Low
Park et al. (2017)		High	Low	Low	Low	Low
Astor et al. (2019)		Low	Low	Low	Low	Low
Fernandez-Ruiz et al. (2019)		High	Low	Low	Unclear	Low
Serum vitamin D levels and the risk of herpes zoster among transplantation patients						
Ban et al. (2017)		High	Low	Moderate	Low	Low
Serum vitamin D levels and the change of serum HHV-8 viral load						
Erlandson et al. (2014)		High	High	Low	Moderate	Low
Vitamin D supplementation and the risk of CMV infection after transplantation						
Moscarelli et al. (2016)		Low	Low	Moderate	Unclear	Low
Vitamin D supplementation and the risk of herpes zoster among dialysis patients						
Chao et al. (2012)		Moderate	Low	Low	Low	Low
Interventional study						
Included Studies	Randomization Process	Deviations From Intended Interventions	Missing Outcome Data	Measurement of the Outcome	Selection of the Reported Result	Overall Judgment
Vitamin D supplementation and change of EBV viral load in blood cells						
Rolf et al. (2018)	Some concerns	High	High	Some concerns	Some concerns	High

Abbreviations: CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HHV-8, Human herpesvirus-8.

Table 4. Quality of Evidence of Outcomes

No. of Studies	Study Design	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Other Considerations	Quality
Outcomes: vitamin D deficiency/insufficiency associates with an increased risk of CMV infection								
6	Observational study	Serious	Serious	Not serious	Serious	Not serious	None	Very low

Abbreviation: CMV, Cytomegalovirus.

[a]Heterogeneity is substantial (I^2 = 55.4%; P = .047).

reduction in EBV viral load among multiple sclerosis patients randomized to receive high-dose vitamin D supplementation, but levels of antibody against EBV were reduced in this intervention group [26]. While this may provide some limited evidence for an immunomodulatory effect of vitamin D, it is unclear how well EBV antibodies reflect infection status, so further studies are needed.

Because of the impairment of renal function in calcium and phosphate homeostasis, many CKD patients take vitamin D supplements for maintaining bone health [40]. One included study showed that taking vitamin D supplements reduced the risk of herpes zoster among CKD patients receiving hemodialysis [24]. In this study, all participants were recruited before the introduction of zoster vaccine in Taiwan [41]. However, as zoster vaccinations can effectively reduce the risk of herpes zoster among older patients with CKD [42], future studies of the relationship between vitamin D and herpes zoster should take zoster vaccinations into account.

The studies included in our review have some limitations. First, 7 observational studies did not adjust for possible confounding factors, and they did not report herpesvirus infections as their main outcomes. In addition, the only included trial did not report the details of randomization or show a clear baseline characteristics table, so it was not possible to assess the effectiveness of the randomization process. These unadjusted confounding factors may lead to a high risk of bias in assessing the association. According to the included studies that adjusted for potential confounders, some evidence existed that vitamin D supplementation may be associated with a decreased risk of CMV disease among transplantation patients and herpes zoster among dialysis patients [24, 25]. In addition, among patients who received organ transplants, vitamin D deficiency was associated with a higher hazard of CMV disease after adjusting for confounders [35].

The inconsistent definition of vitamin D deficiency is another major limitation. Of 7 observational studies assessing vitamin D deficiency, 5 defined vitamin D deficiency as serum 25(OH)D <50 nmol/L (20 ng/mL), 1 defined it as 15 ng/mL (37.5 nmol/L), and the other used the lowest tertile as the exposure group, which was serum 25(OH)D <8.3 ng/mL (20.75 nmol/L). This significant difference in exposure definitions increases the heterogeneity between studies.

Another limitation is generalizability. The populations of the included studies were people with severe underlying conditions,

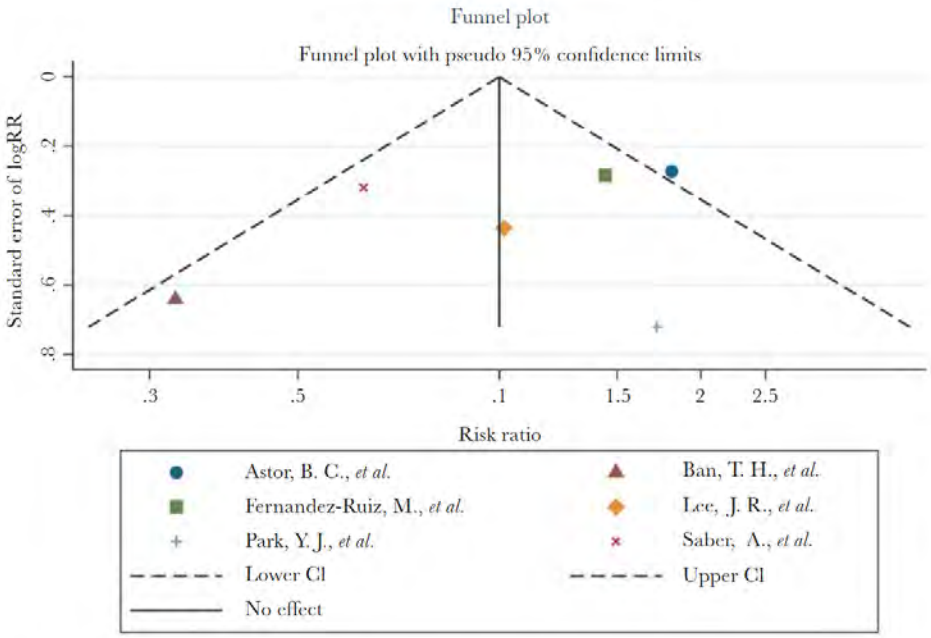

Figure 3. Assessment of publication bias of serum vitamin D levels before transplantation and the risk of CMV infection after transplantation. Abbreviation: CMV, cytomegalovirus.

such as end-stage renal disease, organ transplantation, and HIV. Further, these studies were conducted in single-hospital settings. Their results cannot be extrapolated to other populations with different comorbidities or even the general population. More studies among different populations are needed.

Our review has some strengths. This is the first review systemically examining the existing available evidence about vitamin D and herpesvirus infection. We comprehensively searched 6 major medical databases and 4 gray literature databases, and we summarized the results and assessed the risk of bias using a predefined framework. However, our study has some limitations. First, due to the paucity of studies, we were not able to review the association between vitamin D and some herpesvirus infections, such as HSV-1, HSV-2, HHV-6A, HHV-6B, and HHV-7. This may be caused by their difficulty in diagnosis. Second, because no guideline exists for defining serum 1,25-dihydroxycholecalciferol, an active metabolite of vitamin D, we were unable to include studies assessing this. Third, despite the comprehensiveness of our search, our search strategy may still have missed some eligible studies. Although we did not limit the language for eligible studies, studies in other languages may not be able to be identified. Further reviews of the association between vitamin D and herpesviruses need to consider these limitations.

Based on currently available studies, some limited evidence suggests that vitamin D supplementation may have a protective effect against herpes zoster in hemodialysis patients and CMV disease in renal transplant patients, but insufficient evidence supports any association between serum vitamin D deficiency and the risk of herpesvirus infection or reactivation. However, the current studies have focused solely on immunosuppressed patients with major underlying comorbidities, and some did not adjust for potential confounding factors. As vitamin D deficiency is not uncommon, for future studies, it is important to focus not only on individuals with specific comorbidities but also on the general population. In addition, future studies need to adopt consistent definitions of vitamin D deficiency, and adequately adjust for possible confounding factors, to provide robust evidence of any association between vitamin D and herpesvirus infection or reactivation.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. L.Y.L. contributed to the design of the study, searched for databases, extracted data, assessed the risk of bias, underwent statistical analysis, and drafted and revised the manuscript according to other authors' comments; K.B. contributed to the design of the study, extracted data, assessed the risk of bias, and revised the paper critically; H.J.F. contributed to the design of the study and revised the paper critically; C.W.G. contributed to the design of the study protocol, determined study eligibility, made critical comments on the manuscript, and revised the paper critically; S.M.L. contributed to the design of the study, determined study eligibility, made critical comments on the manuscript, and revised the paper critically; L.S. contributed to the design of the study and revised the paper critically. All authors approved the final version of the manuscript.

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References

1. Iwasaki A, Medzhitov R. Innate responses to viral infections. In: Bernard N Fields, David M Knipe, Howley PM, eds. Fields Virology 6th ed. Philadelphia, USA: Lippincott Williams & Wilkins; **2013**: 189–213.
2. Forbes HJ, Williamson E, Benjamin L, et al. Association of herpesviruses and stroke: systematic review and meta-analysis. PLoS One **2018**; 13:e0206163.
3. Scott FT, Johnson RW, Leedham-Green M, et al. The burden of herpes zoster: a prospective population based study. Vaccine **2006**; 24:1308–14.
4. Kotton CN, Hirsch MS. Cytomegalovirus and Human Herpesvirus Types 6, 7, and 8. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, eds. Harrison's Principles of Internal Medicine. 20th ed. New York: McGraw-Hill Education; **2018**: 1363.
5. Holick MF. Vitamin D deficiency. N Engl J Med **2007**; 357:266–81.
6. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab **2011**; 96:1911–30.
7. Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ **2010**; 340:b5664.
8. The Scientific Advisory Committee on Nutrition. Vitamin D and Health. London: Public Health England; 21 July **2016**.
9. Public Health England. PHE publishes new advice on vitamin D. **2016**. Available at: https://www.gov.uk/government/news/phe-publishes-new-advice-on-vitamin-d. Accessed 5 October 2020.
10. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature **2002**; 415:389–95.
11. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J **2005**; 19:1067–77.
12. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol **2004**; 173:2909–12.
13. Weber G, Heilborn JD, Chamorro Jimenez CI, et al. Vitamin D induces the antimicrobial protein hCAP18 in human skin. J Invest Dermatol **2005**; 124:1080–2.
14. Kumar A, Singh MP, Kumar RS, Ratho RK. 25-hydroxyvitamin D3 and 1,25 dihydroxyvitamin D3 as an antiviral and immunomodulator against herpes simplex virus-1 infection in HeLa cells. Viral Immunol **2018**; 31:589–93.
15. Su G, Liu Z, Qin X, et al. Vitamin D deficiency and treatment versus risk of infection in end-stage renal disease patients under dialysis: a systematic review and meta-analysis. Nephrol Dial Transplant **2018**; 34:146–56.
16. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ **2017**; 356:i6583.
17. Lin LY, Bhate K, Forbes H, et al. Vitamin D deficiency or supplementation and the risk of human herpesvirus infections or reactivation: a systematic review protocol. BMJ Open **2019**; 9:e031867.
18. Bramer WM, Giustini D, de Jonge GB, et al. De-duplication of database search results for systematic reviews in EndNote. J Med Libr Assoc **2016**; 104:240–3.
19. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ **2019**; 366:l4898.
20. Sterne JAC, Egger M, Moher D. Chapter 10: addressing reporting biases. *Cochrane Handbook for Systematic Reviews of Intervention*. Version 5.1.0 (updated March

2011) **2011**. Available at: http://handbook-5-1.cochrane.org/chapter_10/10_4_1_funnel_plots.htm. Accessed 24 April 2019.
21. Guyatt GH, Oxman AD, Vist GE, et al; GRADE Working Group. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ **2008**; 336:924–6.
22. Ban TH, Kim JH, Jang HB, et al. Clinical effects of pre-transplant serum 25-hydroxyvitamin D level on post-transplant immunologic and non-immunologic outcomes in kidney transplant recipients. Transpl Immunol **2017**; 40:51–6.
23. Erlandson KM, Gudza I, Fiorillo S, et al. Relationship of vitamin D insufficiency to AIDS-associated Kaposi's sarcoma outcomes: retrospective analysis of a prospective clinical trial in Zimbabwe. Int J Infect Dis **2014**; 24:6–10.
24. Chao CT, Lai CF, Huang JW. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. Eur J Intern Med **2012**; 23:711–5.
25. Moscarelli L, Antognoli G, Buti E, et al. 1,25 dihydroxyvitamin D circulating levels, calcitriol administration, and incidence of acute rejection, CMV infection, and polyoma virus infection in renal transplant recipients. Clin Transplant **2016**; 30:1347–59.
26. Rolf L, Muris AH, Mathias A, et al. Exploring the effect of vitamin D3 supplementation on the anti-EBV antibody response in relapsing-remitting multiple sclerosis. Mult Scler **2018**; 24:1280–7.
27. Hu YC, Wang WW, Jiang WY, et al. Low vitamin D levels are associated with high viral loads in patients with chronic hepatitis B: a systematic review and meta-analysis. BMC Gastroenterol **2019**; 19:84.
28. Villar LM, Del Campo JA, Ranchal I, et al. Association between vitamin D and hepatitis C virus infection: a meta-analysis. World J Gastroenterol **2013**; 19:5917–24.
29. Kitson MT, Sarrazin C, Toniutto P, et al. Vitamin D level and sustained virologic response to interferon-based antiviral therapy in chronic hepatitis C: a systematic review and meta-analysis. J Hepatol **2014**; 61:1247–52.
30. Visser ME, Durao S, Sinclair D, et al. Micronutrient supplementation in adults with HIV infection. Cochrane Database Syst Rev **2017**; 5:CD003650.
31. Finberg RW, Fingeroth JD. Infections in Transplant Recipients. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill Education; **2018**: 1033–1034.
32. Park YJ, Kim SU, Lee KH, et al. Vitamin D deficiency is associated with increased risk of bacterial infections after kidney transplantation. Korean J Intern Med **2017**; 32:505–13.
33. Saber A, Fotuhi F, Rostami Z, et al. Vitamin D levels after kidney transplantation and the risk of cytomegalovirus infection. Nephrourol Mon **2015**; 7:e29677.
34. Fernández-Ruiz M, Rodríguez-Goncer I, Ruiz-Merlo T, et al. Low 25-hydroxyvitamin D levels and the risk of late CMV infection after kidney transplantation: role for CMV-specific mediated immunity. Transplantation **2019**; 103:e216–7.
35. Astor BC, Djamali A, Mandelbrot DA, et al. The association of 25-hydroxyvitamin D levels with late cytomegalovirus infection in kidney transplant recipients: the Wisconsin Allograft Recipient Database. Transplantation **2019**; 103:1683–8.
36. Ljungman P, Boeckh M, Hirsch HH, et al; Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis **2017**; 64:87–91.
37. Fishman JA. Infection in organ transplantation. Am J Transplant **2017**; 17:856–79.
38. Hodson EM, Ladhani M, Webster AC, et al. Antiviral medications for preventing cytomegalovirus disease in solid organ transplant recipients. Cochrane Database Syst Rev **2013**; 28:CD003774.
39. Chiengthong K, Cheungpasitporn W, Thongprayoon C, et al. Vitamin D deficiency is not associated with graft versus host disease after hematopoietic stem cell transplantation: a meta-analysis. J Evid Based Med **2020**; 13:183–91.
40. National Institute for Health and Care Excellence (NICE). *Chronic Kidney Disease in Adults: Assessment and Management*. **2015**. Available at: www.nice.org.uk/guidance/cg182. Accessed 26 October 2020.
41. Taiwan Centers of Disease Control. Zoster vaccine. **2019**. Available at: https://www.cdc.gov.tw/Category/Page/WzNj0ONng_AdPtlJc4XDhA. Accessed 26 October 2020.
42. Langan SM, Thomas SL, Smeeth L, et al. Zoster vaccination is associated with a reduction of zoster in elderly patients with chronic kidney disease. Nephrol Dial Transplant **2016**; 31:2095–8.
43. Lee JR, Dadhania D, August P, et al. Circulating levels of 25-hydroxyvitamin D and acute cellular rejection in kidney allograft recipients. Transplantation **2014**; 98:292–9.

3.4. Additional literature review

I repeated the literature search using Medline (OVID) in November 2021 using the same search strategy, and I found 61 potentially relevant studies published after August 2019. Among these, one study is relevant to my literature review after detailed screening and full-text examination.

A small Korean hospital-based case-control study recruited 440 people over 15 years old who had HIV/AIDS, serum vitamin D tests at baseline, and at least one year of follow-up. In a subgroup of 237 participants who did not receive antiretroviral therapy, vitamin D deficiency (n=113; defined as serum vitamin D levels ≤ 14 ng/mL or approximately 35 nmol/L) was associated with a higher risk of CMV disease after adjusting for age, comorbidities, and CD4+ T-cell counts (adjusted OR: 10.13, 95% CI: 1.11–92.03) (77).

Compared with other studies included in my meta-analysis, the sample size was small, and the participants were immunosuppressed. However, moreover, Lee et al. reported the outcome of CMV diseases in a subgroup analysis with only a few cases (CMV diseases n=10), and the confidence intervals of the reported results were wide, indicating considerable uncertainty (77). This case-control study did not specify the timing of CMV and vitamin D measurement; thus, the risk of reverse causation is unclear. Moreover, bias due to residual confounding cannot be ruled out because the authors only adjusted for age, comorbidities, and CD4 counts in their model.

3.5. The implication of the systematic review

The systematic review of published papers presented in this chapter demonstrates that the association between vitamin D and herpesviruses is

unclear. Limited evidence indicated that vitamin D supplementation may be associated with a lower risk of HZ among people receiving haemodialysis. All reviewed studies were conducted among people with comorbidities or immunosuppression in hospital settings. These results identified a knowledge gap regarding the association between vitamin D and HZ, especially among the immunocompetent population, which will be addressed in the following chapters.

3.6. Chapter summary

- The meta-analysis of six cohort studies revealed no evidence that vitamin D deficiency is associated with an increased risk of CMV disease among individuals receiving transplantation.
- No evidence indicated that vitamin D deficiency is associated with an increased risk of HZ among individuals receiving transplantation.
- Among people with HIV, some evidence demonstrated that vitamin D deficiency was associated with an increased risk of CMV disease, but vitamin D deficiency was not associated with a change in HHV-8 viral load.
- Vitamin D supplementation was associated with decreased HZ among people with CKD and CMV disease after renal transplantation.
- Vitamin D supplementation was not associated with reduced EBV viral load.
- The existing studies were conducted among people with immunosuppressive conditions in hospital settings. Therefore, a community-based study focusing on the general population is warranted.

Chapter 4. Data source description

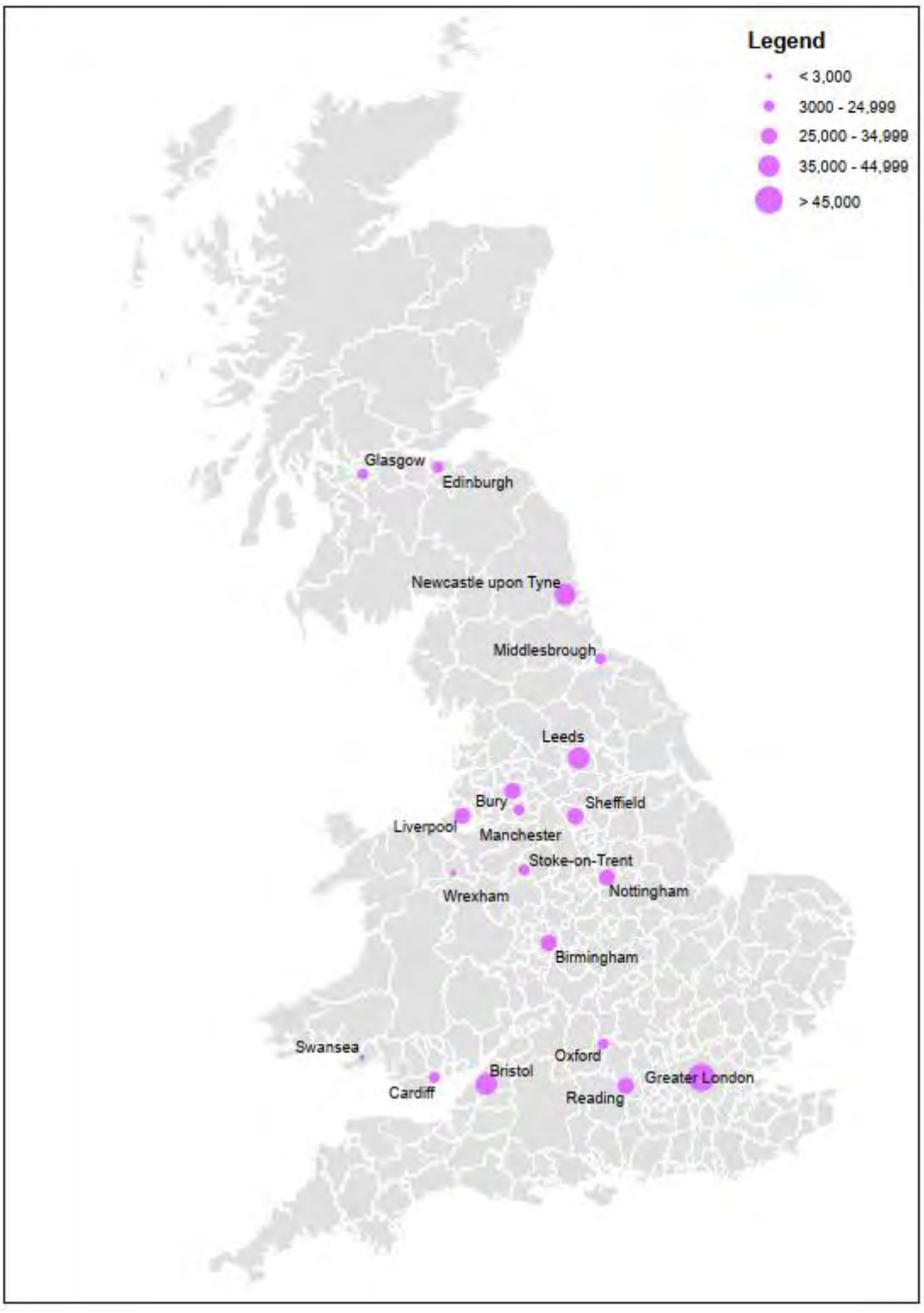
Chapter overview

In the previous chapter, I systematically reviewed existing literature on vitamin D and herpesviruses. Before further investigating the association between vitamin D and HZ, in this chapter, I present an overview of UK Biobank, the data source I use for subsequent analyses. This chapter begins with an introduction of the participants and how measurements were undertaken in the UK Biobank cohort, followed by an explanation of the linked EHR. In the final part of this chapter, I discuss the strengths and limitations of UK Biobank.

4.1. UK Biobank

4.1.1. Participants

UK Biobank is a nationwide prospective cohort that investigates a wide range of risk factors for major diseases among people of middle and older ages. This cohort was compiled from 2006 to 2010, recruiting people throughout England, Wales, and Scotland. The participants were aged 40–69 years and lived within 40 km of one of the 22 UK Biobank assessment centres. The distribution of UK Biobank assessment centres and the number of participants recruited are illustrated in **Figure 1**. Over 9,200,000 individuals registered with the NHS were invited to join the cohort, and 500,000 volunteers were recruited (78).



Chapter 4. Figure 1 The distribution of UK Biobank assessment centres and the numbers of participants recruited. Three centres (Barts, Croydon, and Hounslow) are merged and labelled "Greater London." Plotted by using ArcGIS 10.5.

4.1.2. Measurements

Demographic factors, physical examinations, and personal history

The overall data structure of UK Biobank is summarised in **Figure 2**. The participants visited 22 assessment centres across the UK and received thorough physical examinations, questionnaires, and interviews. Demographic factors, such as sex, age, and ethnicity, were recorded, and physical measurements of weight, height, and body mass index (BMI) were also measured (79). In addition, participants' addresses were recorded.

The Index of Multiple Deprivation (IMD) is a score that estimates the level of deprivation in a small area, and is comprised of several domains such as income, employment, health and disability, education, housing, living environment, and crime. In different countries, these evaluation domains may differ (80). The IMD score can be used to evaluate socioeconomic deprivation (81). For UK Biobank participants, an IMD score closest to the recruitment year was assigned by matching each participant's postcode (82).

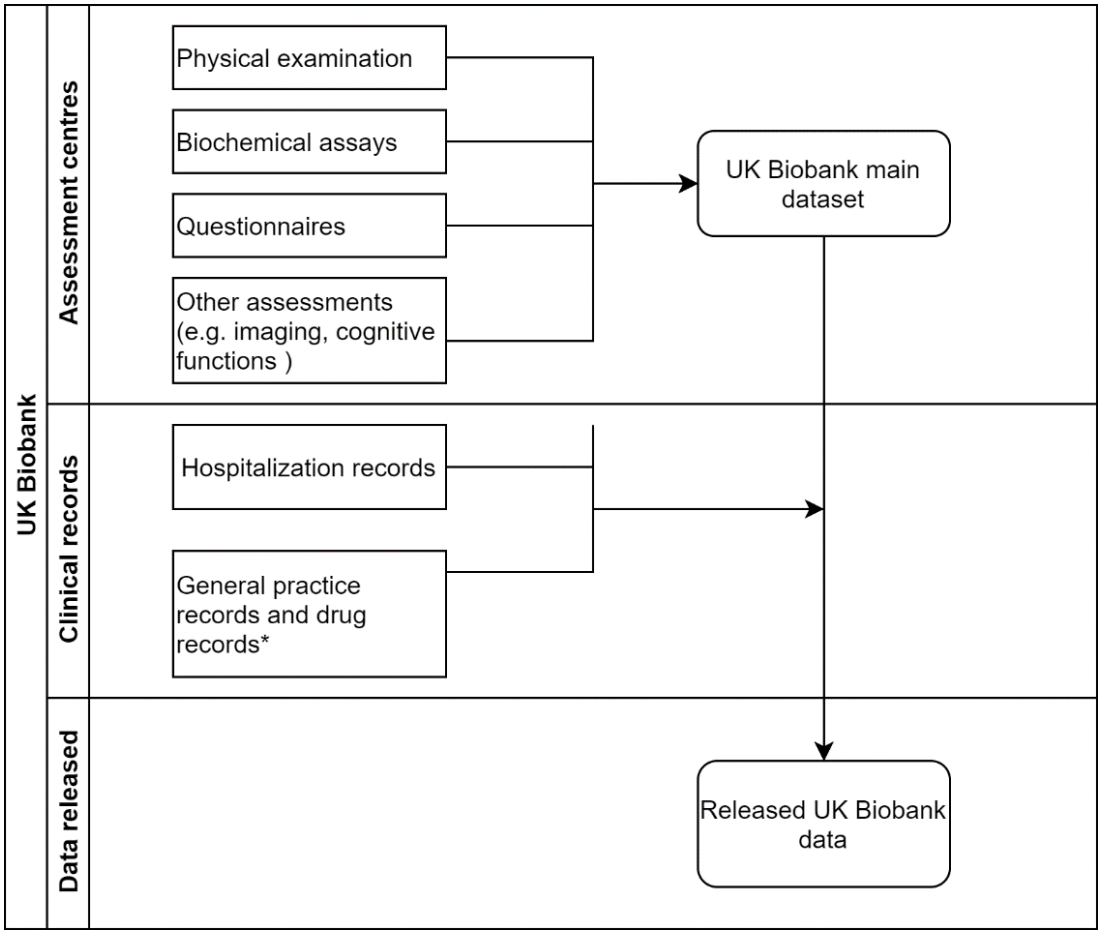
Trained nurses interviewed each participant to obtain their personal medical history. The interviewers recorded and coded any non-cancer diagnoses disclosed to the participants by a physician. For any unclear diagnoses, interviewers recorded a free-text description that was later examined and classified by a doctor. The record would be marked as ‘unclassifiable’ if a diagnosis could not be made from the free-text description (83, 84).

Lifestyle factors

Lifestyle factors, such as alcohol consumption and smoking status, were also recorded at the assessment centres using a touch-screen questionnaire. Information about vitamin and mineral supplementation was also recorded using a touch-screen questionnaire by asking the question, ‘do you regularly take any of the following [vitamin and minerals]?’ (85).

Biochemical assays

In addition to demographic and lifestyle factors, UK Biobank participants also provided samples for biochemical assays, including blood, urine, and saliva (86). The collected biological samples were sent to a central laboratory and handled by an automated dispensing system (87). The biochemical assays of the blood samples were analysed for various biomarkers, including serum vitamin D levels (86).



Chapter 4. Figure 2 The structure of UK Biobank dataset.

4.2. Linked clinical data

4.2.1. Healthcare system in the UK

In the UK, general practitioners (GP) provide a wide range of medical services in the primary care setting, such as vaccination, chronic disease care, and minor surgeries. To receive medical care, residents must register with a GP practice. GPs are the first physicians that manage all non-emergency health issues. If advanced investigation or management is needed, patients are referred to the hospital system for secondary care. After hospital discharge, hospitals provide feedback to GPs regarding the patients'

conditions. Because of their crucial roles in the healthcare system, GPs are regarded as the gatekeepers of health systems (88). Consultation records from GPs were stored and handled by the data providers of GP practices, and information on inpatient care was collected and managed by the NHS.

4.2.2. Linked clinical records of UK Biobank

Inpatient clinical records

The participants of UK Biobank provided their consent to have their clinical records linked, including inpatient care records and GP consultation records (89). The inpatient care records were imported by the data providers from England (NHS Digital), Scotland (Information Services Division of Scotland part of NHS National Services Scotland), and Wales (NHS Wales Informatics Service's Information Services Division). The imported inpatient EHRs include the admission and discharge dates, diagnostic codes, and operation costs. The diagnostic codes for inpatient data are coded according to International Classification of Diseases (ICD) version 10 (ICD-10) or version 9 (ICD-9), and the operation codes are coded in OPCS Classification of Interventions and Procedures version 3 (OPCS-3) or version 4 (OPCS-4) (90). Currently, 440,559 participants already have their inpatient records linked, and the records are updated up to September 2021 (91, 92).

Primary care records

The primary care data of UK Biobank participants were extracted by primary care system suppliers and linked to the main dataset. In brief, UK Biobank securely sent the participants' identifications, including their identifiers, NHS numbers, sex, and date of birth, to the primary care data providers in

England, Scotland, and Wales. These data providers collected matched participants' consultation records, including GP registration, diagnosis codes, diagnosis dates, data providers, prescription codes, drug names, and drug quantities, securely extracted and imported to UK Biobank (93, 94). Before linking to the main dataset, these data were further de-identified by removing personal data fields, such as postcodes and dates of birth (95). Finally, the de-identified and anonymous data were released to eligible researchers for study purposes only.

Different data providers used different coding classification systems for their clinical records, and the censoring dates of the dataset vary; these are summarised in **Table 1.** The clinical diagnoses from the Phoenix Partnership (TPP) system were coded in Clinical Term Version 3 (CTV 3), whereas diagnosis codes from other data providers were coded in Read 2 codes. The GP prescription data from TPP were coded in the British National Formulary (BNF) codes, and the data from the Vision or EMIS Group (EMIS) were recorded using the Dictionary of Medicines and Devices (DM+D) or Read Codes version 2 (**Table 1**). The censoring date of the linked GP dataset was decided based on the completeness of the received data. The censoring date was defined as the last date of a month in which the number of received records in that month was less than 90% of the average number of the previous three months (96). The mean follow-up time calculated from the time of recruitment was approximately 10 years. Approximately 45% of the participants already had linked primary care records.

Chapter 4. Table 1 Data providers and clinical coding of the linked primary care data of the UK Biobank

Country	Data providers	Category	Coding	Earliest year of data	Censoring date
England	Vision	Clinical diagnosis	Read 2	1940	31 May 2017
		Prescriptions	DM+D		
			Read 2		
	TPP	Clinical diagnosis	CTV3	1938	31 May 2016
		Prescriptions	BNF		
Scotland	Vision/EMIS	Clinical diagnosis	Read 2	1939	31 March 2017
		Prescriptions	Read 2		
Wales	Vision/EMIS	Clinical diagnosis	Read 2	1948	31 Aug 2017
		Prescriptions	Read 2		

4.2.4. COVID-19 data release

Because of the COVID-19 pandemic, confidential patient information was permitted to be shared under the Control of Patient Information (COPI) regulations (97) to address priority COVID-19 research questions.

Therefore, beginning in April 2020, UK Biobank began to receive COVID-19 testing results for its participants from Public Health England every week (98). Until late 2021, the COVID-19 testing results from Scotland and Wales were also included. NHS (Pillar 1) or commercial partners (Pillar 2) performed COVID-19 testing using PCR (99, 100). The reported data included the participant ID, testing date, and test results (100). By 8 January 2021, 57,217 UK Biobank participants in England had received COVID-19 testing.

Along with COVID-19 testing results, additional clinical records, including primary care records, hospital inpatient records, and death registries, were also released (101). Unlike the previously released GP data, the primary care data for COVID-19 only includes participants from England. This English primary care data for COVID-19 contain more than 400,000 UK Biobank participants, more than approximately 80% of the study population, covering data from the earliest record until 2020 (92, 102). Notably, these data can only be used for COVID-19 research.

The primary care data for COVID-19 research were coded differently from the previously released data. The data providers and the clinical coding of the primary care data for COVID-19 are summarised in **Table 2**. The clinical diagnoses from TPP and EMIS were coded in CTV3, and Systematized

Nomenclature of Medicine–Clinical Terms (SNOMED–CT), and the prescription records were coded in the DM+D codes (**Table 2**). The death registry data contain the date of death and the cause of death, which were coded according to ICD-10 (103). The structure of the inpatient care records remained the same as the previously released versions.

Chapter 4. Table 2 Data providers and clinical coding of the linked primary care data for COVID-19 research

Country	Data providers	Category	Coding	Last recorded date
England	EMIS	Clinical diagnosis	SNOMED-CT	25 July 2020
		Prescriptions	DM+D	
	TPP	Clinical diagnosis	CTV3	03 June 2020
		Prescriptions	DM+D	

4.4. Strengths and limitations of using UK Biobank for research

Strengths

UK Biobank is a unique, prospective, and large cohort. Using a prospective cohort, researchers can investigate the possible temporality between risk factors and outcomes. UK Biobank measured numerous exposures in detail, and its large sample size provides researchers with the statistical power to study the effects of many exposures on a range of outcomes (89).

Before initiating the UK Biobank study, a series of pilot studies were conducted to ensure the adequacy of the recruitment, assessment, and sample processing (89). The biological samples are systematically stored and processed in a centralised laboratory using an automatic analysis system (87, 104). The analysis of biological samples was further confirmed by quality control and quality assurance schemes (105, 106). These measures can minimise non-differential information bias across different exposures.

Furthermore, the powerful linkage between UK Biobank database and clinical databases can further increase the scope of the cohort. By linking the EHRs, researchers can extend the range of disease outcomes of interest over time (107). In addition, because UK Biobank thoroughly measured a wide range of demographic variables, its linkage with clinical records can improve the completeness of EHR data, reducing bias due to missing data (108).

Limitations

One main limitation of UK Biobank is selection bias. A study comparing the participants to nonparticipating invitees demonstrated that the UK Biobank cohort contained more females and older participants, and fewer obese participants; UK Biobank participants were also less likely to be smokers, had fewer self-reported health issues and cancer incidence rates, and more people were living in less socioeconomically deprived areas (78). Because of the selection bias arising from sampling, the distribution of some crucial confounders or effect modifiers may differ from that of the general population, which could influence the magnitude of the association between exposures and outcomes (109).

The selection bias of sampling also affects the generalisability of UK Biobank. As mentioned above, the response rate of this cohort is only approximately 5.45%, and its participants differ from the general UK population. Therefore, UK Biobank cannot provide a valid estimation of the prevalence or incidence of diseases in the UK population (110). However, a study comparing UK Biobank with 18 other national representative datasets found that the association between known disease risk factors and mortality in both datasets were consistent. This finding supports the generalisability of some associations found in UK Biobank (111).

Second, some data of UK Biobank collected at the baseline assessment may be out of date, especially for some time-dependent variables. UK Biobank participants were assessed between 2006 and 2010, but only 20,000 received repeated complete assessments between 2013 to 2014; these

participants lived within a 30 km radius of the Stockport assessment centre (112). Because the proportion of participants with repeated assessments is small, it is challenging to evaluate the change in variables over time. Using historical information about participants may lead to misclassification bias when assessing the exposure status or covariates. For instance, for studies assessing vitamin D status and COVID-19 outcomes, the 15-year-old vitamin D status may not reflect the actual vitamin D status during the COVID-19 pandemic (113, 114). Because of this limitation, research using UK Biobank relies on a strong untestable assumption regarding the stability of study variables.

Third, another limitation of UK Biobank is that validation of some outcome diagnoses is lacking. Unlike other data sources with many validation studies such as Clinical Practice Research Datalink, the data structure of the clinical data of UK Biobank is different. For instance, laboratory test results are not included in the primary care data of UK Biobank, which may lead to the underestimation of some diseases, such as CKD (115). After UK Biobank primary care data were released in 2019, validation studies were still limited. One study evaluated the diagnostic accuracy of dementia by comparing diagnostic codes with participants' medical records, and indicated that the positive predictive value (PPV) was over 80% for all-cause dementia (116). However, this study only focused on Scottish participants with dementia, and the results may not be generalisable for other regions or diseases.

4.5. Ethics

The UK Biobank project was approved by the Northwest Haydock Research Ethics Committee (reference: 11/NW/0382). Our project was approved by UK Biobank (ID:51265) and by the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (reference: 17158). The protocol for applying LSHTM ethics online and approval documents are attached in **Appendices 2–4.**

Chapter 5. The distribution of vitamin D status in the UK Biobank

Chapter overview

In the previous chapter, I introduced the primary setting of UK Biobank cohort, including the study population and the measurement of variables. In this chapter, I explore the vitamin D status of the UK Biobank participants through a cross-sectional study describing the distribution and factors associated with vitamin D deficiency, insufficiency, and sufficiency in the UK Biobank cohort. This paper has been published in *BMJ Open* (117).

My study focuses on the following two main research questions:

1. What is the distribution of serum vitamin D levels among UK Biobank participants?

2. What are the factors associated with vitamin D deficiency and insufficiency among UK Biobank participants?

By answering these questions, my findings from this cross-sectional study can inform the design of other studies on vitamin D using UK Biobank, such as confounder selection.

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Thesis Title	The association between vitamin D deficiency and the risk of herpes zoster and COVID-19		
Primary Supervisor	Sinéad Langan		

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Distribution of vitamin D status in the UK: a cross-sectional analysis of UK Biobank

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ABSTRACT

Objective No recent large studies have described the distribution of vitamin D status in the UK. Understanding the epidemiology of vitamin D deficiency is important to inform targeted public health recommendations. This study aimed to investigate the distribution of factors associated with serum vitamin D status in a large national cohort.

Design A cross-sectional study.

Setting The UK Biobank, a prospective cohort study following the health and well-being of middle-aged and older adults recruited between 2006 and 2010.

Participants A total of 449 943 participants aged 40–69 years with measured serum vitamin D status were eligible for the analysis. Participants completed a questionnaire about sex, age, ethnic background, vitamin D supplementation, smoking, drinking and socioeconomic status.

Primary and secondary outcome measures We investigated the distribution of serum vitamin D status and the association between demographic factors and vitamin D deficiency or insufficiency. Vitamin D deficiency was defined as a serum 25-hydroxyvitamin D level <25 nmol/L. Multivariable logistic regression was used to assess the association between demographic factors and vitamin D status.

Results Asian (n=4297/8000, 53.7%) and black (n=2459/7046, 34.9%) participants had a higher proportion of vitamin D deficiency than white participants (n=50 920/422 907, 12%). During spring and winter, the proportion of vitamin D deficiency was higher across the UK and higher in the north than in the south. Male sex, abnormal body mass index, non-white ethnic backgrounds, smoking and being more socioeconomically deprived were associated with higher odds of vitamin D deficiency. Increasing age, taking vitamin D supplements and drinking alcohol were associated with lower odds of deficiency.

Conclusions Vitamin D status varied among different ethnic groups and by season and geographical area within the UK. Taking supplements was associated with a lower risk of vitamin D deficiency. These findings support the vitamin D supplementation recommendations of Public Health England.

Strengths and limitations of this study

- This study used a large cohort of British adults in middle and older age, and its large sample size provides more statistical power in assessing associations.
- The findings provide further evidence to support the nutritional supplementation recommendations of Public Health England.
- The cohort is not nationally representative, so the findings cannot be extrapolated to the general population.
- The questionnaire on vitamin supplementation was self-reported and thus cannot be used to estimate the required supplement amount.

INTRODUCTION

Vitamin D is essential to bone formation. It is mainly produced by the skin after sun exposure, and it can also be obtained from food and supplementation. After production or ingestion, it is further metabolised in the liver into 25-hydroxyvitamin D (25(OH)D), which can be measured in the blood. 25(OH)D is further transformed in the kidneys into 1,25-dihydroxyvitamin D, the active form of vitamin D. This active vitamin D metabolite acts on the intestines and kidneys, regulates the absorption and excretion of calcium and phosphate, and facilitates the mineralisation of bone. Vitamin D deficiency may impair bone mineralisation, leading to osteopenia or osteoporosis.[1] Currently, Public Health England suggests that people older than 4 years of age should take 10 µg (400 IU) of vitamin D daily as a supplement during the winter to support musculoskeletal health.[2]

In addition to the classical effects of mineral homeostasis, more recent attention has focused on novel effects of vitamin D. Previous studies have indicated that vitamin D may have potential immunomodulatory effects. Active vitamin D can enhance innate immunity by increasing the production of antimicrobial peptides, and vitamin D can regulate adaptive immunity as well.[3] Epidemiological studies have also indicated that vitamin D is associated with autoimmune diseases such as inflammatory bowel disease, type I diabetes mellitus and multiple sclerosis.[4] In addition,

Table 1 Comparison of included and excluded participants

Characteristics	Included (N=449 943)	Excluded (N=52 550)
Sex		
Female	240 976 (53.6%)	32 402 (61.7%)
Male	208 967 (46.4%)	20 148 (38.3%)
Average age (SD)	56.5 (8.1)	57.0 (7.8)
BMI*		
Underweight	2307 (0.5%)	321 (0.6%)
Healthy weight	145 082 (32.3%)	15 222 (29.7%)
Overweight	190 177 (42.4%)	22 053 (43.1%)
Obese	110 541 (24.7%)	13 600 (26.6%)
Central obesity*		
Not obese	177 869 (39.6%)	17 719 (34.5%)
Central obesity	271 126 (60.4%)	33 642 (65.5%)
Ethnic background		
White	424 213 (94.4%)	47 901 (91.3%)
Mixed	2593 (0.6%)	316 (0.6%)
Asian	8016 (1.8%)	1823 (3.5%)
Black	7050 (1.6%)	984 (1.9%)
Chinese	1407 (0.3%)	167 (0.3%)
Other ethnicity†	6068 (1.4%)	1267 (2.4%)
Tobacco smoking		
Non-smoker	402 337 (89.6%)	45 860 (88.3%)
Current smoker	46 793 (10.4%)	6184 (11.9%)
Alcohol drinking status		
Never drink	35 590 (7.9%)	5071 (9.8%)
Drink occasionally	101 690 (22.7%)	12 208 (23.5%)
Drink weekly	220 709 (49.2%)	24 028 (46.2%)
Drink daily	90 984 (20.3%)	10 713 (20.6%)
Vitamin and mineral supplementation use		
Vitamin D and associated mineral supplement‡	285 524 (63.6%)	31 097 (63.9%)
Other vitamin and mineral supplement§	163 214 (36.4%)	17 537 (36.1%)
Mean outdoor time in summer (SD)	3.8 (2.4)	3.8 (2.4)
Mean outdoor time in winter (SD)	1.9 (1.8)	1.9 (1.8)
Index of Multiple Deprivation (IMD)¶		
1 (Least deprived)	88 516 (20.2%)	9616 (18.7%)
2	88 210 (20.1%)	9782 (19.1%)
3	87 584 (20.0%)	10 249 (20.0%)
4	87 585 (20%)	10 306 (21.0%)
5 (most deprived)	86 539 (19.7%)	11 370 (22.2%)

*The classification is suggested by NICE guidelines.
†Includes any other unlisted ethnic groups, unclear ethnic groups and participants prefer not to answer.
‡Vitamin D and associated mineral: vitamin D, multivitamin, fish oil and calcium supplementation.
§Other vitamin and mineral supplements: vitamin A, B, C, E, folic acid or folate, glucosamine, zinc, iron, selenium and other supplements.
¶IMD scores were by quintile.
BMI, body mass index; NICE, National Institute for Health and Care Excellence.

a meta-analysis using original data from trials suggested that taking vitamin D supplementation may decrease the risk of respiratory infections.[5]

Although 1,25-dihydroxyvitamin D plays an active role in metabolism, its half-life is less than 4 hours, while the half-life of 25(OH)D is around 2–3 weeks. Thus, clinical 25(OH)D levels in the blood have been used to assess vitamin D status.[6] 25(OH)D can be analysed using either chemiluminescence immunoassay or tandem mass spectrometry, which are both recognised by the Royal Osteoporosis Society and Public Health England.[6 7] However, no current consensus exists about the definition of vitamin D deficiency, so each study may use different standards. The Endocrine Society of the USA defined vitamin D deficiency as 25(OH)D below 50 nmol/L, while the criterion of Public Health England is 25(OH)D less than 25 nmol/L.

Using the criterion of a blood vitamin D level less than 25 nmol/L, a cohort of British Caucasians (n=7437) indicated that in winter and spring, the average prevalence of vitamin D deficiency was 15.5% among people who were 45 years old.[8] Similarly, among adults in the National Diet and Nutrition Survey in the UK (n=3450), the prevalence of vitamin D deficiency was approximately 24.0% in men and 21.7% in women aged between 19 and 64 years.[9] However, these studies were relatively small, and no recent large study has described the distribution of vitamin D status in the UK.

The UK Biobank is a prospective, nationwide cohort used to investigate risk factors for major diseases in middle and old age; cohort participants were assessed for various biochemical biomarkers, including serum vitamin D status.[10 11] By using UK Biobank data, we aimed to conduct a cross-sectional study to investigate different demographic, seasonal and regional factors associated with vitamin D deficiency distribution in the UK.

METHODS

Study population

The UK Biobank was compiled from 2006 to 2010 by recruiting participants throughout the UK. People aged 40–69 years who lived within 40 km of 1 of the 22 UK Biobank assessment centres and who were registered with the UK National Health Service were eligible to be included in the cohort; approximately 500 000 volunteers were recruited.[10 12] UK Biobank participants received a wide range of examinations, including questionnaires and physical measures, and blood, urine and saliva samples were assayed.[6] Only participants with results for serum vitamin D status were included in our analyses.

Measurement of covariates

At each participant's first visit to a UK Biobank assessment centre, a touchscreen questionnaire collected basic demographic characteristics; sociodemographic, environmental and lifestyle factors; and the date of assessment.[10] Information such as sex, age, ethnic background, skin

Table 2 Basic characteristics of the study population

Characteristics	Vitamin D deficiency (<25 nmol/L)	Vitamin D insufficiency (25–50 nmol/L)	Vitamin D sufficiency (>50 nmol/L)
Serum vitamin D levels (SD) (nmol/L)	19.2 (3.9)	37.8 (7.1)	67.7 (14.5)
Sex			
Female (N=240 263)	32 180 (13.4%)	100 423 (41.8%)	107 660 (44.8%)
Male (N=208 338)	28 507 (13.7%)	87 368 (41.9%)	92 463 (44.4%)
Age (SD)	54.8 (8.2)	56.2 (8.1)	57.3 (8.0)
BMI*			
Underweight (N=2301)	423 (18.3%)	844 (36.7%)	1034 (45%)
Healthy weight (N=144 591)	15 886 (11.0%)	54 115 (37.4%)	74 590 (51.6%)
Overweight (N=189 583)	22 841 (12.1%)	79 351 (41.9%)	87 391 (46.1%)
Obese (N=110 292)	20 990 (19.0%)	52 704 (47.8%)	36 598 (33.2%)
Central obesity*			
Non-obese (N=177 348)	19 514 (11.0%)	66 998 (37.8%)	90 836 (51.2%)
Central obesity (N=270 306)	40 903 (15.1%)	120 389 (44.5%)	109 014 (40.3%)
Ethnic background			
White (N=422 907)	50 920 (12.0%)	176 195 (41.7%)	195 792 (46.3%)
Mixed (N=2589)	642 (24.8%)	1241 (47.9%)	706 (27.3%)
Asian (N=8000)	4297 (53.7%)	2979 (37.3 %)	724 (9.1%)
Black (N=7046)	2459 (34.9%)	3503 (49.7%)	1084 (15.4%)
Chinese (N=1405)	381 (27.1%)	787 (56.0%)	237 (16.9%)
Other ethnicity† (N=6059)	1860 (30.7%)	2816 (46.5%)	1383 (22.8%)
Tobacco smoking			
Non-smoker (N=401 069)	50 270 (12.5%)	167 496 (41.8%)	183 303 (45.7%)
Current smoker (N=46 721)	10 170 (21.7%)	19 944 (42.7%)	16 607 (35.6%)
Alcohol drinking status			
Never drink (N=35 503)	8419 (23.7%)	15 265 (43.0%)	11 819 (33.3%)
Drink occasionally (N=101 398)	16 655 (16.4%)	45 208 (44.6%)	39 535 (39.0%)
Drink weekly (N=219 988)	24 577 (11.1%)	90 836 (41.3%)	104 575 (47.6%)
Drink daily (N=90 742)	10 730 (11.8%)	36 063 (39.7%)	43 949 (48.4%)
Mean outdoor time in summer (hours) (SD)	3.3 (2.4)	3.6 (2.4)	4.1 (2.4)
Mean outdoor time in winter (hours) (SD)	1.7 (1.8)	1.9 (1.8)	2.1 (1.8)
Vitamin and mineral supplementation use			
Other vitamin or mineral supplement‡ (N=284 768)	48 877 (17.1%)	128 478 (45.1%)	107 413 (37.7%)
Vitamin D and associated mineral supplement§ (N=162 629)	11 368 (7.0%)	58 801 (36.2%)	92 460 (56.9%)
Index of Multiple Deprivation (IMD)¶			
1 (least deprived, N=88 264)	8414 (9.5%)	34 962 (39.6%)	44 888 (50.9%)
2 (N=87 909)	9321 (10.6%)	36 073 (41.0%)	42 515 (48.4%)
3 (N=87 268)	10 480 (12.0%)	36 049 (41.3%)	40 739 (46.7%)
4 (N=87 321)	13 211 (15.1%)	37 646 (43.1%)	36 464 (41.8%)
5 (most deprived, N=86 347)	17 602 (20.4%)	38 369 (44.4%)	30 376 (35.2%)
Seasons			
Spring (N=129 570)	25 912 (20%)	62 378 (48.1%)	41 280 (31.7%)
Summer (N=118 924)	5385 (4.5%)	39 856 (33.5%)	73 683 (62.0%)
Autumn (N=108 888)	8324 (7.6%)	41 496 (38.1%)	59 068 (54.3%)
Winter (N=91 219)	21 066 (23.1%)	44 061 (48.3%)	26 092 (28.6%)

Continued

Table 2 Continued

Characteristics	Vitamin D deficiency (<25 nmol/L)	Vitamin D insufficiency (25–50 nmol/L)	Vitamin D sufficiency (>50 nmol/L)
Regions of UK Biobank assessment centres			
South West (N=38 872)	3068 (7.9%)	14 622 (37.6%)	21 182 (54.5%)
South East (N=39 814)	3245 (8.2%)	15 400 (38.7%)	21 169 (53.2%)
London (N=61 291)	10 232 (16.7%)	27 037 (44.1%)	24 022 (39.2%)
East Midlands (N=30 337)	3001 (9.9%)	12 115 (39.9%)	15 221 (50.2%)
West Midlands (N=40 044)	6785 (17.0%)	17 670 (44.1%)	15 589 (38.9%)
Wales (N=19 142)	2732 (14.3%)	8808 (46.0%)	7614 (39.8%)
Yorkshire and The Humber (N=66 197)	8372 (12.7%)	27 878 (42.1%)	29 947 (45.2%)
North West (N=68 196)	8715 (12.8%)	27 924 (41.0%)	31 557 (46.3%)
North East (N=52 277)	6919 (13.2%)	21 174 (40.5%)	24 184 (46.3%)
Scotland (N=32 419)	7618 (23.5%)	15 163 (46.8%)	9638 (29.7%)

*The classification is suggested by NICE guidelines.
†Includes any other unlisted ethnic groups, unclear ethnic groups and participants prefer not to answer.
‡Vitamin D and associated mineral: vitamin D, multivitamin, fish oil and calcium supplementation.
§Other vitamin and mineral supplements: vitamin A, B, C, E, folic acid or folate, glucosamine, zinc, iron, selenium and other supplements.
¶IMD scores were by quintile.
BMI, body mass index; NICE, National Institute for Health and Care Excellence.

colour, smoking status, drinking status, sun exposure, and vitamin and mineral supplement use were recorded. Physical measurements, including body mass index (BMI) and waist circumference were also taken. The Index of Multiple Deprivation (IMD) of participants was obtained through data linkage.[13]

Defining serum vitamin D status

Biochemical assays were performed on blood samples collected during the baseline evaluation at the assessment centres.[6] Briefly, serum samples were collected in a silica clot accelerator tube and stored at −80°C. These samples were later processed in a central laboratory using an automated dispensing system.[14] Serum 25(OH)D status was measured by chemiluminescence immunoassay (DiaSorin LIAISON XL, Italy), which was certified by the Vitamin D Standardization-Certification Program of the Centers for Disease Control and Prevention.[15] To ensure the precision of analysis, quality control samples at different concentrations were analysed,[16] and the testing assay for vitamin D was verified through the RIQAS Immunoassay Speciality I EQA programme (Randox Laboratories), an external quality assurance scheme.[17]

Statistical analysis

This was a cross-sectional study describing the distribution of serum vitamin D status, so only participants who had at least one available measurement of serum vitamin D status were included. We used the standards of vitamin D deficiency adopted by Public Health England.[6 18] A serum 25(OH)D level less than 25 nmol/L was coded as 'deficiency', and 25–50 nmol/L was coded as 'insufficiency'. A 25(OH)D level greater than 50 nmol/L was coded as 'sufficiency'. Vitamin D was coded as missing in cases of no reportable value, values above or below the reportable limits, or unrecoverable aliquot problems.

To compare the distribution of vitamin D status by these factors associated with vitamin D, continuous covariates, such as BMI and waist circumference, were coded as categorical variables following National Institute for Health and Care Excellence guidelines.[19] According to the guidelines, different BMI and waist circumference standards were applied for Asian and Chinese populations. Self-reported ethnic backgrounds were coded into six groups according to the original questionnaire. Participants with any other unlisted ethnic group, with an unclear ethnic group or preferring not to answer were assigned 'other ethnic group'. The mean outdoor time was recorded as 0.5 hours/day if it was less than 1 hour. Responses regarding vitamin and mineral use were further regrouped as 'vitamin D, multivitamin, fish oil and calcium use' and 'other vitamin and mineral use'. IMD scores were categorised into five quintiles, with the fifth quintile assigned the 'most deprived' group. The locations of 22 Biobank assessment centres were grouped using the geographical regions of the UK.[20] The date of blood collection for vitamin D examination was categorised into four seasons, according to the Met Office definitions.[21]

Demographic factors including sex, age (modelled as a continuous variable), ethnicity, BMI, smoking, drinking alcohol, IMD, vitamin D testing season and geographical location were further analysed in terms of their association with vitamin D insufficiency (<50 nmol/L) and deficiency (<25 nmol/L) using simple and multivariable

Table 3 The association between demographic characteristics and low vitamin D status

Characteristics	OR of vitamin D insufficiency (25(OH)D <50 nmol/L)		OR of vitamin D deficiency (25(OH)D <25 nmol/L)	
	Crude	Adjusted*	Crude	Adjusted*
Sex				
Female	1	1	1	1
Male	1.02 (1.0, 1.03)	0.91 (0.90, 0.93)	1.02 (1.01, 1.04)	0.91 (0.9, 0.93)
Age (SD)	0.98 (0.97, 0.97)	0.98 (0.98, 0.98)	0.97 (0.96, 0.97)	0.98 (0.98, 0.98)
BMI				
Healthy weight	1	1	1	1
Underweight	1.3 (1.20, 1.42)	1.26 (1.14, 1.38)	1.83 (1.64, 2.03)	1.71 (1.51, 1.93)
Overweight	1.25 (1.23, 1.26)	1.24 (1.22, 1.25)	1.11 (1.09, 1.13)	1.04 (1.01, 1.06)
Obese	2.15 (2.11, 2.18)	2.08 (2.04, 2.12)	1.91 (1.86, 1.95)	1.68 (1.64, 1.72)
Ethnic background				
White	1	1	1	1
Mixed	2.30 (2.11, 2.51)	2.24 (2.03, 2.46)	2.42 (2.21. 2.64)	2.31 (2.09, 2.56)
Asian	8.67 (8.02, 9.35)	8.54 (7.87, 9.27)	8.47 (8.10,8.86)	10.99 (10.39, 11.62)
Black	4.74 (4.44, 5.06)	4.14 (3.85, 4.45)	3.93 (3.74, 4.13)	3.6 (3.39, 3.83)
Chinese	4.25 (3.70, 4.89)	4.42 (3.81, 5.14)	2.72 (2.42, 3.06)	2.77 (2.42, 3.18)
Other ethnicity	2.91 (2.74, 3.10)	2.73 (2.55, 2.93)	3.24 (3.07, 3.42)	3.11 (2.9, 3.33)
Tobacco smoking				
Non-smoker	1	1	1	1
Current smoker	1.53 (1.50, 1.56)	1.43 (1.40, 1.46)	1.94 (1.90, 1.99)	1.82 (1.77, 1.87)
Alcohol drinking status				
Never drink	1	1	1	1
Drink occasionally	0.78 (0.76, 0.8)	0.85 (0.84, 0.88)	0.63 (0.61, 0.65)	0.75 (0.73, 0.78)
Drink weekly	0.55 (0.53, 0.56)	0.66 (0.64, 0.68)	0.40 (0.39, 0.42)	0.55 (0.53, 0.57)
Drink daily	0.53 (0.52, 0.55)	0.70 (0.68, 0.72)	0.43 (0.42, 0.45)	0.66 (0.64, 0.69)
Vitamin and mineral supplementation				
Other vitamin and mineral supplement	1	1	1	1
Vitamin D, multivitamin, fish oil and calcium supplement	0.46 (0.45, 0.47)	0.41 (0.41, 0.42)	0.36 (0.35, 0.37)	0.32 (0.31, 0.33)
Index of Multiple Deprivation (IMD)†				
1 (least deprived)	1	1	1	1
2	1.11 (1.09, 1.13)	1.03 (1.01, 1.05)	1.12 (1.09,1.16)	1.02 (0.98, 1.05)
3	1.18 (1.16, 1.2)	1.05 (1.03,1.07)	1.29 (1.26,1.33)	1.11 (1.07, 1.14)
4	1.44 (1.42, 1.47)	1.17 (1.14,1.19)	1.69 (1.64,1.74)	1.27 (1.23, 1.31)
5 (most deprived)	1.91 (1.87, 1.94)	1.34 (1.31,1.37)	2.43 (2.36, 2.50)	1.53 (1.48, 1.58)
Seasons				
Summer	1	1	1	1
Spring	3.48 (3.42, 3.54)	3.86 (3.79, 3.93)	5.26 (5.1, 5.42)	6.43 (6.22, 6.65)
Autumn	1.37 (1.35, 1.40)	1.43 (1.40, 1.45)	1.74 (1.68, 1.80)	1.89 (1.82, 1.96)
Winter	4.06 (3.99, 4.14)	4.56 (4.47, 4.65)	6.31 (6.12, 6.51)	7.82 (7.55, 8.1)
Regions (categorised centres)				
South West	1	1	1	1
South East	1.05 (1.03, 1.08)	1.15 (1.11, 1.18)	1.04 (0.98, 1.09)	1.11 (1.05, 1.18)
London	1.86 (1.81, 1.91)	1.31 (1.28, 1.35)	2.34 (2.24, 2.44)	1.31 (1.25, 1.38)
East Midlands	1.19 (1.15, 1.23)	1.07 (1.04, 1.11)	1.28 (1.22, 1.35)	1.13 (1.07, 1.2)
West Midlands	1.88 (1.82, 1.93)	1.22 (1.18, 1.26)	2.38 (2.27, 2.48)	1.26 (1.19, 1.32)

Continued

Table 3 Continued

Characteristics	OR of vitamin D insufficiency (25(OH)D <50 nmol/L)		OR of vitamin D deficiency (25(OH)D <25 nmol/L)	
	Crude	Adjusted*	Crude	Adjusted*
Wales	1.81 (1.75, 1.88)	1.03 (0.99, 1.07)	1.94 (1.84, 2.05)	1.03 (0.97, 1.09)
Yorkshire and The Humber	1.45 (1.41, 1.48)	1.19 (1.16, 1.23)	1.68 (1.61, 1.76)	1.30 (1.24, 1.36)
North West	1.38 (1.35, 1.42)	1.15 (1.12, 1.18)	1.68 (1.61, 1.76)	1.28 (1.22, 1.34)
North East	1.39 (1.35, 1.43)	1.23 (1.19, 1.27)	1.78 (1.7, 1.86)	1.53 (1.46, 1.61)
Scotland	2.83 (2.74, 2.92)	1.98 (1.91, 2.05)	3.58 (3.43, 3.75)	2.39 (2.28, 2.51)

*Adjusted for sex, age, BMI categories, ethnicity, smoking, drinking, vitamin D supplementation, IMD, seasons, regions of UK Biobank assessment centres.
†IMD scores were by quintile.
BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

logistic regression models. Covariates such as central obesity were not included in the model to avoid probable collinearity with BMI. All statistical analyses were carried out using Stata/MP V.15 (StataCorp, USA).

Patient and public involvement

Patients and/or the public were not involved in this retrospective study.

RESULTS

Comparison of included and excluded participants

We compared the included participants with those who did not have their vitamin D status tested (table 1). Vitamin D values were missing for 52 550 participants, who were excluded. Among excluded participants, the proportion of female participants (61.7%) was slightly higher than among the included participants (53.6%), while the distribution of the other categorical variables was similar across the included and excluded groups. The average age and mean outdoor time in summer were similar among included and excluded participants, while included participants who spent more time outside in winter.

Demographic distribution of vitamin D status

The demographic characteristics according to vitamin D status are shown in table 2. The average vitamin D levels were 19.2 nmol/L in the deficient group, 37.8 nmol/L in the insufficient group and 67.7 nmol/L in the sufficient group. The distribution of vitamin D status was similar in both sexes, and the mean age of the vitamin D sufficiency group was older than other groups. The proportion of vitamin deficiency was lower among people with healthy weight (n=15 886, 11%) and non-obese people (n=177 348, 11%). Asian (n=4297, 53.7%) and black (n=2459, 34.9%) people had a higher proportion of vitamin D deficiency than white participants (n=50 920, 12.0%). Vitamin D deficiency was more prevalent among current smokers (n=10 170, 21.7%) and those who never drank alcohol (n=8419, 23.7%). People deficient in vitamin D spent less outdoor time in winter (mean=1.7 hours). The proportion of vitamin D deficiency was lower among participants taking vitamin D and associated mineral supplements (n=11 368, 7%) compared with people taking any other supplements (n=48 877, 17%). The most deprived people had the highest proportion of vitamin D deficiency (n=17 602, 20.4%), while the least deprived participants had the lowest deficiency proportion (n=8414, 9.5%).

Vitamin D deficiency was more common in winter (n=21 066, 23.1%) and spring (25 912, 20%). Participants who visited assessment centres in Scotland had the highest proportion of vitamin D deficiency (n=7618, 23.5%) compared with other assessment centres in the southern UK (table 2). A map showing the distribution of the proportion of vitamin D deficiency in different seasons by each UK Biobank assessment centre is illustrated in online supplemental figure 1. This shows that in winter and spring, vitamin D deficiency was prevalent across the country, while in summer and autumn it was less common. Furthermore, in spring, autumn and winter, the proportion of vitamin D deficiency in the northern part of the UK was higher than in the southern part of the country (online supplemental figure 1).

Association between demographic factors and vitamin D status

To assess the association of factors related to vitamin D status, the ORs of having vitamin D deficiency (25(OH)D <25 nmol/L) or insufficiency (25(OH)D <50 nmol/L) were summarised in table 3. Male sex, abnormal BMI, smoking, non-white ethnicity, and being more deprived had greater odds of vitamin D deficiency or insufficiency. Increasing age, drinking alcohol, and taking vitamin D, multivitamin, fish oil or calcium supplements were associated with lower odds of vitamin D deficiency or insufficiency. Compared with summer, receiving testing in spring or winter had greater odds of vitamin D deficiency or insufficiency. For the regions with assessment centres, Scotland had higher odds of vitamin D deficiency and insufficiency compared with other regions in the UK. After adjusting for the variables listed in table 3, these associations remained (table 3).

DISCUSSION

In this cross-sectional study, we found that vitamin D deficiency was more prevalent among people of colour, in spring and winter, and in the northern UK. Male sex, abnormal BMI, non-white ethnic background, smoking and being more deprived increased the odds of vitamin D deficiency. Taking vitamin D supplements, drinking alcohol and increasing age were associated with decreased odds of vitamin D deficiency.

Our study showed that vitamin D status was strongly associated with seasonality and geographical distribution, which is similar to previous studies.[8 9] Other studies have also indicated that vitamin D deficiency is more prevalent among non-white ethnic groups, such as south Asians. A small longitudinal study (n=140) in the UK showed that more than 90% of south Asian women in the cohort had vitamin D insufficiency (25(OH)D <50 nmol/L) throughout the year, while the proportion of insufficiency was much lower in the white comparison groups (20.3% in summer and 70% in winter).[22] A systematic review of 19 cross-sectional studies and 1 cohort study indicated that serum hydroxyvitamin D levels were lower among black, African American and non-Hispanic black groups, compared with Caucasian groups.[23] These findings indicate the importance of differences in ethnicity, latitude and season, which should always be considered in dietary guidelines and future nutritional studies of vitamin D.

We found that abnormal BMI, tobacco smoking, and deprivation increased the odds of vitamin D deficiency or insufficiency, while taking vitamin D and associated mineral supplements was associated with a lower risk of vitamin D deficiency or insufficiency. These results are consistent with other research about factors associated with vitamin D.[1 24 25] The common vitamin D supplements contain around 400–2000 IU of vitamin D_2 or D_3, meeting the official daily intake guide. Our findings provide some evidence about the effect of vitamin D supplements as well as the Public Health England recommendation for vitamin D supplementation in winter and for some ethnic backgrounds.[2]

Curiously, age and drinking alcohol were inversely associated with vitamin D status. Ageing has been regarded as a risk factor for vitamin D deficiency; however, our results showed that increasing age slightly decreased the odds of deficiency. Nevertheless, the mean age of our study population was 56.5 years, and age-related vitamin D deficiency tends to manifest at a more advanced age.[26] Another possible explanation is that our study population focused on middle-aged or older participants, who may be more likely to receive prescriptions containing vitamin D. However, this information was not included in our study. Regarding drinking alcohol, although a previous systematic review reported no consistent correlation between alcohol intake and serum vitamin D levels,[27] recent studies have shown a negative association. A study using the National Health and Nutrition Examination Survey database in the USA from 2001 to 2010 showed the prevalence of vitamin D deficiency among current alcohol drinkers was 38% lower than in non-drinkers.[25] Similarly, a cross-sectional survey in Portugal among 1500 participants aged over 65 years showed that alcohol drinkers had lower odds of vitamin D deficiency compared with non-drinkers (moderate drinker: OR=0.49 (CI: 0.32 to 0.73); excessive drinker: OR=0.48 (CI: 0.27 to 0.85)).[24] The mechanism behind this possible association is still unclear, and future studies about vitamin D should take alcohol into account.

The key strengths of our study are its large sample size and wide range of measurements, which provide more statistical power in analysing the factors associated with vitamin D deficiency. Moreover, information about the exposure and outcomes was collected following a predefined protocol, and samples were processed systematically, which minimises potential differential or non-differential misclassification bias. Our analysis about the association between demographic factors and vitamin D deficiency is solid. However, several important limitations need to be considered. Despite the large size of the UK Biobank, this cohort is not nationally representative. Compared with non-participants, the participants of the UK Biobank were more likely to be older, women, and with a higher socioeconomic status and fewer health conditions.[12] Due to this healthy volunteer effect, our findings on vitamin D status cannot be generalised to the UK population, and we may have underestimated the prevalence of vitamin D deficiency. Nevertheless, as the healthy volunteer effect does not affect the validity of exposure–outcome relationships, our findings on the factors associated with vitamin D status are likely to be valid.

This analysis has concentrated on vitamin D status using serum vitamin D levels obtained from a single blood test, which may not reflect long-term vitamin D status. Additionally, the questionnaires collecting information about vitamin D supplementation and outdoor physical activity could not precisely quantify the exposure. In further research, a more representative sample should be considered to assess the prevalence of vitamin D deficiency in the UK. Researchers may consider using repeated measurements to assess long-term vitamin D status more precisely or including clinical diagnosis or vitamin D prescription to capture clinical status. In addition, collecting information to quantify vitamin D supplementation and outdoor activities in different seasons can further validate the recommendation of Public Health England.

CONCLUSION

Vitamin D deficiency was more common in winter and spring, and its prevalence was higher in the northern UK than the southern UK. Male sex, abnormal BMI, Asian and black ethnic backgrounds, and tobacco smoking were associated with higher odds of vitamin D deficiency. Taking vitamin D supplements and drinking alcohol were associated with lower odds of vitamin D deficiency. These results provide some evidence supporting the Public

Health England recommendation for taking vitamin D supplementation in winter and for people with black or Asian ethnic backgrounds.

Contributors L-YL contributed to the design and analysis of the study, drafted and revised the manuscript according to other authors' comments. CW-G contributed to the design of the study, made critical comments on the manuscript and revised the paper critically. SL contributed to the design of the study, made critical comments on the manuscript and revised the paper critically. LS contributed to the design of the study and revised the paper critically. All authors approved the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The UK Biobank obtained ethics approval from its Research Ethics Committee (reference: 11/NW/0382). Our study received ethics approval from the UK Biobank (ID:51265) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (reference: 17158). Our study followed the principles of the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Other researchers can apply for UK Biobank data to answer specific research questions. We will upload our analysis codes to LSHTM Data Compass, an open data repository for research outputs from LSHTM (https://datacompass.lshtm.ac.uk/).

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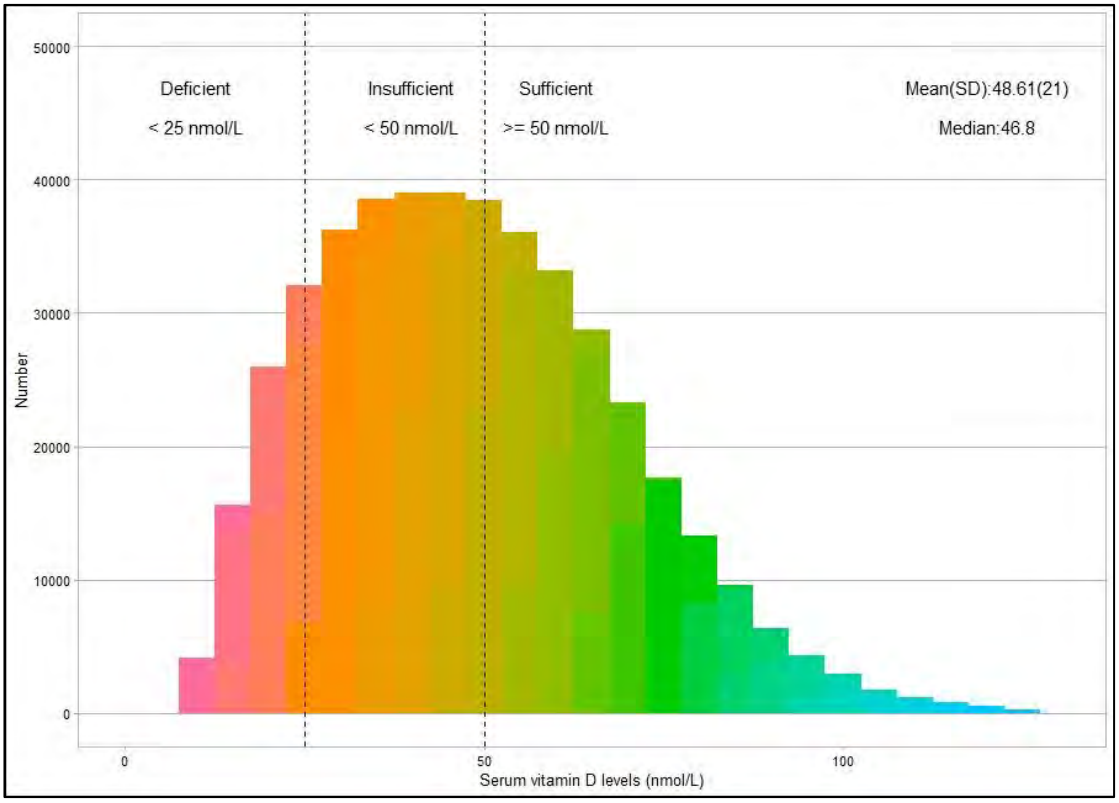
REFERENCES

1 Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.
2 Public Health England. PHE publishes new advice on vitamin D, 2016. Available: https://www.gov.uk/government/news/phe-publishes-new-advice-on-vitamin-d [Accessed 05 October 2020].
3 Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. *Nutrients* 2018;10:1656. doi:10.3390/nu10111656
4 Mann EH, Pfeffer PE, Hawrylowicz CM. Chapter 104 - Vitamin D and Adaptive Immunology in Health and Disease. In: Feldman D, ed. *Vitamin D*. Fourth Edition. Academic Press, 2018: 937–49.
5 Martineau AR, Jolliffe DA, Hooper RL, *et al*. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;356:i6583.
6 The Scientific Advisory Committee on Nutrition. Vitamin D and health: public health England 2016.
7 Royal Osteoporosis Society. Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management.. In: . 10. 2 ed. Royal Osteoporosis Society, 2018.
8 Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 Y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* 2007;85:860–8.
9 Public Health England. National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012). In: Bates B, Lennox A, Prentice A, eds. *National Diet and Nutrition Survey (NDNS)*. Public Health England, 2017.
10 Sudlow C, Gallacher J, Allen N, *et al*. Uk Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12:e1001779.
11 UK Biobank. Uk Biobank biomarker panel 2015.
12 Fry A, Littlejohns TJ, Sudlow C, *et al*. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol* 2017;186:1026–34.
13 UK Biobank. Index of multiple deprivation scores 2018.
14 Elliott P, Peakman TC, Biobank UK, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. *Int J Epidemiol* 2008;37:234–44.
15 CDC. CDC vitamin D Standardization-Certification program (CDC VDSCP) 2020.
16 UK Biobank. *Biomarker assay quality procedures: approaches used to minimise systematic and random errors*. 1.2 ed, 2019.
17 UK Biobank. *Companion document for serum biomarker data*. 1 ed, 2019.
18 Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;340:b5664.
19 National Institute for Health and Care Excellence (NICE). Bmi: preventing ill health and premature death in black, Asian and other minority ethnic groups: National Institute for health and clinical excellence 2013.
20 Office for National Statistics. Regions (December 2017) map in United Kingdom, 2019. Available: https://geoportal.statistics.gov.uk/datasets/0758ee9479044ecc9547ebab340c84a8 [Accessed 09/23 2020].
21 Met Office. Seasons 2019. Available: https://www.metoffice.gov.uk/weather/learn-about/weather/seasons accessed 25/11 2019
22 Darling AL, Hart KH, Macdonald HM, *et al*. Vitamin D deficiency in UK South Asian women of childbearing age: a comparative longitudinal investigation with UK Caucasian women. *Osteoporos Int* 2013;24:477–88.
23 Renzaho AMN, Halliday JA, Nowson C. Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: a systematic review. *Nutrition* 2011;27:868–79.
24 Santos A, Amaral TF, Guerra RS, *et al*. Vitamin D status and associated factors among Portuguese older adults: results from the nutrition up 65 cross-sectional study. *BMJ Open* 2017;7:e016123.
25 Liu X, Baylin A, Levy PD. Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. *Br J Nutr* 2018;119:928–36.
26 Aspell N, Laird E, Healy M, *et al*. The prevalence and determinants of vitamin D status in community-dwelling older adults: results from the English longitudinal study of ageing (ELSA). *Nutrients* 2019;11:1253.
27 Tardelli VS, Lago MPPdo, Silveira DXda, *et al*. Vitamin D and alcohol: a review of the current literature. *Psychiatry Res* 2017;248:83–6.

5.2. Further analysis on vitamin D level distributions in the UK Biobank

I further explored the distribution of the serum vitamin D levels as continuous variables. **Chapter 5. Figure 1** is a histogram showing the distribution of vitamin D levels in the UK Biobank. As can be seen in the plot, the overall distribution of serum vitamin D levels is slightly right-skewed, and the mean (48.6 nmol/L) is slightly larger than the median (46.8 nmol/L). Overall, majority of people in the UK Biobank were insufficient or deficient in vitamin D, and the data distribution is still close to normal. This result is similar to a previous study, indicating that 58.7% of 50-year-old participants of a nationally representative cohort from England had a serum vitamin D level less than 50 nmol/L (118).



Chapter 5. Figure 1. The distribution of vitamin D levels in the UK Biobank

As mentioned in Chapter 1, there is currently no universal consensus about vitamin D deficiency. In this thesis, serum vitamin D levels were categorized as deficient (<25 nmol/L), insufficient (25-49 nmol/L), and sufficient (≥50 nmol/L) following Public Health England's definition, which is appropriate within the clinical context in the UK. However, the definition of vitamin D deficiency varies in different countries. Therefore, the findings of this thesis may not be applied to countries using different definitions of vitamin D deficiency (**Chapter 1. Table 3**). Future studies may consider analysing vitamin D as a continuous variable so that the results would be more easily applied to different countries using different standards. Utilising continuous data would also optimally use all the data, beyond the focus in this thesis.

5.3. Chapter summary

- Vitamin D deficiency was more common among Asian (n=4297/8000, 53.7%) and black (n=2459/7046, 34.9%) ethnic groups.
- In the UK, vitamin D deficiency was more common during spring and winter, and the proportion of vitamin D deficiency was higher in the north and the south.
- Male sex, abnormal BMI, non-white ethnicity, smoking and higher levels of socioeconomic deprivation were associated with increased odds of vitamin D deficiency.
- I found that increased age, supplementation, and alcohol consumption were associated with lower odds of vitamin D deficiency.
- My findings support Public Health England's recommendation on vitamin D supplementation in the winter and among people with black and Asian ethnic backgrounds.

Chapter 6. The association between vitamin D and the risk of herpes zoster

Chapter overview

In Chapter Three, my systematic review indicated that the association between vitamin D and HZ remains unclear, especially among the general population; in the previous chapter, I identified demographic and lifestyle factors associated with vitamin D deficiency or insufficiency, which are potential confounders to adjust for in the following analysis. This chapter explores the association between vitamin D deficiency or insufficiency and the HZ risk using UK Biobank cohort data.

The research question of this chapter is: d*oes vitamin D deficiency increase the risk of herpes zoster?*

This paper has been submitted to *the British Journal of General Practice* (119).

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SECTION A – Student Details

Student ID Number	1803144	**Title**	Mr
First Name(s)	Liang-Yu		
Surname/Family Name	Lin		
Thesis Title	The association between vitamin D deficiency and the risk of herpes zoster and COVID-19		
Primary Supervisor	Sinéad Langan		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Please list the paper's authors in the intended authorship order:	Liang-Yu Lin, Rohini Mathur, Amy Mulick, Liam Smeeth, Sinéad M Langan, Charlotte Warren-Gash
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Liang-Yu contributed to the design of the study, underwent statistical analysis, drafted and revised the manuscript according to other authors' comments; Rohini Mathur contributed to the design of the study and revised the paper critically; Amy Mulick contributed to the design of the study, examined the statistical analysis, and revised the paper critically; Charlotte Warren-Gash contributed to the design of the study, made critical comments on the manuscript and revised the paper critically; Sinéad Langan contributed to the design of the study, made critical comments on the manuscript and revised the paper critically; Liam Smeeth contributed to the design of the study and revised the paper critically. All authors approved the final version of the manuscript.

SECTION E

Student Signature	[redacted]
Date	14/11/2021

Supervisor Signature	[redacted]
Date	22.11.2021

Title: The Association between Vitamin D and Incident Herpes Zoster: A UK Biobank Study

List title: Vitamin D and Herpes Zoster

Authors:

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Competing interest:

None declared.

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Data sharing statement:

Other researchers can apply for UK Biobank data to answer specific research questions. We have uploaded our analysis codes to GitHub (https://github.com/liang-yu12/ukb_vd_hz_publish).

Abstract

Background: Although vitamin D has immunomodulatory effects, any association with herpes zoster (HZ) is unclear.

Aim: To explore the association between vitamin D status and the risk of incident HZ in adults in the UK.

Design and setting: We conducted a cohort study including participants from UK Biobank, who had at least one vitamin D testing result with linked primary care electronic health records.

Methods: The primary exposure was vitamin D status, categorised as deficient (< 25 nmol/L), insufficient (25–50 nmol/L) or sufficient (≥ 50 nmol/L). The secondary exposures were self-reported vitamin D supplementation at baseline assessment and vitamin D prescription records. The outcome was diagnosed incident HZ, identified from linked primary care or hospital inpatient records. We used Weibull regression, adjusting for potential confounders including demographic factors, comorbidities and immunosuppression.

Results: We included 177,572 eligible participants in our analysis with mean follow-up time of 10.1 (SD=1.9) years. No evidence showed that low vitamin D was associated with a higher incidence of HZ, compared with people with sufficient vitamin D (deficient: adjusted hazard ratio [HR] = 0.99, 95% confidence interval [CI] = 0.90–1.10; insufficiency: RR = 1.03, CI = 0.96–1.10.) We found no evidence that vitamin D supplementations or receiving vitamin D prescription was associated with HZ incidence (supplementation: HR = 0.88, CI = 0.67–1.16; prescription: HR = 1.11, CI = 0.91–1.34.)

Conclusion: We observed no association of vitamin D status, supplementation or prescription with incident HZ. No evidence supported vitamin D supplementation as a strategy to prevent HZ.

Keywords: Vitamin D, Herpes zoster, Electronic Health Records, primary health care, UK Biobank

How this fits in:

Vitamin D is regarded as having some antimicrobial effects. We used large nationwide cohort data to explore the association between vitamin D status and the risk of herpes zoster. Our results showed that neither serum vitamin D status, vitamin D supplementation, nor vitamin D prescriptions in primary care was associated with incident herpes zoster. Based on currently available evidence, vitamin D supplements are not an effective intervention to prevent herpes zoster.

Introduction

Herpes zoster is a common disease among adults. In the UK, its annual incidence is around 5 per 1,000 person-years, and its average lifetime risk is around 30% in people without vaccination (38, 120). The typical symptoms are unilateral painful vesicular rashes in a dermatomal distribution, lasting about seven to ten days. Herpes zoster significantly decreases patients' quality of life and substantially increases medical and social costs (38). It may also be associated with a range of neurological, ocular, cutaneous and visceral complications (121). The most important risk factor for herpes zoster is ageing because immunity wanes over time (35). Immunosuppression and some comorbidities such as chronic kidney disease (CKD) and systematic lupus erythematosus (SLE) are also associated with increased herpes zoster risk (39). Vaccines are effective for reducing the risk of herpes zoster (122). However, in the UK, the vaccination programme is only available for people aged 70 years or greater (123). Studying other possible preventive measures for herpes zoster is important, especially for people under the age of 70.

The musculoskeletal protection effects of vitamin D have been well-established because it regulates calcium and phosphate homeostasis (2). In addition, the non-skeletal effects of vitamin D have been recently studied, such as immunomodulation. In vitro studies have shown that vitamin D could stimulate the expression of antimicrobial peptides, protecting against infections (22, 23). However, epidemiological studies have shown inconsistent associations between vitamin D and infections. A systematic review and meta-analysis published in 2021, combining 37 clinical trials of

vitamin D supplementation, showed that taking vitamin D slightly decreased the risk of respiratory infections (odds ratio = 0.92, 95% CI = 0.86–0.99) (24). Our previous systematic review and meta-analysis found inconclusive evidence for any association between vitamin D and herpesviruses in studies conducted primarily among immunosuppressed individuals (124). One case-control study among people with CKD showed that vitamin D supplementation may decrease the odds of herpes zoster (125).

If vitamin D deficiency is associated with an increased risk of herpes zoster in the general population, taking vitamin D supplements may become a cheap public health measure for its prevention. Therefore, we aimed to explore the association between serum vitamin D status or supplementation and the risk of herpes zoster using the UK Biobank cohort.

Methods

Data source

Our data source was UK Biobank, a nationwide cohort recruited between 2006 and 2010, consisting of approximately half a million participants aged 40 to 69 years from England, Wales and Scotland. At recruitment, participants visited 22 UK Biobank assessment centres, in which they received physical examinations, completed questionnaires and gave biological samples, including blood, urine and saliva (89). Participants also consented to have their clinical data linked, including diagnosis codes for inpatient and outpatient visits, the dates of diagnosis, the dates of each hospitalisation episode or consultation, and prescribing records from primary care (126, 127). Nearly all participants have linked hospital inpatient records, and around 230,000 participants also have their primary care records linked (128).

Primary exposure: vitamin D status

The primary exposure of our study was serum vitamin D status recorded between 2006 and 2010. The detailed methods for measuring serum vitamin D levels are described in **Supplementary Box 1**. Participants' with serum 25-hydroxyvitamin D levels less than 25 nmol/L were coded as deficient, between 25 and 50 nmol/L as insufficient, and greater than or equal to 50 nmol/L as sufficient following Public Health England's definition (3).

Secondary exposure: vitamin D supplementation

A secondary exposure, vitamin D supplementation, was recorded using a self-reported questionnaire during participants' baseline visits between 2006 and 2010. This included self-reported use of over-the-counter supplements, such as vitamin D, multivitamins, fish oil and calcium. Another secondary exposure was a general practitioner (GP)-prescribed vitamin D supplementation, obtained from the GP prescription data within two years before the baseline assessment. The detailed data management of secondary exposures is summarised in **Supplementary Box 2**.

Outcome

The outcome of our study was incident herpes zoster, defined through clinical diagnosis recorded in the linked primary care and inpatient records. We developed diagnosis code-lists for herpes zoster in Read 2, Clinical Terms Version 3 (CTV3), and International Classification of Diseases version 10 (ICD-10) codes to identify incident herpes zoster from the dataset (126, 127). Incident herpes zoster diagnoses were defined as participants with a herpes zoster diagnosis occurring at least one day after the start of follow-up from the baseline assessment to 31 July 2019 (**Figure 1**).

Study eligibility

The study design is summarized in **Figure 1**. UK Biobank participants were eligible if they had at least one vitamin D record, with both primary care and inpatient care records. Participants with no vitamin D record, with no linked electronic health records (EHR) or with previous herpes zoster or post-herpetic neuralgia within five years before follow-up were excluded (**Figure 1**).

Measurement of covariates

Some demographic factors associated with vitamin D deficiency and insufficiency were recorded at baseline assessment, including sex, age, ethnicity, body mass index (BMI), smoking status, drinking frequency, Index of Multiple Deprivation (IMD), regions of UK Biobank assessment centres and the seasons when vitamin D was tested (117). We also identified comorbidities associated with an increased risk of herpes zoster, such as CKD and SLE, from the linked EHR and self-reported health conditions (39). Severe immunosuppressive conditions, including organ transplantation, chemoradiotherapy, cell-mediated immunosuppression, HIV, blood cancers, chemotherapy (biological and non-biological agents), bone marrow transplantation and long-term oral steroid use were identified solely from the clinical datasets. Long-term oral steroid use was defined as at least two steroid prescriptions within 90 days. We defined these clinical covariates as ever had a diagnostic code within five years before the follow-up. For blood cancer, bone marrow transplantation and steroid use, the covariate assessment time windows were up to two years before follow-up (**Figure 1**.)

Statistical analysis

This was a historical cohort study. We followed participants from the date when they visited the assessment centre. The end of follow-up was defined as the date that herpes zoster was diagnosed, the date of death or loss to follow-up, or 31 July 2019, whichever was first. The demographic characteristics of the included participants were compared by their vitamin D status, and the included and excluded participants were further compared.

We assessed the association between the primary and secondary exposures and incident herpes zoster using Weibull regression models, adjusting for possible confounders selected by using a directed acyclic graph approach, summarised in **Supplementary Figure 1**. Our models included sex, age, BMI, ethnicity, smoking status, drinking frequency, IMD scores, regions of UK Biobank assessment centres, vitamin D testing seasons, underlying comorbidities and immunosuppression, which are described in **Supplementary Box 3**. Because the proportion of missingness was low (less than 3%), and the chance of being a complete case was not dependent on our outcome, we performed a complete case analysis (129). All statistical analysis and plotting were performed using R Statistical Software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analysis

We performed various sensitivity analyses, and the justification is summarised in **Table 1**. We excluded records after September 2013, following the introduction of the vaccination program, and we compared the effects of different covariate definitions. We also used Poisson regression assuming baseline hazards is constant. To eliminate the potential effect of time-varying hazards, we also reran analyses using the Cox proportional-hazards regression model. (**Table 1**).

Results

Study population

The selection of the study population is summarised in **Figure 2**. After excluding people without vitamin D records, without clinical records, or with previous herpes zoster episodes, we included 177,572 participants in our analysis. A comparison of the included and excluded participants is summarised in **Supplementary Table 1**. The distribution of demographic factors was similar between the included and excluded participants, and more people from Yorkshire and the Humber, Scotland and East Midlands area were included in our analysis. The proportion of missingness across demographic factors was below 3%, and 54.5% of self-reported vitamin D supplementations were missing (**Supplementary Table 2**)**.**

The distribution of demographic factors by vitamin D status is summarised in **Table 2**. Across different vitamin D statuses, the distributions of sex, age, comorbidities and immunosuppression were similar. More participants with Asian or black ethnic backgrounds were vitamin D deficient at baseline. Participants in the vitamin D deficient group were more likely to be obese, smoked more and lived in more deprived areas, and they drank less frequently and were less likely to receive vitamin D prescriptions. More people with vitamin D deficiency were tested during winter, and more vitamin D deficient participants were from Scotland than from other countries. The mean follow-up periods of people with different vitamin D statuses were similar, with an average of 10 years (**Supplementary Table 2**).

Association between vitamin D and risk of herpes zoster

The associations between vitamin D status and the risk of incident herpes zoster are summarised in **Figure 3**. Compared with people with sufficient vitamin D status, some evidence existed that vitamin D deficiency was associated with a decreased risk of incident herpes zoster in the crude Weibull regression model (hazard ratio [HR] = 0.86, 95% confidence interval [CI] = 0.79–0.95). However, in models adjusted for sex and age, as well as models fully adjusted for all covariates, no evidence showed that vitamin D deficiency or insufficiency were associated with incident herpes zoster (partially adjusted model: insufficiency HR = 1.01, CI = 0.95–1.08; deficient HR = 0.96, CI = 0.87–1.05; fully adjusted model: insufficiency HR = 1.03, CI = 0.90–1.10; deficient HR = 0.99, CI = 0.90–1.10; **Figure 3).**

Association between vitamin D supplementation and risk of incident herpes zoster

Figure 4a shows the association between self-reported vitamin D supplementation and the risk of incident herpes zoster. We found no evidence that self-reported vitamin D supplement use was associated with incident herpes zoster in the subgroup of participants for whom this information was recorded. Some evidence existed that ever having received vitamin D prescriptions was associated with an increased risk of herpes zoster in the crude (HR = 1.59, 95% CI= 1.33–1.91) and partially adjusted models (HR = 1.27, 95% CI= 1.06–1.52). However, such association disappeared after fully adjusting for potential confounders (HR = 1.11, 95% CI = 0.91–1.34) (**Figure 4b**).

Sensitivity analyses

After excluding records after 1 September 2013, the main findings remained similar. Neither vitamin D deficiency nor insufficiency (**Supplementary Figure 2**), nor vitamin D supplementation (**Supplementary Figure 3a)**, nor receiving vitamin D prescription (**Supplementary Figure 3b**) provided evidence of an association with herpes zoster. We compared different covariate definitions, and the results did not differ from the initial model (**Supplementary Figure 4**). The stratified Cox regression model showed no evidence of an association between vitamin D status and incident herpes zoster, either before or after the vaccination program was initiated (**Supplementary Figure 5**). The Cox proportional-hazards model showed no evidence of an association between vitamin D supplementation and herpes zoster (**Supplementary Figure 6a**), whereas weak evidence existed that vitamin D prescription was associated with a higher risk of herpes zoster (adjusted HR = 1.17, 95%CI=1.00-1.37, **Supplementary Figure 6b.**) The Poisson regression model showed no evidence of an association of vitamin D status, supplementation or prescription with herpes zoster (**Supplementary Figure 7.** and **Supplementary Figure 8.**)

Discussion

Summary

We found no evidence of an association between vitamin D deficiency or insufficiency and incident herpes zoster after adjusting for potential confounders. Self-reported vitamin D supplementation or receiving vitamin D prescriptions showed no evidence of an association with incident herpes zoster. The results were robust across a range of sensitivity analyses, such as excluding records during the shingles vaccination period and adjusting for differing definitions of confounding factors.

Strengths and limitations

Our study has several strengths. First, compared to previous small studies conducted with clinically high-risk individuals, our large study of a general population provides greater statistical power and generalisability. Second, the vitamin D levels were measured systematically, and the proportion of missingness of covariates was relatively low. Third, the linkage between UK Biobank and the primary and secondary care records enabled us to follow up with participants for a long time and identify incident cases.

Nevertheless, some limitations of our study also need to be stressed. First, the exposure and some covariates are probably time-dependent, but we used the measures taken at baseline. In our previous analysis, the proportion of vitamin D deficiency was lowest in summer, and it was more prevalent in winter and spring (117). In another study measuring vitamin D repeatedly, the intraclass correlation coefficient between two vitamin D measurements

after five years was only 0.59, which was moderately reliable (130). In our analysis, we used Weibull regression which assumes hazards increase during follow-up, and we adjusted for vitamin D testing seasons in our model to minimise the effect of seasonal variation. In sensitivity analysis, we used the Cox model regression to adjust for potentially time-varying hazards, and the results remained similar. Second, despite the completeness of most covariates, for self-reported vitamin D supplementation, more than half of the data were missing. Therefore, this variable may not reflect the real vitamin D supplementation use, and its association with the outcome needs to be interpreted carefully.

Third, the outcomes might be under-ascertained. We defined herpes zoster using EHR, but people with more comorbidities may visit their primary care physicians more frequently. Thus, herpes zoster among these people is more likely to be diagnosed, while mild shingles among a younger or healthier population might not be noticed (131). In our study population, although the proportions of people with diabetes and chronic obstructive pulmonary disease were slightly higher in the vitamin D deficiency group, the overall distributions of comorbidities and immunosuppression were similar across different vitamin D statuses. Any ascertainment bias in our study should be non-differential.

Lastly, residual confounding effects cannot be ruled out. Using diagnostic codes from the linked records may underestimate the true prevalence of some diseases, such as CKD. In studies using EHR, serum creatinine levels are more often used to diagnose CKD (115). However, laboratory test results are not available in the linked EHR of UK Biobank. To enhance the sensitivity

of detecting comorbidities, we included self-reported non-cancer health conditions in our analysis, but the overall prevalence of CKD was still much lower than the national prevalence during the same period (132).

Comparison with existing literature

Ours is the first published study assessing the association between vitamin D status and incident herpes zoster in the general population. Previous studies on this topic have been conducted among people with immunosuppression. For instance, a small case-control study among CKD patients showed that patients taking vitamin D supplements had lower odds of having herpes zoster (125). Compared to previous studies, our study population was largely immunocompetent.

We found no evidence of an association between self-reported or prescribed vitamin D supplements and incident herpes zoster, although the great proportion of missing data in self-reported vitamin D supplements may have biased the results. A positive trend of association between GP-prescribed vitamin D supplementation and herpes zoster was noted in the crude, partially adjusted model and in the sensitivity analysis. This association may be due to confounding by indication, as well as the underestimation of unreported food fortification. People receive vitamin D prescriptions to prevent or treat vitamin D deficiency, but we did not consider the indication for the prescription. Vitamin D food fortification is another main source of vitamin D supplementation in the UK primary care setting (133). However, due to the limitation of data availability, this was not included in our analysis.

Implications for research and/or practice

Our cohort study showed no evidence to support an association between vitamin D status or supplementation and incident herpes zoster. Based on currently available evidence, vitamin D testing, supplementation or fortification cannot be recommended to prevent herpes zoster.

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Ethical approval

The UK Biobank project was approved by the Northwest Haydock Research Ethics Committee (reference: 11/NW/0382). Our project was approved by UK Biobank (ID:51265) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (reference: 17158).

Competing interests

Professor Liam Smeeth is an expert that has been consulted by UK Biobank for biomarkers to be included. All authors declare that they have no known conflicts of interest.

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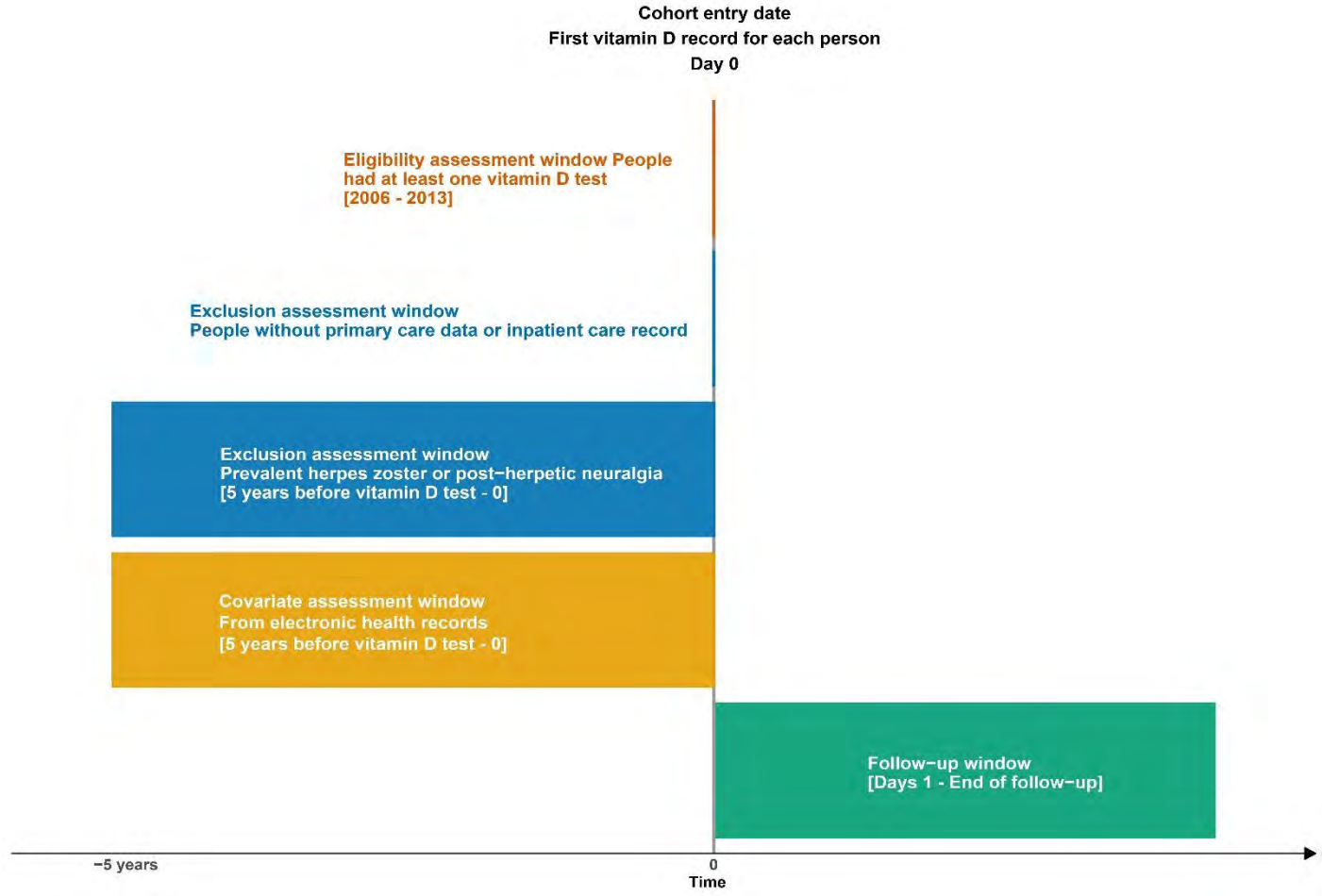
References

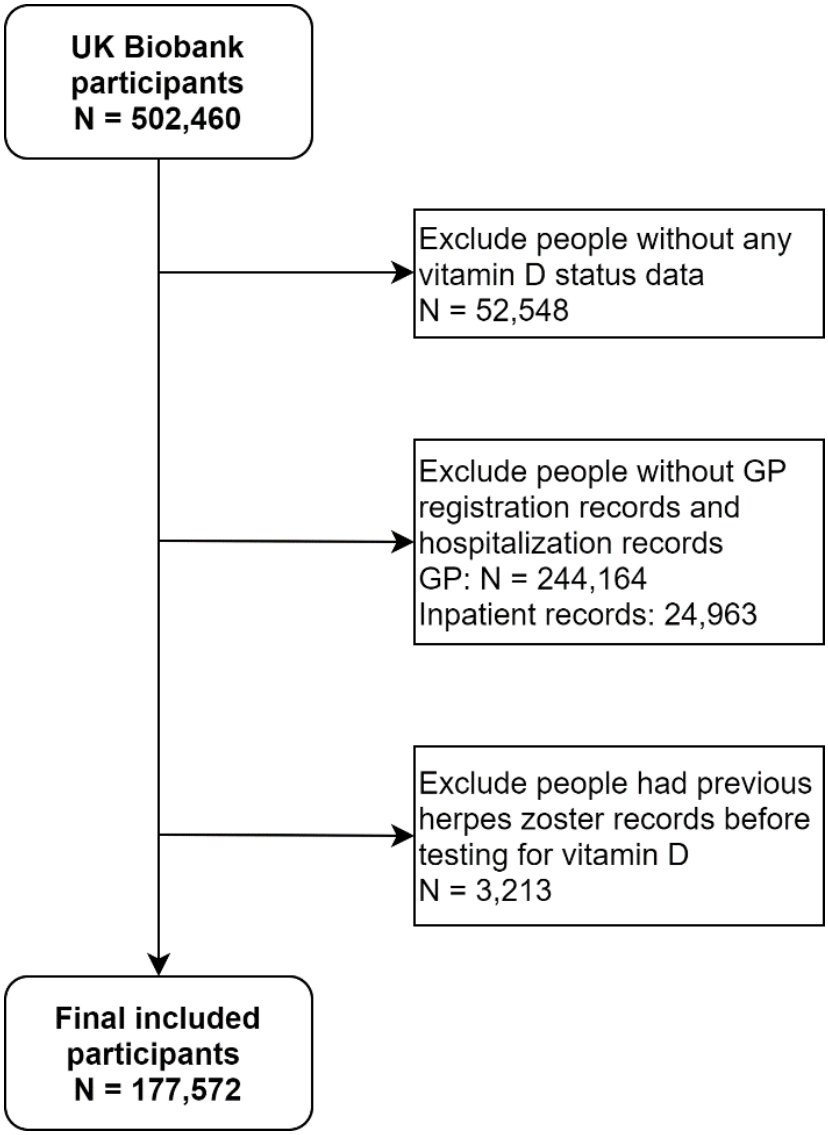
1. Gauthier A, Breuer J, Carrington D, Martin M, Remy V. Epidemiology and cost of herpes zoster and post-herpetic neuralgia in the United Kingdom. Epidemiology and infection. 2009;137(1):38-47.
2. Brisson M, Edmunds WJ, Law B, Gay NJ, Walld R, Brownell M, et al. Epidemiology of varicella zoster virus infection in Canada and the United Kingdom. Epidemiology and infection. 2001;127(2):305-14.
3. Forbes HJ, Bhaskaran K, Grint D, Hu VH, Langan SM, McDonald HI, et al. Incidence of acute complications of herpes zoster among immunocompetent adults in England: a matched cohort study using routine health data*. British Journal of Dermatology. 2021;184(6):1077-84.
4. Cohen JI. Herpes Zoster. New England Journal of Medicine. 2013;369(3):255-63.
5. Forbes HJ, Bhaskaran K, Thomas SL, Smeeth L, Clayton T, Langan SM. Quantification of risk factors for herpes zoster: population based case-control study. Bmj. 2014;348:g2911.
6. Amirthalingam G, Andrews N, Keel P, Mullett D, Correa A, de Lusignan S, et al. Evaluation of the effect of the herpes zoster vaccination programme 3 years after its introduction in England: a population-based study. The Lancet Public Health. 2017;2667:1-9.
7. Public Health England. Shingles: guidance and vaccination programme 2013 [updated 13 March 2020. Available from: https://www.gov.uk/government/collections/shingles-vaccination-programme.
8. Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.
9. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770-3.
10. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsjo A, Törmä H, Stahle M. Vitamin D induces the antimicrobial protein hCAP18 in human skin. The Journal of investigative dermatology. 2005;124(5):1080-2.
11. Jolliffe DA, Camargo CA, Jr., Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. The Lancet Diabetes & Endocrinology. 2021.
12. Lin L-Y, Bhate K, Forbes H, Smeeth L, Warren-Gash C, Langan SM. Vitamin D Deficiency or Supplementation and the Risk of Human Herpesvirus Infections or Reactivation: A Systematic Review and Meta-analysis. Open Forum Infectious Diseases. 2020;8(1).

13. Chao CT, Lai CF, Huang JW. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. Eur J Intern Med. 2012;23(8):711-5.
14. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.
15. UK Biobank. Primary Care Linked Data 2019 [cited 2021 10 August]. Available from: https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=591.
16. UK Biobank. Hospital inpatient data 2020 [Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=138483.
17. UK Biobank. Health-related outcomes data 2021 [updated April 1st 2021. Available from: https://www.ukbiobank.ac.uk/enable-your-research/about-our-data/health-related-outcomes-data.
18. The Scientific Advisory Committee on Nutrition. Vitamin D and Health. London: Public Health England 2016 21 July 2016.
19. Lin L-Y, Smeeth L, Langan S, Warren-Gash C. Distribution of vitamin D status in the UK: a cross-sectional analysis of UK Biobank. BMJ open. 2021;11(1):e038503.
20. Hughes RA, Heron J, Sterne JAC, Tilling K. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. International journal of epidemiology. 2019;48(4):1294-304.
21. Meng JE, Hovey KM, Wactawski-Wende J, Andrews CA, LaMonte MJ, Horst RL, et al. Intraindividual Variation in Plasma 25-Hydroxyvitamin D Measures 5 Years Apart among Postmenopausal Women. Cancer Epidemiology Biomarkers & Prevention. 2012;21(6):916.
22. Batram M, Witte J, Schwarz M, Hain J, Ultsch B, Steinmann M, et al. Burden of Herpes Zoster in Adult Patients with Underlying Conditions: Analysis of German Claims Data, 2007-2018. Dermatol Ther (Heidelb). 2021;11(3):1009-26.
23. McDonald HI, Shaw C, Thomas SL, Mansfield KE, Tomlinson LA, Nitsch D. Methodological challenges when carrying out research on CKD and AKI using routine electronic health records. Kidney Int. 2016;90(5):943-9.
24. Public Health England. Chronic kidney disease (CKD) prevalence model: Public Health England,; 2014 [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/612303/ChronickidneydiseaseCKDprevalencemodelbriefing.pdf.
25. Wan M, Patel A, Patel JP, Rait G, Jones SA, Shroff R. Quality and use of unlicensed vitamin D preparations in primary care in England: Retrospective review of national prescription data and laboratory analysis. British journal of clinical pharmacology. 2021;87(3):1338-46.

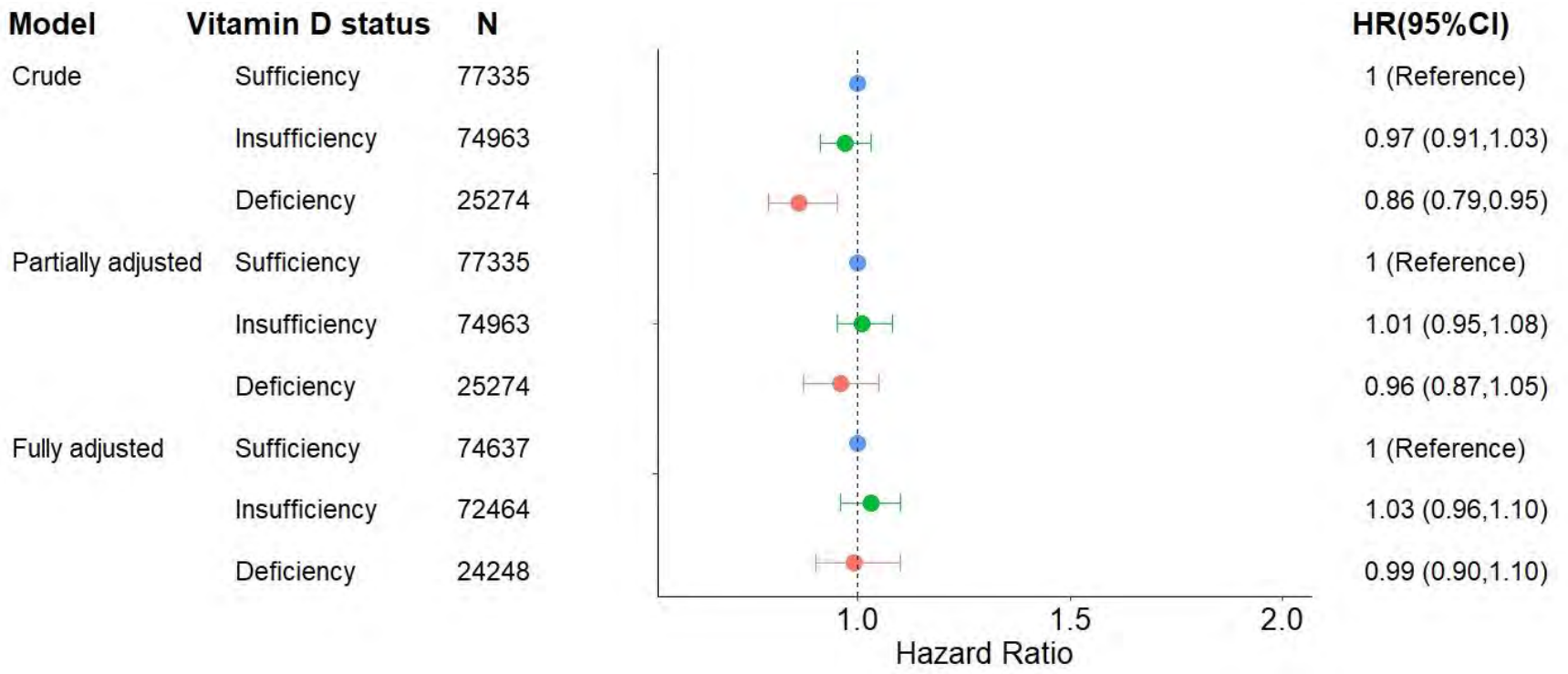
Figures



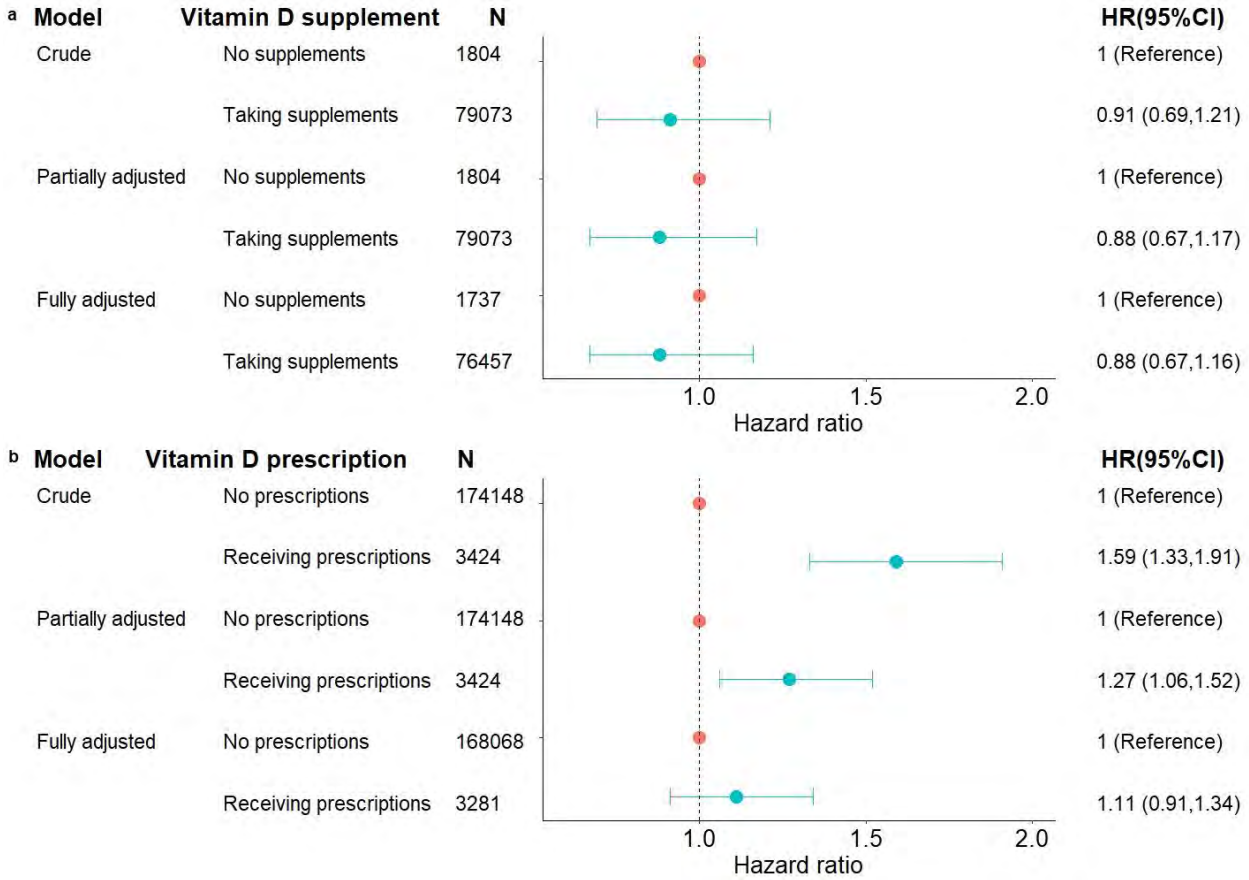
Chapter 6. Figure 1 Study design diagram



Chapter 6. Figure 2 The diagram of selecting eligible participants



Chapter 6. Figure 3 The association between vitamin D status and the risk of herpes zoster. Crude: simple Weibull regression model without adjustment; Partially adjustment: Weibull regression model adjusted for sex and age; Fully adjusted: multivariable Weibull regression



Chapter 6. Figure 4 a. The association between self-reported vitamin D supplementation and the risk of herpes zoster; **b**. the association between receiving vitamin D prescriptions and the risk of herpes zoster. Model explanation: Crude: simple Weibull regression model without adjustment; Partially adjustment: Weibull regression model adjusted for sex and age; Fully adjusted: multivariable Weibull regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Tables

Chapter 6. Table 1 Sensitivity Analysis

Sensitivity analysis		Justification
Model 1	Stop follow-up by 31 August 2013	Because in the UK, a universal herpes zoster vaccination program has initiated since September 2013, excluding these records could minimize the interference of vaccination (123).
Model 2	Only identify comorbidities from clinical records	Because self-reported non-cancer health conditions may not be accurate, we only defined comorbidities by using linked clinical records
Model 3	Only include high dose steroid users in the immunosuppressive conditions	In the herpes zoster immunization guidance, moderate to high dose steroid use were defined as 20mg per day.
Model 4	Comorbidities: exclude self-reported health conditions	To assess the effects using different covariates definitions
	Immunosuppressive conditions: in steroid use, only include high dose users	
Model 5	Use Cox-regression to analyse the association between exposure and outcomes	Because some covariates are time dependent, we used cox regression model with the time scale as age. If the model violated the proportional hazard assumption, we would stratify the follow-up time on 1 September 2013, which was the date that the vaccination program initiated.
Model 6	Use Poisson regression to analyse the association between exposure and outcomes	Assuming the hazard of herpes zoster remains constant over follow-up, we used Poisson regression model to examine our hypothesis.

6.2. Chapter summary

- A total of 177,572 UK Biobank participants with vitamin D records and linked primary care and inpatient care records were included in this study, with a mean follow-up of 10 years.
- After fully adjusting for potential confounders using Weibull regression, I found no evidence that vitamin D deficiency or insufficiency were associated with incident HZ (insufficient HR = 1.03, CI = 0.90–1.10; deficient HR = 0.99, CI = 0.90–1.10.)
- I found no evidence that self-reported vitamin D supplementation or GP prescribed vitamin D supplementation were associated with incident HZ (self-reported supplementation: HR = 0.88, CI = 0.67–1.16; GP prescribed supplementation: HR = 1.11, CI = 0.91–1.34.)
- I performed a range of sensitivity analyses, including adjusting for differing definitions of confounding factors and using different statistical models. The results remained similar in the sensitivity analyses.
- According to the results, vitamin D supplementation or fortification cannot be recommended to prevent HZ in the general population.

Chapter 7. The association between vitamin D deficiency and the risk of COVID-19

Chapter overview

In the previous chapter, I found no evidence of an association between vitamin D deficiency or supplementation and the risk of HZ. However, a previous systematic review and meta-analysis indicated that vitamin D supplementation is associated with a decreased risk of acute respiratory infection (24). As the COVID-19 pandemic has caused a global pandemic, if vitamin D deficiency is associated with an increased risk of COVID-19, taking vitamin D supplementations may be a cheap public health intervention. Therefore, in the last part of this research project, I examined the association between vitamin D deficiency and COVID-19. The manuscript of this project has been submitted to *PLOS ONE* (134).

The research question for this chapter is:

Does vitamin D deficiency increase the risk of COVID-19 infection, hospitalisation, or mortality?

Several new studies on vitamin D and COVID-19 using the UK Biobank cohort were published after the submission of the manuscript, and these studies were summarised and discussed after the manuscript as submitted.

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SECTION A – Student Details

Student ID Number	1803144	**Title**	Mr
First Name(s)	Liang-Yu		
Surname/Family Name	Lin		
Thesis Title	The association between vitamin D deficiency and the risk of herpes zoster and COVID-19		
Primary Supervisor	Sinéad Langan		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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Where is the work intended to be published?	PLOS ONE
Please list the paper's authors in the intended authorship order:	Liang-Yu Lin, Amy Mulick, Rohini Mathur , Liam Smeeth1, Charlotte Warren-Gash, Sinéad M Langan
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Liang-Yu contributed to the design of the study, underwent statistical analysis, drafted and revised the manuscript according to other authors' comments; Rohini Mathur contributed to the design of the study and revised the paper critically; Amy Mulick contributed to the design of the study, and revised the paper critically; Charlotte Warren-Gash contributed to the design of the study, made critical comments on the manuscript and revised the paper critically; Sinéad Langan contributed to the design of the study, made critical comments on the manuscript and revised the paper critically; Liam Smeeth contributed to the design of the study and revised the paper critically. All authors approved the final version of the manuscript.

SECTION E

Student Signature	[redacted]
Date	14/11/2021

Supervisor Signature	[redacted]
Date	22.11.2021

Title: The association between vitamin D status and COVID-19 in England: a cohort study using UK Biobank

List title: Vitamin D and COVID-19 in England

Authors:

Liang-Yu Lin[1], Amy Mulick[1], Rohini Mathur[1], Liam Smeeth[1], Charlotte Warren-Gash[1], Sinéad M Langan[1]

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Competing interest:

None declared.

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Data sharing statement:

Other researchers can apply for UK Biobank data to answer specific research questions. We will upload our analysis codes to GitHub (https://github.com/liang-yu12/vd_covid).

Abstract

Background:

Recent studies indicate that vitamin D supplementation may decrease respiratory tract infections, but the association between vitamin D and COVID-19 is still unclear.

Objective:

To explore the association between vitamin D status and infections, hospitalisation and mortality due to COVID-19.

Methods:

We used UK Biobank, a nationwide cohort of 500,000 individuals aged between 40 and 69 years, at recruitment between 2006 and 2010. We included people with at least one serum vitamin D test, living in England with linked primary care and inpatient records. The primary exposure was serum vitamin D status measured at recruitment, defined as deficiency at <25 nmol/L, insufficiency at 25-49 nmol/L and sufficiency at ≥ 50 nmol/L. Secondary exposures were self-reported or prescribed vitamin D supplements. The primary outcome was laboratory-confirmed or clinically diagnosed SARS-CoV-2 infections. The secondary outcomes included hospitalisation and mortality due to COVID-19. We used multivariable Cox regression models stratified by British summertime (BST) months and non-BST months, adjusting for demographic factors and underlying comorbidities.

Results:

We included 307,512 participants (54.9% female, 55.9% over 70 years old) in our analysis. During BST, weak evidence existed that the vitamin D deficiency group had a lower hazard of being diagnosed with COVID-19 (hazard ratio [HR]=0.86, 95% confidence interval [CI]=0.77–0.95). During non-BST, the vitamin D deficiency group had a higher hazard of COVID-19 diagnosis compared with the vitamin D sufficient group (HR=1.14, 95% CI=1.01–1.30). No evidence was found that vitamin D deficiency or insufficiency was associated with either hospitalisation or mortality due to COVID-19 in any time strata.

Conclusion:

We found no evidence of an association between historical vitamin D status and hospitalisation or mortality due to COVID-19, along with inconsistent results for any association between vitamin D and COVID-19 diagnosis. However, studies using more recent vitamin D measurements and systematic COVID-19 testing are needed.

Keywords:

Vitamin D, COVID-19, Electronic Health Records, UK Biobank

Abstract word count: 297

Introduction

The COVID-19 global pandemic is one of the biggest public health crises in recent history. The rapid spread of SARS-CoV-2 infection has caused serious casualties, overwhelming healthcare systems and disrupting societies. In the UK, more than 160,000 deaths due to COVID-19 within 28 days of a positive test were reported in the first year (135), planned surgeries and care have been delayed or cancelled (136), and prolonged lockdown measures along with the pandemic have worsened mental health (54). Despite the introduction and distribution of COVID-19 vaccines by the end of 2020, controlling this pandemic at a global scale remains extremely difficult. Studying the aetiology of SARS-CoV-2 is important to inform effective prevention strategies in public health.

Vitamin D is essential to bone health for its ability in regulating calcium and phosphate homeostasis, and recent studies indicate it may have some immunomodulatory effects. At the cellular level, vitamin D can increase the production of antimicrobial peptides (23, 137) and regulate adaptive immunity response (138). Clinically, a systematic review of observational studies indicated that vitamin D deficiency might be associated with a longer duration of acute respiratory tract infection (139). Another systematic review and meta-analysis, including data from 37 original trials, showed that vitamin D supplementation may protect against respiratory tract infections (pooled odds ratio = 0.92, 95% CI = 0.86–0.99) (24). Because of its potential for preventing respiratory infections, vitamin D supplementation and fortification

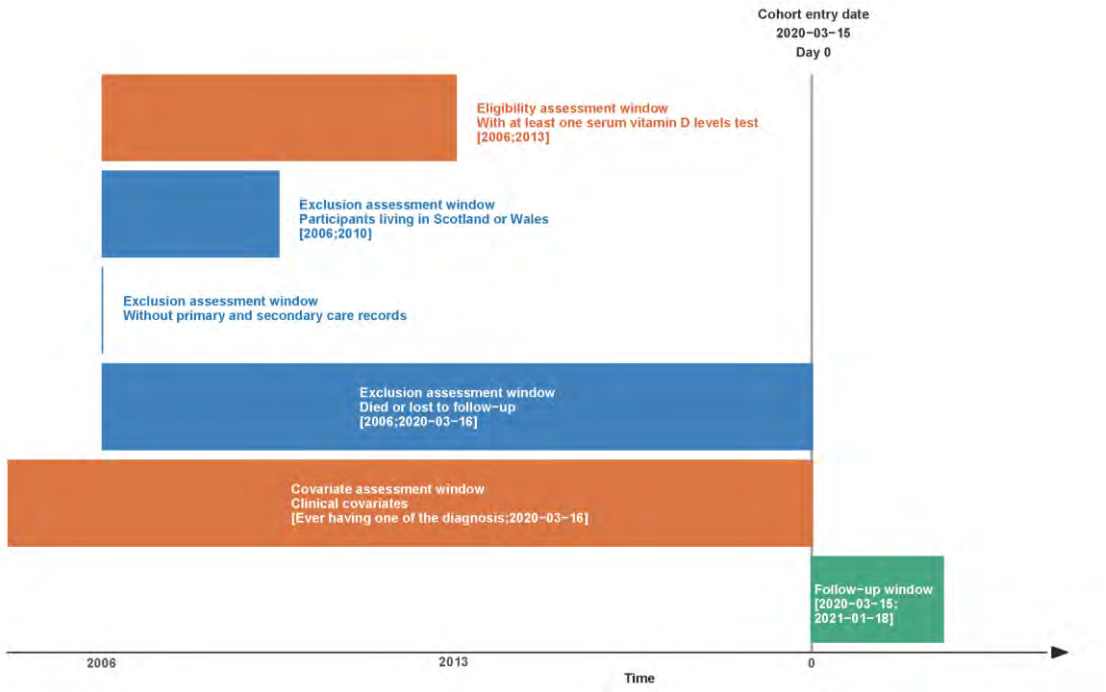
of food have been discussed as possible cheap public health interventions against COVID-19 (140).

Despite this potential, the association between vitamin D and COVID-19 is still unclear. Consequently, we aimed to conduct a historical cohort study using UK Biobank dataset and linked electronic health records, to better understand the association between serum vitamin D status, vitamin D supplementation and COVID-19 diagnosis and outcomes.

Methods

Study population and eligibility

The study population was from UK Biobank, a nationwide cohort established between 2006 and 2010 (89). In brief, participants aged 40 to 69 were recruited to 22 assessment centres around the UK. Their demographic information was collected through a touch screen questionnaire, and they received serum biochemical tests, including vitamin D analysis. UK Biobank participants also gave their consent to have electronic health records linked, including primary care and inpatient care records, and death certificates. The primary care data were provided by data system suppliers TPP and EMIS in England, and the inpatient care records and death records were provided by NHS digital. The external data providers extracted the health records by matching participant identifiers, including unique participant identifiers, NHS number, date of birth, gender and postcode. These health records were further processed and checked by UK Biobank before importing into the database (94). We only included participants in England who had at least one serum vitamin D test, primary care registration records and inpatient care records. Those who lacked serum vitamin D test records, were not registered in England, did not have both inpatient and primary care registration records, were lost to follow-up, or died before 16 March 2020 were excluded. The distribution of demographic factors of the included and excluded participants was compared. The design of the cohort and inclusion and exclusion criteria are depicted in **Figure 1**.



Chapter 7. Figure 1 Graphical depiction of the inclusion and exclusion criteria, cohort entry date and follow-up period

Primary exposure: vitamin D status

The primary exposure was serum vitamin D status. The measurement of vitamin D levels in UK Biobank has been described previously (117). In brief, serum vitamin D levels were measured when a participant visited a UK Biobank assessment centre, where their blood samples were collected and stored at -80 °C. Serum 25-hydroxyvitamin D status was measured using chemiluminescence immunoassay (DiaSorin Ltd. LIASON XL, Italy) in a centralised laboratory (87). The testing process has been verified by quality control samples and through an external quality assurance scheme(106, 141). Currently, no global consensus exists for determining vitamin D deficiency. We defined serum vitamin D status using Public Health England's definition (deficiency: <25 nmol/L; insufficiency: 25–50 nmol/L; sufficiency: >=

50 nmol/L) (3). Participants who had their serum vitamin D levels tested between April and October were labelled as ‘during British summertime (BST),’ and those who were had been tested between November and March were assigned as ‘during non-British summertime(non-BST).’

Secondary exposure: vitamin D supplementation and vitamin D prescription

The secondary exposures for this study were 1. taking vitamin D supplementation, or 2. receiving a vitamin D prescription from a GP. Information about vitamin D and other mineral supplementations was collected through a self-reported questionnaire using touch panels at the assessment centre between 2006 and 2010. We defined vitamin D supplementation as people who were taking vitamin D and associated minerals, including vitamin D, multivitamins, fish oil and calcium supplementation. Information about vitamin D supplementation was coded as ‘taking vitamin D supplement’ and ‘not taking vitamin D supplement,’ and it was coded as missing if a participant did not respond to the questionnaire.

Vitamin D prescriptions included all medications listed in British National Formula section 9.6.4, and we further compiled a prescription code list in Dictionary of Medicines and Devices (DM+D) using an existing mapping tool published by the NHS (142). By using the DM+D code list, we identified participants who had ever received vitamin D prescriptions from the primary care prescription datasets. Vitamin D prescription was coded as ‘had vitamin D prescriptions’ and ‘not receiving prescriptions.’

Primary outcome: COVID-19 diagnosis

The primary outcome of our study was SARS-CoV-2 infection, which was defined through laboratory testing or by clinical diagnosis of COVID-19. The laboratory tests for SARS-CoV-2 were performed using PCR, which was performed by the NHS (Pillar 1) or commercial partners (Pillar 2) (99, 100). These testing results were reported to Public Health England and automatically imported into UK Biobank weekly (98). Clinically diagnosed COVID-19 was defined as participants having COVID-19 diagnosis codes in their electronic health records, either in primary care or inpatient care, or on the death certificate. We used existing code lists in CTV3 codes, SNOMED-CT and ICD-10 to identify COVID-19 diagnosis.

Secondary outcome ascertainment: hospitalisation and mortality due to COVID-19

Hospitalisation due to COVID-19 was defined as COVID-19 related diagnosis (ICD-10 codes U071 or U072) recorded in the inpatient care dataset, and the admission date of each record was extracted. Mortality due to COVID-19 was defined as a participant having a COVID-19 diagnosis (ICD-10 codes U071 or U072) in the death registry data and being diagnosed as COVID-19 within 28 days, and the date of death was also recorded.

Measurement of covariates

We included basic demographic factors associated with vitamin D deficiency or insufficiency in our model, described in our previous paper (117). Demographic variables recorded between 2006 and 2010, such as sex, age, ethnicity, body mass index (BMI), alcohol drinking frequency, cigarette smoking, index of multiple deprivations (IMD), the time receiving serum

vitamin D tests and the region of the UK Biobank assessment centre, were included in our analysis. The current age at the start of the pandemic was calculated from participants' year of birth, which was coded as 'under 70 years old' and 'greater than and equal to 70 years old.' Other continuous covariates were further grouped into categorical variables. Self-reported ethnicity was classified as 'white,' 'black,' and 'Asian and others' according to the original questionnaire. BMI was grouped following National Institute for Health and Care Excellence guidelines for different sexes and ethnicities (143). IMD scores were classified by five quintiles, and the quintile with the highest scores was assigned as 'most deprived.' We categorised the location of 22 UK Biobank assessment centres by the regions of England. The smoking status was coded as 'non-smoker', 'ex-smoker', or 'current-smoker' according to the original questionnaire. Regarding drinking frequency, participants were recoded as 'weekly' if participants reported drinking three or four times a week, and monthly if drinking one to three times a month was reported. Participants reported with 'prefer not to say' were labelled as missing value.

In addition, we included clinical covariates such as clinically extreme vulnerability and underlying chronic diseases. Participants who were clinically extremely vulnerable to COVID-19 were defined by Public Health England (144). Underlying chronic diseases included hypertension, cardiovascular diseases, diabetes mellitus and asthma. Clinical covariates were assessed as a history of ever having one of the medical conditions of interest recorded in linked primary or secondary care records from the start of GP registration or HES recording until 16th March 2020. For health

conditions such as chemoradiotherapy, blood cancer and bone marrow transplantation, we only included people who had a recent history in less than six months before the index date.

Statistical analysis

The follow-up time of our study began on 15th March 2020. Because the availability of clinical datasets varied, the end of follow-up was defined differently for each outcome. For the primary outcome, SARS-CoV-2 infections, the event dates were the dates of COVID-19 diagnosis, and the censoring dates were the date of death or 18th January 2021. For hospitalisation due to COVID-19, the event dates were the dates of admission, and the censoring dates were the date of death or 30th November 2020. For mortality due to COVID-19, the event dates were the dates of death due to COVID-19, and the censoring dates were the dates of death due to other causes or 18th December 2020. In addition, among all UK Biobank participants with vitamin D testing data, we analysed the association between testing for vitamin D during the BST and vitamin D status using logistic regression.

The proportional hazard assumption was examined using log(-log[survival]) plots. Due to the overlapping of survival curves, the assumptions about vitamin D status and COVID-19 diagnosis (**Supplementary Figure 1a**), hospitalisation (**Supplementary Figure 1b**), and mortality (**Supplementary Figure 1c**) due to COVID-19 were violated. Therefore, we used stratified Cox regression to assess the association between vitamin D exposure and COVID-19 outcomes. The follow-up time of our models was stratified pre and

post 25th October 2020, which was the end date of the BST. We carried out a crude analysis, then generated a partially adjusted model controlling sex and age, as well as a full model adjusting for all covariates. All statistical analysis was performed by using R Statistical Software (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analysis and model checking

The sensitivity analysis and justifications are summarised in **Table 1**. We repeated our analysis while changing the outcome to laboratory-confirmed SARS-CoV-2 infections, and we redid the analysis for hospitalisation and mortality among the subgroup with COVID-19 diagnosis. In addition, the association between receiving vitamin D tests during BST and vitamin D status was analysed using logistic regression adjusting for covariates among all participants with at least one vitamin D level test.

Chapter 7. Table 1 Sensitivity analysis

Sensitivity analysis	Justification
1. The analysis was repeated on patients with laboratory-confirmed COVID-19.	The laboratory method is the gold standard for diagnosing COVID-19.
2. The analysis of hospitalisation and mortality was repeated on a redefined cohort, which only included patients with laboratory-confirmed and clinically diagnosed COVID-19.	Because the strategies for testing COVID-19 have been changing over time, the COVID-19 diagnosis may be established in a different context. In the main analysis, we assessed the hospitalisation and mortality in the whole population at risk. To compare the severity of COVID-19 among patients with a confirmed diagnosis, we confined the analysis among subgroups with COVID-19 diagnosis, which had been made through clinical diagnosis or laboratory methods.

Ethics

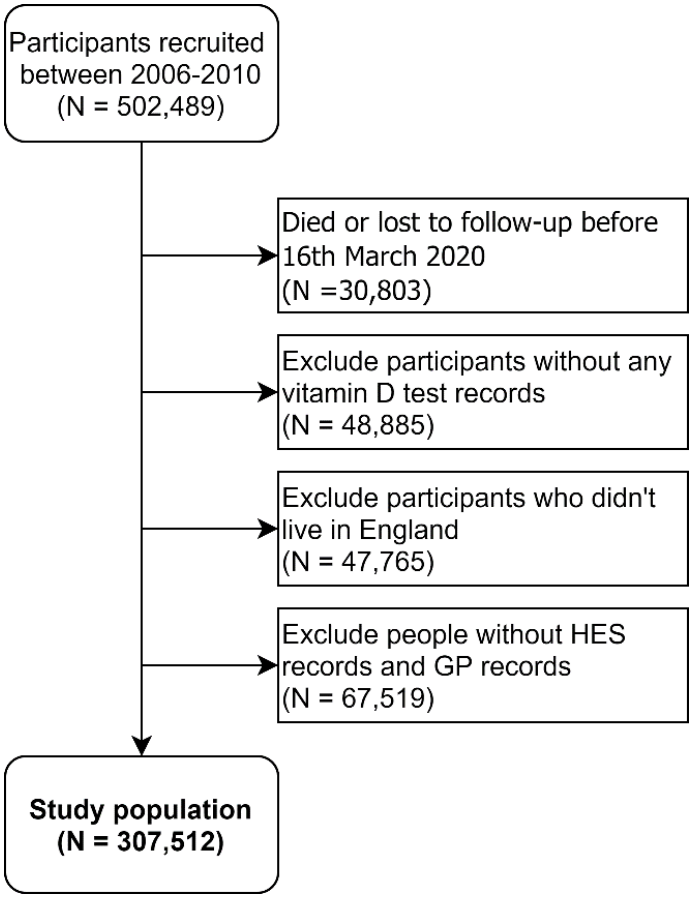
The UK Biobank project was approved by the North West Haydock Research Ethics Committee (reference: 11/NW/0382). Our project was approved by UK Biobank (ID:51265) and by the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (reference: 17158). We followed the principles of the Declaration of Helsinki(145).

Results

Study population

The selection of eligible participants is shown in **Figure 2**. After excluding ineligible people, a total of 307,512 participants were included in our analysis. The comparison of included and excluded participants is summarised in **Supplementary Table 1**. The distribution of sex, age, ethnicity, BMI, drinking behaviour, smoking, IMD, region and taking vitamin D supplementation was similar between included and excluded participants. More participants were clinically extremely vulnerable to COVID-19 or had underlying comorbidities among included participants with their electronic health records linked than those who were not eligible for inclusion.

Among eligible participants, more people were vitamin D sufficient (142,947; 46%) or insufficient (126,802; 41%) compared with vitamin D deficient participants (37,763, 12%). 65% received their vitamin D levels checked during BST months, while 35% were measured in non-BST months. The distribution of demographic factors by vitamin D status is summarised in **Table 2**. The distribution of sex, taking vitamin D supplementation, region of residency, clinical vulnerability to COVID-19 and underlying chronic comorbidities was similar across different vitamin D groups. Compared to participants with insufficient or sufficient vitamin D levels, the vitamin D deficiency group had more participants under than 70 years, non-white, obese and more deprived. Furthermore, the proportions of alcohol drinking and taking vitamin D supplements were lower among the vitamin D deficiency group.



Chapter 7. Figure 2 Diagram of selecting study participants

Chapter 7. Table 2 The distribution of demographic characteristics by vitamin D status

	Total (N=307,512)	Vitamin D deficiency (<25 nmol/L) (N=37,763)	Vitamin D insufficiency (25 – 50 nmol/L) (N=126,802)	Vitamin D sufficiency (≥ 50 nmol/L) (N=142,947)
The time when vitamin D sample was collected				
- During non-British summer time (November - March)	108140 (35.2%)	22312 (59.1%)	51295 (40.5%)	34533 (24.2%)
- During British summer time (April - October)	199372 (64.8%)	15451 (40.9%)	75507 (59.5%)	108414 (75.8%)
Sex				
- Female	169,018 (55.0%)	20,706 (54.8%)	69,860 (55.1%)	78,452 (54.9%)
- Male	138,494 (45.0%)	17,057 (45.2%)	56,942 (44.9%)	64,495 (45.1%)
Age[1]				
- Under 70 years old	150,428 (48.9%)	22,701 (60.1%)	64,727 (51.0%)	63,000 (44.1%)
- Greater and equal to 70 years old	157,084 (51.1%)	15,062 (39.9%)	62,075 (49.0%)	79,947 (55.9%)
Ethnicity				
- White	289,165 (94.0%)	30,790 (81.5%)	118,478 (93.4%)	139,897 (97.9%)
- Black	5,310 (1.7%)	1,812 (4.8%)	2,694 (2.1%)	804 (0.6%)
- Asian and others	13,037 (4.2%)	5,161 (13.7%)	5,630 (4.4%)	2,246 (1.6%)
BMI[2]				
- Healthy weight	97,499 (31.8%)	9,443 (25.2%)	35,560 (28.2%)	52,496 (36.8%)
- Underweight	1,480 (0.5%)	228 (0.6%)	516 (0.4%)	736 (0.5%)
- Overweight	130,370 (42.6%)	14,087 (37.6%)	53,492 (42.4%)	62,791 (44.0%)

- Obese	76,989 (25.1%)	13,661 (36.5%)	36,732 (29.1%)	26,596 (18.6%)
Drinking frequency				
- Never	24,394 (8.0%)	5,504 (14.7%)	10,492 (8.3%)	8,398 (5.9%)
- Sometimes	70,806 (23.1%)	10,637 (28.3%)	31,453 (24.9%)	28,716 (20.1%)
- Weekly	149,866 (48.8%)	14,874 (39.6%)	60,519 (47.8%)	74,473 (52.2%)
- Daily	61,770 (20.1%)	6,533 (17.4%)	24,054 (19.0%)	31,183 (21.8%)
Drinking status				
- Never	13,434 (4.4%)	3,487 (9.3%)	5,806 (4.6%)	4,141 (2.9%)
- Previous	10,867 (3.5%)	1,979 (5.3%)	4,651 (3.7%)	4,237 (3.0%)
- Current	282,442 (92.1%)	32,044 (85.4%)	116,026 (91.7%)	134,372 (94.1%)
Smoking status				
- Non-smoker	167,513 (54.8%)	20,201 (54.0%)	69,386 (55.0%)	77,926 (54.8%)
- Ex-smoker	108,326 (35.4%)	11,315 (30.2%)	43,916 (34.8%)	53,095 (37.3%)
- Current-smoker	30,105 (9.8%)	5,926 (15.8%)	12,875 (10.2%)	11,304 (7.9%)
IMD[3]				
- Least deprived	59,870 (20.0%)	4,945 (13.5%)	23,069 (18.7%)	31,856 (22.9%)
- 2 deprived	59,219 (19.8%)	5,525 (15.1%)	23,876 (19.3%)	29,818 (21.4%)
- 3 deprived	60,261 (20.1%)	6,551 (17.9%)	24,501 (19.9%)	29,209 (21.0%)
- 4 deprived	60,255 (20.1%)	8,313 (22.7%)	25,560 (20.7%)	26,382 (19.0%)
- Most deprived	59,490 (19.9%)	11,230 (30.7%)	26,414 (21.4%)	21,846 (15.7%)
Vitamin D and mineral supplementation[4]				
- Not taking supplement	22417 (21.2%)	2791 (32.6%)	9252 (24.0%)	10374 (17.8%)

- Taking vitamin D supplement	83131 (78.8%)	5773 (67.4%)	29364 (76.0%)	47994 (82.2%)
Vitamin D prescription[5]				
- Not receiving prescriptions	235431 (76.6%)	25567 (67.7%)	97094 (76.6%)	112770 (78.9%)
- Had vitamin D prescriptions	72081 (23.4%)	12196 (32.3%)	29,708 (23.4%)	30177 (21.1%)
Region of UK Biobank assessment centres				
- East Midlands	43707 (14.2%)	5,609 (14.9%)	17,643 (13.9%)	20,455 (14.3%)
- London	24467 (8.0%)	2,350 (6.2%)	9,711 (7.7%)	12,406 (8.7%)
- North East	44374 (14.4%)	7,446 (19.7%)	19,774 (15.6%)	17,154 (12.0%)
- North West	50808 (16.5%)	5,916 (15.7%)	20,569 (16.2%)	24,323 (17.0%)
- South East	28859 (9.4%)	2,329 (6.2%)	11,192 (8.8%)	15,338 (10.7%)
- South West	29445 (9.6%)	2,271 (6.0%)	11,103 (8.8%)	16,071 (11.2%)
- West Midlands	31522 (10.3%)	5,249 (13.9%)	13,919 (11.0%)	12,354 (8.6%)
- Yorkshire and The Humber	54330 (17.7%)	6,593 (17.5%)	22,891 (18.1%)	24,846 (17.4%)
Clinically vulnerable to COVID-196[6,7]				
- Not vulnerable	249,944 (81.3%)	29,903 (79.2%)	103,063 (81.3%)	116,978 (81.8%)
- Clinically extremely vulnerable	57,568 (18.7%)	7,860 (20.8%)	23,739 (18.7%)	25,969 (18.2%)
Other comorbidities[6,8]				
- No chronic diseases	94,237 (30.6%)	10,466 (27.7%)	38,186 (30.1%)	45,585 (31.9%)
- With Chronic diseases	213,275 (69.4%)	27,297 (72.3%)	88,616 (69.9%)	97,362 (68.1%)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. Vitamin D supplement includes vitamin D, multivitamin, fish oil and calcium supplementation. 5. Vitamin D prescription included all drugs in BNF section 9.6.4, which were identified by using code lists in DM+D codes from linked GP prescription records. 6. Health conditions were identified from linked electronic health records. 7. The clinically extremely vulnerable groups were defined by using Public Health England’s definition. 8. Including hypertension, cardiovascular diseases, diabetes mellitus, or asthma.

Description of outcomes

The distribution of COVID-19 diagnosis, hospitalisation, and mortality due to COVID-19 over time is summarised in **Supplementary Figure 1**. As can be seen in **Supplementary Figure 1a,** among 10,165 participants with SARS-CoV-2 infection, more participants were diagnosed with COVID-19 in spring (13.8%), autumn (51.4%), and winter (31%), while fewer cases were reported in summer (3.8%). Despite the shorter follow-up period, similar distributions were also noted for hospitalisation (**Supplementary Figure 1b**) and mortality (**Supplementary Figure 1b**). In the larger cohort containing all participants with vitamin D records, we found that participants visiting the UK Biobank assessment centre during BST months had around 60% lower odds of vitamin D deficiency or insufficiency than those receiving tests during non-BST months (**Supplementary Table 2**).

Association between vitamin D status and COVID-19 diagnosis

Table 3 summarises the association between vitamin D status and being diagnosed with COVID-19. In crude analysis, in BST months, people with vitamin D insufficiency or deficiency had a higher hazard of being diagnosed with COVID-19 than sufficient participants (insufficiency: crude HR=1.18, CI=1.07–1.31; deficiency: crude HR=1.11, CI=1.03–1.19), but in non-BST months, only the vitamin D insufficiency group had a higher hazard of COVID-19 (insufficiency: crude HR=1.15, CI=1.02–1.31.) The results of the partially adjusted model were similar: only vitamin D deficiency in non-BST was associated with an increased hazard of COVID-19 (HR=1.15, CI=1.02–1.31). After adjusting for all covariates, participants with vitamin D deficiency

had a 14% lower hazard of being diagnosed with COVID-19 compared with people with sufficient vitamin D status in BST (HR=0.86, CI=0.77–0.95). In non-BST months, the hazard of being diagnosed with COVID-19 was 14% higher among the vitamin D deficiency group (HR=1.14, CI=1.01–1.30). No evidence showed that vitamin D insufficiency was associated with COVID-19 during either BST or non-BST. Being male, non-white, overweight or obese, more deprived, clinically vulnerable or with underlying comorbidities was associated with an increased hazard of being diagnosed with COVID-19, while people who were older than 70 years had a lower hazard of being diagnosed (**Table 3**).

Chapter 7. Table 3 The association between vitamin D status and COVID-19 diagnosis

		HR (95% CI) (crude)	HR (95% CI) (adjusted for sex and age)	HR (95% CI) (adjusted for all covariates)
British summertime (15 March to 25 October 2020)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	1.18 (1.07-1.31)	1.07 (1.00-1.15)	0.96 (0.90-1.04)
	Vitamin D deficiency	1.11 (1.03-1.19)	1.08 (0.98-1.20)	0.86 (0.77-0.95)
Non-British summertime (26 October to 18 January 2021)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	0.93 (0.86-1.02)	0.93 (0.85-1.02)	0.93 (0.85-1.02)
	Vitamin D deficiency	1.15 (1.02-1.31)	1.15 (1.02-1.31)	1.14 (1.01-1.30)
Sex	Female	Reference	-	Reference
	Male	1.10 (1.06-1.15)	-	1.08 (1.04-1.13)
Age[1]	Under 70 years old	Reference	-	Reference
	Greater and equal to 70 years old	0.59 (0.56-0.61)	-	0.57 (0.54-0.59)
Ethnicity	White	Reference	-	Reference
	Black	1.79 (1.60-2.01)	1.58 (1.41-1.78)	1.36 (1.20-1.53)
	Asian and others	1.72 (1.60-1.86)	1.57 (1.45-1.70)	1.43 (1.31-1.56)
BMI[2]	Healthy weight	Reference	Reference	Reference
	Underweight	1.10 (0.81-1.49)	1.08 (0.80-1.47)	1.06 (0.78-1.44)
	Overweight	1.23 (1.17-1.29)	1.26 (1.19-1.32)	1.20 (1.14-1.26)
	Obese	1.60 (1.52-1.69)	1.62 (1.54-1.71)	1.44 (1.36-1.52)
Drinking frequency	Never	Reference	Reference	Reference

	Sometimes	0.87 (0.81-0.94)	0.86 (0.80-0.92)	0.94 (0.87-1.01)
	Weekly	0.80 (0.74-0.85)	0.77 (0.72-0.82)	0.93 (0.86-1.00)
	Daily	0.65 (0.60-0.70)	0.64 (0.60-0.70)	0.81 (0.75-0.89)
Smoking status	Non-smoker	Reference	Reference	Reference
	Ex-smoker	1.09 (1.04-1.14)	1.16 (1.11-1.21)	1.15 (1.10-1.20)
	Current smoker	1.23 (1.15-1.31)	1.15 (1.08-1.23)	1.06 (0.99-1.13)
Vitamin D status testing time	During non-British summertime	Reference	Reference	Reference
	During British summer time	0.98 (0.94-1.02)	0.99 (0.95-1.03)	1.00 (0.96-1.04)
IMD[3]	Least deprived	Reference	Reference	Reference
	2 deprived	1.18 (1.10-1.27)	1.18 (1.10-1.27)	1.09 (1.02-1.18)
	3 deprived	1.38 (1.28-1.48)	1.36 (1.27-1.46)	1.21 (1.13-1.30)
	4 deprived	1.63 (1.53-1.75)	1.59 (1.49-1.70)	1.35 (1.26-1.45)
	Most deprived	2.16 (2.03-2.30)	2.05 (1.92-2.19)	1.59 (1.48-1.70)
Regions	North East	Reference	Reference	Reference
	East Midlands	0.87 (0.80-0.95)	0.88 (0.80-0.96)	0.92 (0.84-1.01)
	London	1.04 (0.97-1.12)	1.01 (0.94-1.09)	0.92 (0.85-0.99)
	North West	1.30 (1.22-1.39)	1.31 (1.22-1.39)	1.24 (1.16-1.32)
	South East	0.57 (0.52-0.63)	0.57 (0.52-0.63)	0.66 (0.60-0.73)
	South West	0.66 (0.60-0.73)	0.65 (0.59-0.71)	0.70 (0.64-0.77)
	West Midlands	1.03 (0.96-1.12)	1.02 (0.94-1.10)	0.95 (0.88-1.03)
	Yorkshire and The Humber	0.97 (0.91-1.04)	0.96 (0.90-1.03)	0.96 (0.90-1.03)
Clinically vulnerable to COVID-19[4]	Not vulnerable	Reference	Reference	Reference

	Extremely vulnerable	1.28 (1.23-1.35)	1.42 (1.36-1.49)	1.29 (1.23-1.35)
Underlying comorbidities[5]	No chronic diseases	Reference	Reference	Reference
	Chronic diseases	1.07 (1.02-1.12)	1.21 (1.15-1.26)	1.02 (0.97-1.07)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Association between vitamin D status and hospitalisation due to COVID-19

Table 4 summarises the association between serum vitamin D status and hospitalisation due to COVID-19 stratified by BST. In the crude and partially adjusted models, in BST, vitamin D insufficiency or deficiency was associated with a higher hazard of hospitalisation due to COVID-19, while in non-BST, such an association was not seen. We found either during or after BST, after adjusting for covariates and compared with people with sufficient vitamin D status, no evidence existed that vitamin D insufficiency or deficiency was associated with a higher hazard of hospital admission due to COVID-19 (during BST: insufficiency adjusted HR= 0.94, CI= 0.82–1.08, deficiency adjusted HR= 1.08, CI= 0.89–1.31; during non-BST: insufficiency adjusted HR=1.11, CI= 0.83–1.49, deficiency adjusted HR=0.92, CI= 0.61–1.37). Other covariates such as male sex, age older than 70 years, non-white ethnicity, overweight or obesity, cigarette smoking, and being more deprived, clinically vulnerable or having underlying comorbidities increased the hazard of hospitalisation due to COVID-19. Compared with participants who never drink alcohol, more frequent alcohol drinking was associated with a decreased hazard of hospitalisation (**Table 4**).

Chapter 7. Table 4 The association between vitamin D status and hospitalisation due to COVID-19

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summertime (15 March to 25 October 2020)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	1.13 (0.99-1.29)	1.18 (1.03-1.34)	0.94 (0.82-1.08)
	Vitamin D deficiency	1.50 (1.26-1.79)	1.66 (1.40-1.98)	1.08 (0.89-1.31)
Non-British summertime (26 October to 30 November 2020)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	1.05 (0.79-1.39)	1.05 (0.79-1.39)	1.11 (0.83-1.49)
	Vitamin D deficiency	0.92 (0.63-1.35)	0.92 (0.63-1.35)	0.92 (0.61-1.37)
Sex	Female	Reference	-	Reference
	Male	1.96 (1.76-2.18)	-	1.72 (1.53-1.93)
Age[1]	Under 70 years old	Reference	-	Reference
	Greater and equal to 70 years old	1.80 (1.61-2.01)	-	1.50 (1.33-1.69)
Ethnicity	White	Reference	Reference	Reference
	Black	2.22 (1.67-2.95)	2.75 (2.07-3.66)	2.17 (1.59-2.94)
	Asian and others	1.59 (1.28-1.97)	1.77 (1.43-2.20)	1.39 (1.08-1.79)
BMI[2]	Healthy weight	Reference	Reference	Reference
	Underweight	2.00 (0.94-4.23)	2.31 (1.09-4.89)	1.97 (0.93-4.19)
	Overweight	1.80 (1.55-2.10)	1.56 (1.33-1.82)	1.43 (1.22-1.68)
	Obese	3.05 (2.61-3.55)	2.76 (2.37-3.22)	2.05 (1.74-2.42)
Drinking frequency	Never	Reference	Reference	Reference
	Sometimes	0.67 (0.56-0.80)	0.70 (0.58-0.83)	0.79 (0.65-0.96)

	Weekly	0.53 (0.45-0.63)	0.48 (0.41-0.57)	0.68 (0.57-0.82)
	Daily	0.53 (0.43-0.64)	0.43 (0.35-0.52)	0.63 (0.51-0.78)
Smoking status	Non-smoker	Reference	Reference	Reference
	Ex-smoker	1.61 (1.44-1.81)	1.41 (1.26-1.59)	1.29 (1.14-1.46)
	Current smoker	1.94 (1.65-2.28)	1.88 (1.60-2.22)	1.42 (1.19-1.69)
Vitamin D status testing time	During non-British summertime	Reference	Reference	Reference
	During British summer time	0.99 (0.89-1.11)	1.01 (0.90-1.13)	1.04 (0.92-1.17)
IMD[3]	Least deprived	Reference	Reference	Reference
	2 deprived	1.19 (0.96-1.46)	1.19 (0.96-1.47)	1.06 (0.85-1.31)
	3 deprived	1.37 (1.12-1.68)	1.39 (1.13-1.70)	1.12 (0.91-1.38)
	4 deprived	1.82 (1.50-2.20)	1.88 (1.55-2.28)	1.41 (1.15-1.72)
	Most deprived	2.87 (2.39-3.43)	3.04 (2.54-3.64)	1.78 (1.47-2.16)
Regions	North East	Reference	Reference	Reference
	East Midlands	1.18 (0.94-1.48)	1.17 (0.93-1.47)	1.30 (1.03-1.65)
	London	0.88 (0.71-1.09)	0.92 (0.74-1.13)	0.79 (0.63-0.99)
	North West	1.54 (1.28-1.84)	1.52 (1.27-1.83)	1.39 (1.16-1.68)
	South East	0.49 (0.37-0.66)	0.49 (0.37-0.66)	0.67 (0.49-0.90)
	South West	0.58 (0.44-0.76)	0.60 (0.45-0.78)	0.69 (0.51-0.91)
	West Midlands	1.34 (1.09-1.65)	1.33 (1.08-1.64)	1.21 (0.97-1.50)
	Yorkshire and The Humber	1.20 (1.00-1.45)	1.21 (1.00-1.46)	1.27 (1.05-1.55)
Clinically vulnerable to COVID-19[4]	Not vulnerable	Reference	Reference	Reference
	Extremely vulnerable	3.50 (3.14-3.89)	3.20 (2.87-3.57)	2.55 (2.28-2.86)

Underlying comorbidities[5]	No chronic diseases	Reference	Reference	Reference
	Chronic diseases	2.84 (2.43-3.31)	2.41 (2.06-2.82)	1.61 (1.36-1.90)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Association between vitamin D status and COVID-19 mortality (Table 5)

Table 5 summarises the association between vitamin D status and mortality due to COVID-19. In the crude and partially adjusted model, no association was found between vitamin D status and COVID-19 mortality, except vitamin D deficiency during BST had a higher risk of dying from COVID-19 after adjusting for sex and age (partially adjusted HR=1.64, CI=1.06–2.54.) Compared with people with sufficient vitamin D status and after adjusting for covariates, no evidence existed that the hazard of COVID-19 mortality was higher among participants with vitamin D insufficiency or deficiency, either during or after BST (during BST: insufficiency adjusted HR= 0.84, CI= 0.60–1.17, deficiency adjusted HR= 1.08, CI= 0.68–1.72; during non-BST: insufficiency adjusted HR=1.35, CI= 0.79–2.30, deficiency adjusted HR=1.46, CI= 0.73–2.91). In addition, male sex, age over 70 years, black ethnicity, underweight and obesity, cigarette smoking, being most deprived, clinical vulnerability and having underlying comorbidities were associated with an increased hazard of COVID-19 mortality. Frequent alcohol drinking was associated with a decreased hazard of COVID-19 mortality (**Table 5**).

Chapter 7. Table 5 The association between vitamin D status and COVID-19 mortality

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summertime (15 March to 25 October 2020)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	0.95 (0.69-1.31)	1.06 (0.77-1.46)	0.84 (0.60-1.17)
	Vitamin D deficiency	1.26 (0.82-1.95)	1.64 (1.06-2.54)	1.08 (0.68-1.72)
Non-British summertime (26 October to 18 December 2020)	Vitamin D sufficiency	Reference	Reference	Reference
	Vitamin D insufficiency	1.22 (0.72-2.05)	1.22 (0.72-2.05)	1.35 (0.79-2.30)
	Vitamin D deficiency	1.34 (0.67-2.65)	1.34 (0.68-2.65)	1.46 (0.73-2.91)
Sex	Female	Reference	-	Reference
	Male	2.86 (2.22-3.68)	-	2.39 (1.83-3.13)
Age[1]	Under 70 years old	Reference	-	Reference
	Greater and equal to 70 years old	6.50 (4.60-9.18)	-	5.60 (3.86-8.12)
Ethnicity	White	Reference	-	Reference
	Black	2.25 (1.23-4.11)	3.93 (2.14-7.21)	3.39 (1.78-6.46)
	Asian and others	0.83 (0.44-1.57)	1.12 (0.60-2.11)	0.84 (0.40-1.76)
BMI[2]	Healthy weight	Reference	Reference	Reference
	Underweight	4.72 (1.46-15.24)	6.38 (1.97-20.63)	5.04 (1.55-16.36)
	Overweight	2.05 (1.44-2.92)	1.59 (1.11-2.27)	1.35 (0.94-1.95)
	Obese	3.75 (2.64-5.32)	3.17 (2.23-4.50)	2.16 (1.50-3.12)
Drinking frequency	Never	Reference	Reference	Reference

	Sometimes	0.51 (0.34-0.76)	0.55 (0.37-0.81)	0.56 (0.37-0.84)
	Weekly	0.49 (0.34-0.69)	0.43 (0.30-0.61)	0.58 (0.40-0.85)
	Daily	0.58 (0.39-0.86)	0.41 (0.28-0.62)	0.60 (0.40-0.92)
Smoking status	Non-smoker	Reference	Reference	Reference
	Ex-smoker	2.05 (1.59-2.64)	1.54 (1.19-1.99)	1.36 (1.04-1.77)
	Current smoker	2.13 (1.48-3.06)	2.16 (1.50-3.12)	1.53 (1.04-2.25)
Vitamin D status testing time	During non-British summertime	Reference	Reference	Reference
	During British summer time	0.97 (0.76-1.23)	0.99 (0.78-1.26)	1.07 (0.83-1.39)
IMD[3]	Least deprived	Reference	Reference	Reference
	2 deprived	1.16 (0.73-1.86)	1.17 (0.74-1.87)	1.00 (0.62-1.61)
	3 deprived	1.36 (0.86-2.12)	1.40 (0.89-2.19)	1.12 (0.71-1.77)
	4 deprived	1.78 (1.16-2.72)	1.94 (1.27-2.97)	1.36 (0.88-2.12)
	Most deprived	3.21 (2.17-4.74)	3.75 (2.53-5.55)	2.11 (1.39-3.20)
Regions	North East	Reference	Reference	Reference
	East Midlands	1.02 (0.64-1.60)	0.98 (0.62-1.55)	1.16 (0.73-1.84)
	London	0.46 (0.28-0.75)	0.51 (0.31-0.82)	0.46 (0.28-0.77)
	North West	0.81 (0.55-1.20)	0.80 (0.54-1.18)	0.72 (0.48-1.07)
	South East	0.18 (0.08-0.41)	0.18 (0.08-0.41)	0.22 (0.09-0.55)
	South West	0.32 (0.17-0.61)	0.34 (0.18-0.65)	0.44 (0.23-0.85)
	West Midlands	1.11 (0.74-1.68)	1.12 (0.74-1.69)	0.97 (0.64-1.50)
	Yorkshire and The Humber	1.21 (0.85-1.73)	1.24 (0.87-1.77)	1.29 (0.90-1.85)
Clinically vulnerable to COVID-19[4]	Not vulnerable	Reference	Reference	Reference

	Extremely vulnerable	4.29 (3.40-5.40)	3.32 (2.63-4.20)	2.58 (2.02-3.29)
Underlying comorbidities[5]	No chronic diseases	Reference	Reference	Reference
	Chronic diseases	4.64 (3.08-7.00)	2.97 (1.96-4.49)	1.87 (1.21-2.88)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, and diabetes mellitus

Association between vitamin D prescription or supplementation and COVID-19

Some evidence existed that during BST, people who had been ever prescribed vitamin D supplementation from a GP had a higher hazard of being diagnosed with COVID-19 (**Supplementary Table 3,** adjusted HR=1.22, CI=1.13–1.32), hospitalisation (**Supplementary Table 4,** adjusted HR=1.59, CI=1.39–1.82) and mortality (**Supplementary Table 5,** adjusted HR=2.31, CI=1.68–3.17). During BST, no evidence showed self-reported vitamin D supplementation was associated with a lower hazard of COVID-19 diagnosis (adjusted HR=0.88, CI=0.76–1.01), while the hazard was higher during non-BST (**Supplementary Table 6,** adjusted HR=1.23, CI=1.03–1.47). No evidence was found that self-reported vitamin D supplementation was associated with hospitalisation (**Supplementary Table 7**) or mortality (**Supplementary Table 8**) due to COVID-19 either during or after BST.

Sensitivity analysis

The repeated analysis of vitamin D status and laboratory-confirmed COVID-19 was similar to the original model (**Supplementary Table 9**). Similarly, the subgroup analysis of hospitalisation and mortality among patients with COVID-19 diagnosis showed no evidence that vitamin D status was associated with the hazard of COVID-19 hospitalisation or mortality (**Supplementary Table 10** and **Supplementary Table 11**).

Discussion

In this large cohort study, we found no consistent evidence that historical vitamin D status was associated with COVID-19. No evidence showed that historical evidence of vitamin D deficiency or insufficiency was associated with hospitalisation or mortality due to COVID-19. During BST, weak evidence existed that vitamin D deficiency was associated with a lower hazard of being diagnosed with COVID-19, while during non-BST, the association was reversed. In the secondary analysis, during BST, people who ever received vitamin D prescription had a higher hazard of having COVID-19 diagnosis, hospitalisation and mortality due to COVID-19. No association was found between self-reported vitamin D supplementation and hospitalisation or mortality due to COVID-19.

Our study has some strengths. First, compared to previous studies using UK Biobank datasets early in the pandemic, the follow-up period of our study was longer, and therefore we were able to cover more than one wave of COVID-19 infections (114, 146-148). Second, our analysis adjusted for more clinical covariates using the latest electronic health records, allowing us to estimate the effect of vitamin D status more accurately. Third, despite the variation of COVID-19 testing strategies, the clinical outcomes of hospitalisation and mortality were collected systematically, which minimised the misclassification bias of these outcomes. The large sample size of our study also provides more statistical power than previous studies using single-hospital records. Finally, our analysis showed that some known factors were also associated with COVID-19 hospitalisation and mortality, including male

sex, older age, non-white ethnicity, abnormal BMI, cigarette smoking, being more deprived, being clinically vulnerable and having underlying comorbidities. These findings were similar to previous studies using a large electronic health records database (149), implying that our analysis regarding hospitalisation and mortality is valid.

Nevertheless, the study has some limitations. First, the data regarding historical vitamin D status and vitamin D supplementation were collected between 2006 and 2013. The distribution of vitamin D status and supplementation behaviour may be very different now. A previous study among postmenopausal women repeatedly measured vitamin D levels after five years, and the results showed the intraclass correlation coefficient between the two results was only 0.59 (0.54–0.64), which was suboptimal (130). In our study, the vitamin D levels were measured seven to 15 years ago, introducing a possible misclassification bias of exposure. In addition, information about self-reported vitamin D supplementation was only available for 54% of participants, which further reduced the statistical power of our analysis and may result in misclassification. Future studies should consider using more recent data about vitamin D status and more complete vitamin D supplementation information.

Second, the diagnosis of COVID-19 was influenced by testing strategies, which is likely to have led to outcome misclassification. At the early stage of the pandemic in the UK, the testing capacity was limited to people who required inpatient care. Therefore, only participants with relatively severe symptoms were tested, and people who were asymptomatic or had mild symptoms had to stay at home instead of seeking medical care (150). As the

COVID-19 testing capacity increased, more people with mild or no symptoms were able to access testing and classified as cases. Since the COVID-19 testing was not systematic, the outcome of the COVID-19 diagnosis was misclassified. For future studies, the COVID-19 outcomes should be ascertained systematically.

Third, despite a large number of our population, the external validity of UK Biobank is limited. The participants of UK Biobank are not nationally representative, and they are wealthier, older, and more likely to be white and women, which may introduce healthy volunteer bias (78). However, in our model, we adjusted for demographic covariates, and we also included IMD scores as a proxy for socioeconomic status. Our results regarding exposure and outcomes remain internally valid.

Previous small, single-hospital studies have shown an association between pre-hospitalised vitamin D levels and mortality (151, 152), while other hospital-based studies enrolling more participants have indicated no evidence of such an association (153-155). We found no evidence that historical vitamin D status was associated with inpatient admission or mortality due to COVID-19. This finding similar to another study using UK Biobank data with a shorter follow-up period (147), while we adjusted for more clinical covariates and had a longer follow-up time. However, because the information on vitamin D status from UK Biobank was mainly collected between 2006 and 2010, this may not reflect participants' current vitamin D status. This finding of no association may be biased by misclassification of vitamin D exposure, so results should be interpreted cautiously.

Our study showed inconsistent associations between vitamin D deficiency and COVID-19 diagnosis during the different follow-up times. During the BST months, vitamin D deficiency was negatively associated with having a COVID-19 diagnosis after adjusting for covariates. This result is similar to previous small studies performed early in the pandemic (156, 157) and consistent with other studies using UK Biobank data (114, 146-148). A possible explanation is that people in the northern hemisphere are less likely to be vitamin D deficient during this period of the year, and during this time, people normally spend less time indoors, which also decreases the risk of being infected with SARS-CoV-2. However, in addition to the misclassification bias of vitamin D exposure, the changing testing strategies for SARS-CoV-2 introduced misclassification bias in COVID-19 diagnosis. These marked biases may lead to inaccurate estimation in the association between vitamin D and COVID-19.

Our study showed no evidence that vitamin D prescription or supplementation was associated with COVID-19 admission or mortality. Previously, a single-hospital study also showed no association between vitamin D supplementation and COVID-19 admission or mortality (153), and a recent meta-analysis also indicated that vitamin D supplements were not associated with COVID-19 mortality reduction (158). However, the information about vitamin D supplementation of UK Biobank was collected at least 10 years ago, which may not accurately reflect current vitamin D intake. Furthermore, despite adjusting for various clinical covariates, we still cannot exclude probable residual confounding effects such as confounding by

indication. These results should be interpreted carefully in light of these likely biases.

Conclusion

Our study shows no association between historical vitamin D status and the risk of hospital admission and mortality due to COVID-19, as well as inconsistent results for any association between vitamin D status and COVID-19 diagnosis. To precisely investigate the possible role of vitamin D in COVID-19 prevention, more studies using recent vitamin D status data and systemic COVID-19 surveillance will be needed. There is currently insufficient evidence to support prioritizing vitamin D supplementation or fortification over other preventive strategies for COVID-19, such as mass vaccination programmes.

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References

1. GOV.UK. Daily summary: Coronavirus in the UK 2021 [updated 25 March 2021. Available from: https://coronavirus.data.gov.uk/.
2. Iacobucci G. How is the pandemic affecting non-covid services? Bmj. 2021;372:n215.
3. Office for National Statistics. Coronavirus and the social impacts on behaviours during different lockdown periods, Great Britain up to February 2021 2021 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandwellbeing/articles/coronavirusandthesocialimpactsonbehavioursduringdifferentlockdownperiodsgreatbritain/uptofebruary2021#optimism-about-when-life-will-return-to-normal.
4. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004;173(5):2909-12.
5. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsjo A, Törmä H, Stahle M. Vitamin D induces the antimicrobial protein hCAP18 in human skin. The Journal of investigative dermatology. 2005;124(5):1080-2.
6. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, Hormone, and Immunomodulator. Nutrients. 2018;10(11).
7. Wang MX, Koh J, Pang J. Association between micronutrient deficiency and acute respiratory infections in healthy adults: a systematic review of observational studies. Nutr J. 2019;18(1):80-.
8. Jolliffe DA, Camargo CA, Jr., Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. The Lancet Diabetes & Endocrinology.
9. Martineau AR, Forouhi NG. Vitamin D for COVID-19: a case to answer? The Lancet Diabetes & Endocrinology. 2020;8(9):735-6.
10. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.
11. UK Biobank. Integrating electronic health records into the UK Biobank Resource. 2014.
12. Lin L-Y, Smeeth L, Langan S, Warren-Gash C. Distribution of vitamin D status in the UK: a cross-sectional analysis of UK Biobank. BMJ open. 2021;11(1):e038503.
13. Elliott P, Peakman TC, Biobank UK. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International journal of epidemiology. 2008;37(2):234-44.
14. UK Biobank. Biomarker assay quality procedures: approaches used to minimise systematic and random errors. 2019.
15. UK Biobank. Companion document for serum biomarker data. 2019 11/03/2019.
16. The Scientific Advisory Committee on Nutrition. Vitamin D and Health. London: Public Health England 2016 21 July 2016.

17. NHS Business Services Authority. BNF SNOMED mapping 2021 [Available from: https://www.nhsbsa.nhs.uk/prescription-data/understanding-our-data/bnf-snomed-mapping.
18. Public Health England. PHE data series on deaths in people with COVID-19: technical summary - 12 August update. 2020 12 August 2020.
19. UK Biobank. COVID-19 test results data 2021 [Available from: https://biobank.ctsu.ox.ac.uk/crystal/exinfo.cgi?src=COVID19_tests.
20. Armstrong J, Rudkin JK, Allen N, Crook DW, Wilson DJ, Wyllie DH, et al. Dynamic linkage of COVID-19 test results between Public Health England's Second Generation Surveillance System and UK Biobank. Microbial Genomics. 2020;6(7).
21. National Institute for Health and Care Excellence (NICE). BMI: preventing ill health and premature death in black, Asian and other minority ethnic groups: National Institute for Health and Clinical Excellence; 2013 3 July 2013. 13-4 p.
22. Public Health England. Guidance on shielding and protecting people who are clinically extremely vulnerable from COVID-19 2020 [Available from: https://www.gov.uk/government/publications/guidance-on-shielding-and-protecting-extremely-vulnerable-persons-from-covid-19/guidance-on-shielding-and-protecting-extremely-vulnerable-persons-from-covid-19#who-is-clinically-extremely-vulnerable.
23. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
24. Amin HA, Drenos F. No evidence that vitamin D is able to prevent or affect the severity of COVID-19 in individuals with European ancestry: a Mendelian randomisation study of open data. BMJ Nutrition, Prevention & Health. 2021:bmjnph-2020-000151.
25. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr. 2020;14(4):561-5.
26. Hastie CE, Pell JP, Sattar N. Vitamin D and COVID-19 infection and mortality in UK Biobank. Eur J Nutr. 2021;60(1):545-8.
27. Raisi-Estabragh Z, McCracken C, Bethell MS, Cooper J, Cooper C, Caulfield MJ, et al. Greater risk of severe COVID-19 in Black, Asian and Minority Ethnic populations is not explained by cardiometabolic, socioeconomic or behavioural factors, or by 25(OH)-vitamin D status: study of 1326 cases from the UK Biobank. Journal of Public Health. 2020;42(3):451-60.
28. Elliott J, Bodinier B, Whitaker M, Delpierre C, Vermeulen R, Tzoulaki I, et al. COVID-19 mortality in the UK Biobank cohort: revisiting and evaluating risk factors. Eur J Epidemiol. 2021:1-11.
29. Meng JE, Hovey KM, Wactawski-Wende J, Andrews CA, LaMonte MJ, Horst RL, et al. Intraindividual Variation in Plasma 25-Hydroxyvitamin D Measures 5 Years Apart among Postmenopausal Women. Cancer Epidemiology Biomarkers & Prevention. 2012;21(6):916.
30. Public Health England. New guidance for households with possible COVID-19 infection 2020 [Available from: https://www.gov.uk/government/news/new-guidance-for-households-with-possible-covid-19-infection.

31. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. American journal of epidemiology. 2017;186(9):1026-34.
32. Infante M, Buoso A, Pieri M, Lupisella S, Nuccetelli M, Bernardini S, et al. Low Vitamin D Status at Admission as a Risk Factor for Poor Survival in Hospitalized Patients With COVID-19: An Italian Retrospective Study. J Am Coll Nutr. 2021:1-16.
33. Angelidi AM, Belanger MJ, Lorinsky MK, Karamanis D, Chamorro-Pareja N, Ognibene J, et al. Vitamin D Status Is Associated With In-Hospital Mortality and Mechanical Ventilation: A Cohort of COVID-19 Hospitalized Patients. Mayo Clinic proceedings. 2021.
34. Cereda E, Bogliolo L, Lobascio F, Barichella M, Zecchinelli AL, Pezzoli G, et al. Vitamin D supplementation and outcomes in coronavirus disease 2019 (COVID-19) patients from the outbreak area of Lombardy, Italy. Nutrition. 2021;82:111055.
35. Gavioli EM, Miyashita H, Hassaneen O, Siau E. An Evaluation of Serum 25-Hydroxy Vitamin D Levels in Patients with COVID-19 in New York City. J Am Coll Nutr. 2021:1-6.
36. Lohia P, Nguyen P, Patel N, Kapur S. Exploring the link between vitamin D and clinical outcomes in COVID-19. Am J Physiol Endocrinol Metab. 2021;320(3):E520-e6.
37. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. JAMA Network Open. 2020;3(9):e2019722-e.
38. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, et al. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. The FEBS Journal. 2020;287(17):3693-702.
39. Shah K, Saxena D, Mavalankar D. Vitamin D supplementation, COVID-19 & Disease Severity: A meta-analysis. Qjm. 2021.

7.2. Additional literature review and discussion

7.2.1. Summary of the additional literature review

Three additional studies are summarised in **Table 6.** Two studies explored the association between vitamin D status and COVID-19 diagnosis, and one study investigated the association between vitamin D supplementation and the risk of having COVID-19 diagnoses. Despite using the same English sub-cohort, the study populations were defined differently in these studies. One study on vitamin D status only included English people with complete data (159), and another study further excluded people with negative COVID-19 tests (160). The study on vitamin D supplementation only included people who received COVID-19 tests (161). The follow-up periods of these studies were between mid-March and May/June 2020.

Of the two studies on vitamin D status and COVID-19, one study indicated that vitamin D deficiency or insufficiency was associated with increased odds of receiving a COVID-19 diagnosis (deficiency: OR = 1.09, 95% CI = 0.919, 1.294), hospitalisation (deficiency: OR = 1.29, 95% CI = 1.18–1.42), and severe COVID-19 (deficiency: OR = 1.23, 95% CI = 1.00–1.51) (159). However, another study on vitamin D status found no evidence that people with insufficient or sufficient vitamin D had lower odds of receiving a COVID-19 diagnosis (sufficiency OR = 0.97, 95% CI = 0.81–1.16), hospitalisation (sufficiency OR = 0.90, 95%CI = 0.63–1.28) or mortality (sufficiency OR = 0.92, 95% CI = 0.54–1.56) (160). In the study on vitamin D supplementation and COVID-19, after adjusting for possible confounders, vitamin D supplementation was associated with a 34% lower risk of COVID-19 (OR = 0.66, 95% CI = 0.45–0.97) (161).

7.2.2. Discussion of additional literature

In my previous analysis, I found an inconsistent association between vitamin D status and the risk of COVID-19 and no association between vitamin D status and hospitalisation or mortality due to COVID-19. These results are similar to those Li X et al. but different from those of Li S et al. (159, 160).

Several possible explanations may account for this discrepancy. First, compared with these studies, my follow-up period was longer, covering more than one wave of the pandemic. Second, the exposures were defined differently. Li S et al. defined their exposure of vitamin D as deficiency (< 25 nmol/L) and insufficiency (< 50 nmol/L), whereas my study and the study by Li X et al. defined vitamin D insufficiency as 25–50 nmol/L. Third, the outcomes ascertainment are different. In these published studies, the outcomes of COVID-19 were purely from laboratory test results. The hospitalisation outcome was defined by using the source of the testing sample using an algorithm (98), but after September 2020, the team that developed this algorithm recommended using inpatient care data as the gold standard to identify COVID-19 inpatients (162). In my study, the outcome of COVID-19 was defined using both testing results and the linked clinical records, and the hospitalisation outcome was obtained from the inpatient dataset. In addition, my data were analysed using a stratified Cox regression model adjusting for time-varying covariates, which may have also led to different results.

My analysis found no evidence that self-reported vitamin D supplementation was associated with a lower hazard of COVID-19 diagnosis during BST

months. However, during the non-BST follow-up period, some evidence indicated that supplementation was associated with a higher hazard of having COVID-19. By contrast, Ma et al. reported that vitamin D supplementation was associated with a decreased COVID-19 risk (161). Several differences in settings may explain the different results. First, Ma et al. only followed the participants for two months (16 March 2020 to 18 May 2020), whereas my study followed the participants longer (16 March 2020 to 18 January 2021). As mentioned earlier, the longer follow-up period in my study covered more waves of the pandemic, and the testing strategies for COVID-19 changed. Another key difference is the definition of vitamin D supplementation. In the paper by Ma et al., the authors defined habitual vitamin D supplementation as participants who solely used vitamin D (161). However, in previous nutritional studies regarding vitamin D supplementations, vitamin D supplements were more commonly defined as 'vitamin D containing supplements', including vitamin D, fish oils, multivitamins, and even calcium (13, 163). Therefore, I used this broader definition of vitamin D supplementation in my study to include those taking vitamin D and associated minerals, including multivitamins, fish oil, and calcium.

Chapter 7. Table 6 Additional literature review on vitamin D and COVID-19 using UK Biobank

Authors and publication year	Study population (N); Follow-up period	Definition of exposure	Definition of outcome	Statistical analysis	Adjusted covariates	Main findings (OR (95% CI))
Li S et al., (2021) (160)	• English people with complete data (N=353,299) • 16th March 2020 – 31st May 2020	Vitamin D status: • Deficiency (< 25 nmol/L) • Insufficiency (< 50 nmol/L)	• Lab-confirmed COVID-19 (at least one positive test) • COVID-19 hospitalisation: hospital originated specimen • Severe COVID-19: hospital originated with at least one positive test result	Logistic regression	• Adjusted for sex, age, deprivation, education, employment, ethnicity, smoking status, metabolic / obesity phenotypes.	• Confirmed COVID-19: Deficiency = 1.090 (0.919, 1.294); insufficiency = 1.204 (1.059, 1.368) • COVID-19 hospitalization: Deficiency = 1.292 (1.175, 1.420); insufficiency = 1.214 (1.132, 1.303) • Severe COVID-19: Deficiency = 1.227 (1.000, 1.505); insufficiency = 1.206 (1.030, 1.412) •
Li X et al., (2021) (159)	• English people with complete	Vitamin D status:	• Lab-confirmed COVID-19 (at least one positive test)	Logistic regression	• Age, gender, BMI, month, ethnicity, physical activity, smoking, alcohol status,	• COVID-19 diagnosis: Deficiency: reference; Insufficiency = 1.03

	data (N=417,342) • 16th March 2020 – 29th June 2020 • Excluded people with negative COVID-19 tests	• Deficiency (< 25 nmol/L) • Insufficiency (25–50 nmol/L) • Sufficient (> 50 nmol/L)	• COVID-19 hospitalisation • COVID-19 death		sunshine exposure, vitamin D supplement, deprivation index, cardiovascular diseases, diabetes, asthma, and malignancy	(0.87–1.20); Sufficiency = 0.97 (0.81–1.16) • COVID-19 hospitalization: Deficiency: reference; Insufficiency = 0.94 (0.64–1.39); Sufficiency = 0.90 (0.63–1.28) • COVID-19 death: Deficiency: reference; Insufficiency = 0.93 (0.57–1.52); Sufficiency = 0.92 (0.54–1.56)
Ma H et al., (2021) (161)	• People received the COVID-19 test (N=4,510) • 16th March 2020 – 18th May 2020	• Self-reported vitamin D supplementation • Vitamin D status (deficiency, insufficiency, sufficiency)	• Lab confirmed Covid19	Logistic regression	• Age, sex, race, research centres, laboratory, origin, blood-type, education, deprivation index, smoking, drinking, physical activity, healthy diet score, obesity, diabetes, hypertension, high cholesterol, cardiovascular diseases, cancer, asthma and COPD, vitamin D level	• Lab confirmed Covid19: Unadjusted model = 0.78 (0.57–1.05) Fully adjusted model = 0.66 (0.45–0.97)

7.3. Further analysis on vitamin D status and COVID-19 diagnosis

To further investigate the effects of sequential confounder adjustment on the association between vitamin D status and COVID-19 diagnosis, I used a stepwise approach to compare the effect measure change occurring with adjustment for different covariates. In brief, I applied a backward selection approach to exclude covariates with a p-value greater than 0.2, followed by adding the remaining covariates to the crude model according to the changing of the hazard ratio between vitamin D and COVID-19 diagnosis during different follow-up periods. The results of this stepwise approach were summarised in **Chapter 7 Table 7**. As shown in **Chapter 7 Table 7**, in steps 7 and 12, removing body mass index (BMI) and adding age groups to the model lead to the most significant change in hazard ratio and the p-value. The outcome obtained from the last model in step 13, which only adjusted for age, BMI, socioeconomic status as measured using the index of multiple deprivation (IMD), ethnicity, sex, and immunosuppression, is close to the complete model in step 1 (**Chapter 7 Table 7**).

In the stepwise analysis, the covariates age and obesity demonstrated the greatest confounding effects on the association between vitamin D and COVID-19 diagnosis. Previous research indicates that older age and obesity are risk factors for COVID-19 mortality, and they were also associated with vitamin D deficiency (6, 7). Therefore, their potential confounding effects should be considered when analyzing the association between vitamin D levels and COVID-19. However, as shown in **Chapter 7 Table 3**, there was evidence that people greater than 70 years old had a lower risk of being diagnosed with COVID-19 (adjusted HR=0.57, 95%CI= 0.54-0.59.) A

possible explanation for this reverse association is that older people were suggested to shield, making them less likely to be infected. However, this association can also be explained by the misclassification of COVID-19 diagnosis, because in the secondary outcome, older people were more likely to be admitted (**Chapter 7 Table 4**) or die from COVID-19 (**Chapter 7 Table 5**). In the stepwise model, BMI showed a strong confounding effect on the association between vitamin D deficiency and diagnosis of COVID-19. However, the possible misclassification of BMI needs to be considered, because this information was recorded more than ten years ago. Because of the misclassification of covariates, exposure, and the primary outcome, the association between vitamin D status and COVID-19 diagnosis remained unclear.

Chapter 7. Table 7. Effect size changes of selecting models using a stepwise approach

Step	Stepwise approach[1]	British summertime		Non-summertime	
		Vitamin D deficiency (<25 nmol/L)	Vitamin D insufficiency (25 – 50 nmol/L)	Vitamin D deficiency	Vitamin D insufficiency
Backward steps[2]					
1	Complete model adjusting for all covariates[3]	0.86 (0.77-0.95, p=0.005)	0.96 (0.90-1.04, p=0.324)	1.14 (1.01-1.30, p=0.038)	0.93 (0.85-1.02, p=0.130)
2.	Exclude British summertime	0.86 (0.77-0.95, p=0.005)	0.96 (0.90-1.04, p=0.326)	1.14 (1.01-1.30, p=0.038)	0.93 (0.85-1.02, p=0.130)
3	Further exclude underlying comorbidities[4]	0.86 (0.77-0.95, p=0.005)	0.96 (0.90-1.04, p=0.327)	1.14 (1.01-1.30, p=0.038)	0.93 (0.85-1.02, p=0.130)
4	Further exclude regions	0.87 (0.78-0.96, p=0.008)	0.97 (0.90-1.04, p=0.365)	1.14 (1.01-1.30, p=0.038)	0.93 (0.85-1.02, p=0.130)
5	Further exclude smoking	0.86 (0.77-0.96, p=0.006)	0.97 (0.90-1.04, p=0.374)	1.15 (1.01-1.30, p=0.033)	0.93 (0.85-1.02, p=0.128)
6	Further exclude drinking frequency[5]	0.86 (0.78-0.96, p=0.006)	0.97 (0.90-1.04, p=0.354)	1.15 (1.02-1.31, p=0.027)	0.94 (0.86-1.03, p=0.157)
7	Further exclude BMI	0.91 (0.82-1.01, p=0.075)	1.16 (1.02-1.32, p=0.021)	1.01 (0.94-1.08, p=0.880)	0.93 (0.85-1.02, p=0.120)

Forward steps[6]					
8	Crude model without any adjustment	1.18 (1.07-1.31, p=0.001)	1.11 (1.03-1.19, p=0.004)	1.15 (1.02-1.31, p=0.023)	0.93 (0.86-1.02, p=0.124)
9	Adjusted for sex and clinically vulnerable	1.17 (1.06-1.30, p=0.002)	1.11 (1.03-1.19, p=0.004)	1.15 (1.02-1.31, p=0.023)	0.93 (0.86-1.02, p=0.124)
10	Add ethnicity[7]	1.06 (0.95-1.17, p=0.286)	1.08 (1.00-1.15, p=0.040)	1.16 (1.02-1.31, p=0.022)	0.93 (0.86-1.02, p=0.125)
11	Add IMD	0.97 (0.87-1.08, p=0.587)	1.04 (0.97-1.11, p=0.316)	1.16 (1.02-1.32, p=0.020)	0.93 (0.85-1.02, p=0.121)
12	Add age groups	0.91 (0.82-1.01, p=0.075)	1.01 (0.94-1.08, p=0.880)	1.16 (1.02-1.32, p=0.021)	0.93 (0.85-1.02, p=0.120)
13	Add BMI[8]	0.86 (0.78-0.96, p=0.006)	0.97 (0.90-1.04, p=0.354)	1.15 (1.02-1.31, p=0.027)	0.94 (0.86-1.03, p=0.157)

1. Effect measure are presented as hazard ratio, 95% confidence interval, p-value; 2. Backward steps: exclude covariates if its p-value is less than 0.2; 3. Including sex, age, ethnicity, BMI, drinking frequency, smoking status, vitamin D status testing during British summertime, Index for multiple deprivation (IMD), Regions, clinically vulnerable to COVID-19, underlying comorbidities. 4. including hypertension, cardiovascular diseases, diabetes mellitus, and asthma; 5. drinking frequency were categorized into never, sometimes, weekly, and daily; 6. forward steps: add covariates by increasing influence on the effect measures; 7. ethnicity were categorized into white, black, and Asian and others; 8. In the last model, covariates including age, BMI, IMD, ethnicity, sex, and clinically vulnerable were adjusted.

7.4. Chapter summary

- Among 307,512 UK Biobank participants with primary and inpatient care records, 10,165 were diagnosed with COVID-19 from 16 March 2020 to 18 January 2021.
- My results showed an inconsistent association between vitamin D status and COVID-19 diagnosis. During BST months, people with vitamin D deficiency had a lower risk of being diagnosed with COVID-19 (HR = 0.86, 95% CI = 0.77–0.95), whereas in non-BST months, the deficiency group had a 14% higher hazard of being diagnosed with COVID-19 (HR = 1.14, CI = 1.01–1.30).
- I found no evidence that vitamin D status was associated with a higher risk of hospitalisation or mortality due to COVID-19 during or after BST months.
- During BST months, I found some evidence that people who received vitamin D prescriptions from a GP had a higher risk of receiving a COVID-19 diagnosis, hospitalisation, and mortality.
- No evidence showed that vitamin D supplementation was associated with COVID-19 diagnosis, hospitalisation, or mortality during or after BST months.
- According to the results, vitamin D testing, supplementation and fortification cannot be recommended to prevent COVID-19.

Chapter 8. Overall discussion

Chapter overview

In the previous chapters, I reviewed existing literature on vitamin D and herpesviruses, described the distribution and the risk factors of vitamin D in UK Biobank, and conducted two cohort studies exploring the associations between vitamin D deficiency HZ and COVID-19. In this final discussion chapter, I briefly summarise the main results and compares my findings with those of previously published studies. Finally, I discuss the strengths and limitations of my studies and their implication for public health policy and research.

8.1. Summary of main findings

In my systematic review and meta-analysis, I summarised 10 eligible studies on serum vitamin D status, supplementation, and the risk of herpesviruses infections or reactivation. Regarding vitamin D status and herpesviruses, no evidence indicated that vitamin D deficiency was associated with an increased risk of CMV disease or HZ among people receiving organ transplantations or with increased HHV-8 viral load among people with HIV. No evidence indicated that vitamin D supplementation was associated with reduced EBV viral load among people with multiple sclerosis. However, two studies indicated that vitamin D supplementation was associated with a decreased risk of HZ among people receiving haemodialysis and those who acquired CMV disease after transplantation.

In my cross-sectional study of 449,943 UK Biobank participants with serum vitamin D records, the proportion of vitamin D deficiency was higher among

people with Asian and black backgrounds, people who lived in the north, and those whose vitamin D was measured during winter or spring. Male sex, abnormal BMI, non-white ethnicities, smoking and higher levels of deprivation were associated with a higher risk of vitamin D deficiency or insufficiency.

My cohort study on vitamin D and HZ included 177,572 UK Biobank participants with vitamin D records and linked clinical records. After adjusting for potential confounders, neither vitamin D status nor vitamin D supplementation or prescriptions were associated with incident HZ.

Finally, in the cohort investigating the association between vitamin D status and the COVID-19, vitamin D status was inconsistently associated with COVID-19 diagnosis during different follow-up periods. People with deficient vitamin D levels had a lower risk of being diagnosed with COVID-19 during the BST months, but their risk increased after BST ended. No evidence indicated that vitamin D deficiency was associated with increased COVID19 hospitalisation or mortality risk.

8.2. Comparison with existing literature

8.2.1. Risk factors of vitamin D deficiency

Some risk factors of vitamin D deficiency in adults have been well established, such as seasons, latitude, black ethnicity, and obesity, which were also observed in my study (2, 117). In addition, I observed that tobacco smoking and socioeconomic deprivation were associated with vitamin D deficiency, whereas supplementation and alcohol consumption were related

to a decreased risk of vitamin D deficiency. These findings were similar to those of other cross-sectional studies (164, 165).

Age is also regarded as a risk factor for vitamin D deficiency, and clinical guidelines recommend higher doses of vitamin D supplementation for people older than 50 years (5). However, my cross-sectional analysis revealed a reverse trend, which suggested that ageing was associated with a marginally decreased risk of vitamin D deficiency or insufficiency. A possible reason is that the UK Biobank participants were relatively young when they were recruited, and these participants were also relatively healthier than the general public. Therefore, this healthy volunteer effect could diminish the association between age and vitamin D status (78, 117).

8.2.2. Vitamin D deficiency and herpes zoster

In previous studies, the association between vitamin D deficiency and HZ risk was uncertain. In Taiwan, a small hospital-based case-control study compared 88 patients with postherpetic neuralgia aged over 50 years with 264 sex- and age-matched healthy controls. The authors reported that people with low serum vitamin D (25(OH)D < 75 nmol/L) had three times the risk of developing postherpetic neuralgia than healthy controls after matching by sex, age, and seasons (adjusted OR = 3.12, 95% CI = 1.73–5.61) (166). However, selection bias may have influenced these results because the controls were selected from healthy people visiting the hospital for health examinations. In addition, residual confounding should be considered because the authors only adjusted for sex, age, and seasons in the matched analysis. In my cohort study analysing 177,572 UK Biobank participants, I

found no evidence of any association between vitamin D deficiency and incident HZ (119). My results were similar to those of another cohort study included in my systematic review, which revealed no evidence that people with vitamin D deficiency have a higher risk of HZ after kidney transplantation (167).

8.2.3. Vitamin D supplementation and herpes zoster

The association between vitamin D status, supplementation, and HZ risk was assessed by some small studies. A hospital-based case-control study in Taiwan showed that taking active vitamin D supplementation was associated with a lower risk of HZ among 126 people receiving haemodialysis (adjusted OR = 0.06, 95% CI= 0.0–0.4) (168). By contrast, such an association was not found in my analysis. My cohort study on vitamin D and HZ using UK Biobank database observed no evidence supporting an association between vitamin D status, vitamin D supplementation, and incident HZ (119). In addition to different study designs and sample sizes, the study population was the main difference between the two studies. Chao et al. conducted their case-control study among people with end-stage kidney diseases (168), whereas the UK Biobank participants were generally healthy.

8.2.4. Vitamin D deficiency and COVID-19 diagnosis, hospitalisation, or mortality

The association between vitamin D deficiency and COVID-19 has been a controversial issue. My analysis showed an inconsistent association between vitamin D status and COVID-19 diagnosis and no association between vitamin D status and hospitalisation or mortality. Previous studies using UK

Biobank data also reported inconsistent results. Among five UK Biobank studies exploring serum vitamin D levels and COVID-19, four studies found no association between vitamin D status and COVID-19 diagnosis, hospitalisation, or mortality (114, 147, 148, 160). Only one study using UK Biobank showed a positive association between vitamin D deficiency/insufficiency and an increased risk of confirmed COVID-19, hospitalisation and severe COIVD-19 (159). However, in these previous studies, the follow-up periods were shorter, covering only the first four months of the pandemic. In addition, these studies only relied on the laboratory test data from Public Health England to define their COVID-19 outcomes, without considering information from linked clinical records. This may explain the difference in findings. Furthermore, these studies were conducted at the beginning of the first wave of the pandemic, so their results were likely influenced by the outcome ascertainment bias due to the changing testing strategies. This would also lead to differences between findings (as discussed in Chapter 7 and section 8.4.3).

From 2020 to 2021, several observational studies showed some evidence that vitamin D deficiency could be associated with a higher risk of COVID-19 diagnosis or hospitalisation (156, 157, 169, 170), although some studies indicated no evidence of an association between vitamin D and SARS-CoV-2 seropositivity (171). A systematic review summarising 17 observational studies indicated that vitamin D deficiency was associated with a higher risk of hospitalisation due to COVID-19 (OR = 2.18, 95% CI: 1.48–3.21; three studies) and increased mortality (OR = 2.47; 95% CI=1.5–4.05, 17 studies) (172). Nevertheless, most previous studies with positive findings were

conducted mainly in single hospital settings with small sample sizes and without sufficiently adjusting for confounders. Therefore, the association between vitamin D deficiency and the risk of COVID-19 remains uncertain.

8.2.5. Vitamin D supplementation and COVID-19

Previously, some evidence suggested a protective effect of vitamin D supplementations against respiratory infections. An updated systematic review and meta-analysis of 43 clinical trials demonstrated that vitamin D supplementation had modest protective effects against respiratory infections (OR = 0.92, 95% CI = 0.86–0.99; 37 studies) (24). However, studies on the effect of vitamin D supplements on COVID-19 revealed a mixed picture. In a previous study using UK Biobank data, Ma et al. stated that vitamin D supplementation was associated with a decreased COVID-19 risk (161), whereas my study found no association between vitamin D supplements or prescriptions COVID-19. These inconsistent results can be explained by the difference in the definitions of vitamin D supplementation, identification of COVID-19 outcomes, follow-up periods, and covariate adjustment.

A non-randomised interventional study (n=77) in France indicated that on day 14, after admission due to COVID-19, people who received long-term high-dose vitamin D bolus had a lower risk of mortality and a greater improvement in clinical improvement scores (173). However, another randomised trial (n=240) in the US indicated that comparing people receiving a single high dose of vitamin D and placebo group, no differences in hospital length of stay, mortality, admission to the intensive unit, or the need for mechanical ventilation was noted (174). Because of the lack of results from

large randomised controlled trials, it is still uncertain whether vitamin D supplementation can treat or prevent COVID-19.

8.3. Strengths

8.3.1. Large sample size selected from the general public

A key strength of my studies is the large number of participants in the study population (UK Biobank). Previous studies on vitamin D and viral infections were mainly conducted in hospital-based settings. The study populations were relatively small, and the participants were more likely to have comorbidities. Studies on vitamin D and the risk of HZ were undertaken among people with immunosuppressive conditions and had smaller sample sizes. By contrast, UK Biobank recruited nearly half-million participants from the general population, providing greater statistical power than hospital-based studies.

8.3.2. Prospectively and systematically collected data

Information about demographic factors, exposure, covariates, and several outcomes of my studies was collected prospectively, facilitating the assessment of temporality. For instance, my study measured the exposure vitamin D status prospectively and systematically before outcome measurement. Because the exposure was not ascertained by retrospective recall or by observers related to the outcomes, the exposure is unlikely to be misclassified because of recall bias or observer bias.

8.3.3. Complete data with few missing demographic and lifestyle data

Another key strength of my studies using UK Biobank is the completeness of data. Most key demographic factors included in my analysis were complete, with less than 3% missing. For the primary exposure vitamin D status, the proportion of people without any vitamin D measurements was less than 10%. Therefore, my primary analysis is less likely to be biased due to missing demographic and lifestyle data.

8.3.4. Linkage to electronic health records for follow-up

In my studies, the outcomes and covariates were identified from the linked EHRs of UK Biobank. Because the UK health system routinely collected the linked EHRs, and clinicians recorded the diagnoses without awareness of this research question as part of routine care, the observer bias in outcome ascertainment is minimised. In addition, more clinical covariates could also be identified if they were recorded in the linked EHR. For instance, in Chapter 7, I adjusted for more clinical comorbidities and immunosuppressive conditions identified from the more recent linked primary care and hospitalised records, whereas most previous studies only relied on information from the self-reported questionnaires recorded more than 10 years ago.

8.4. Limitations

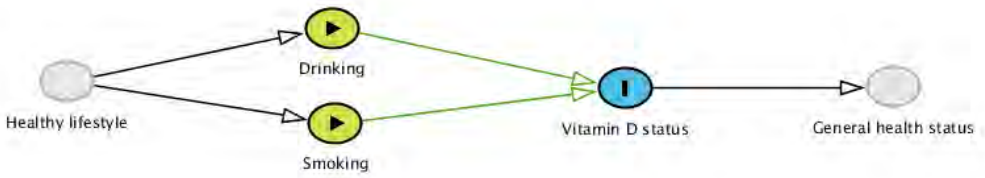
8.4.1. Selection of participants and collider bias

A fundamental issue of using UK Biobank data is selection bias in participants. As previously mentioned, the response rate of UK Biobank was only 5%, and the participants were healthier and wealthier than non-participants. In addition, participants without linked primary care and

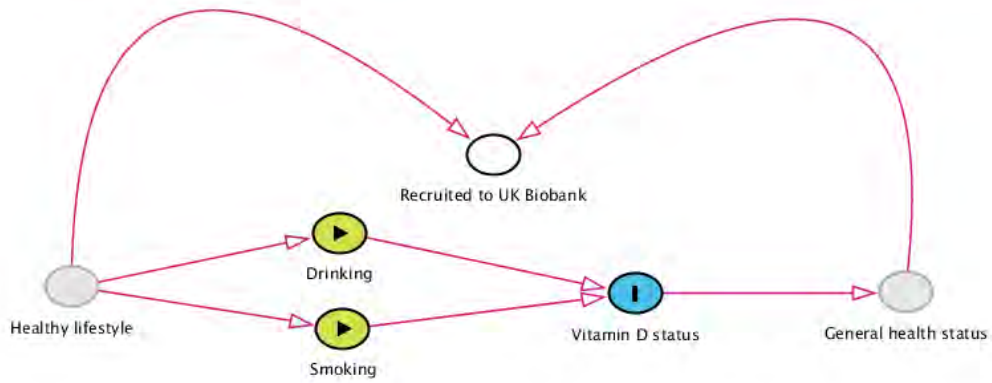
hospitalisation records were excluded from my analysis. For instance, only 170,000 people with electronic health records were included in the analyses in Chapter 6. In Chapter 7, people living in Scotland and Wales were excluded because their COVID-19 testing data were not available. The selection of participants limits the external validity of my findings.

Furthermore, the selection bias due to healthy volunteer effects may further influence the estimation between exposure and outcomes by inducing collider bias (175). **Chapter 8. Figure 1** is a simplified illustration of the DAGs between exposure and outcomes in Chapter 5, in which I was exploring the association between alcohol drinking and smoking and the risk of vitamin D deficiency (**Chapter 8. Figure 1. a**). Assuming that vitamin D status is associated with individuals' health status, therefore, healthy lifestyles and general health status influence participants' recruitment to the study. In this case, being recruited to UK Biobank became a collider (**Chapter 8. Figure 1. b**). Therefore, analysing data from UK Biobank is equal to adjusting for a collider, making the pathway between exposure and outcomes biased (**Chapter 8. Figure 1. b**). Consequently, the interpretation of UK Biobank results should remain cautious. Some associations observed from my findings in UK Biobank, such as the negative association between drinking and vitamin D deficiency, should not be extrapolated to the general population, given the inherent limitation related to collider bias.

a.



b.



Chapter 8. Figure 1 DAGs showing the possible collider bias in UK Biobank studies. The healthy lifestyle and general health status are unobserved variables. Drinking and smoking are exposure, and the vitamin D status is the outcome. Green lines indicate potential causal pathways and red arrows indicate biasing pathways. **a**. a hypothetical study scenario without considering the recruitment of UK Biobank; **b**. actual study scenario of a UK Biobank study. In this case, recruiting to UK Biobank becomes a collider, and the association between exposure and outcome is biased.

8.4.2. Misclassification bias of exposures

UK Biobank participants were first recruited and assessed between 2006 and 2010; therefore, some time-dependent variables, such as vitamin D status, may change markedly over time. Vitamin D synthesis varies across seasons

(176), as noted in Chapter 5. However, Chapter 7 assessed the association between vitamin D and COVID-19, and the index date of the study was 15 March 2020, which was at least 10 years after vitamin D assessment. Therefore, using outdated vitamin D information as an exposure introduces misclassification bias, although it was non-differential.

8.4.3. Misclassification of outcomes

Misclassification of outcomes cannot be ruled out. First, in Chapter 6, the outcome of HZ was identified by the diagnostic codes in the linked EHRs of UK Biobank, and these diagnostic codes have not yet been validated. These unvalidated codes could introduce non-differential ascertainment bias in outcomes. In addition, the HZ among people with comorbidities is more likely to be diagnosed because of more frequent primary care visits (131). Regarding possible ascertainment bias, a study in the US reported that using the ICD-10 code for HZ (code 053) could identify 98% of herpes zoster cases (sensitivity = 98%), and the PPV was also very high (PPV = 93%) (177). Furthermore, the financial barrier to access to healthcare in the UK is generally lower than in the US, which may increase the sensitivity of HZ diagnoses in the EHR databases.

For an observational study about COVID-19, the misclassification of outcomes is a more severe issue because COVID-19 was not tested systematically throughout the study period. In Chapter 7, the outcome of COVID-19 diagnoses was defined using laboratory test results provided by Public Health England. However, the COVID-19 testing strategy was changing during the first wave. At the beginning of the pandemic, only people

admitted to the hospital could be able to be tested because of o limited testing capacity. The testing capacity expanded later, and some people were tested for surveys. This change in testing strategies drastically influenced the ascertainment of COVID-19 diagnoses, which may bias the results.

8.4.4. Residual confounding

Despite adjusting for covariates, residual confounding cannot be ruled out in my studies. First, using diagnostic codes alone may not be sensitive enough to identify the true prevalence of some clinical covariates. For instance, CKD is a risk factor of HZ and severe COVID-19, and it is also closely related to vitamin D deficiency. Using laboratory tests results (e.g. creatinine levels) to identify individuals with CKD is more accurate than using diagnostic codes alone (115). According to Public Health England, the estimated prevalence of CKD (stages 3–5) in England among people aged 64 and younger was 1.9%. The prevalence was 13.5% among people aged 65–74 years (132). However, only 0.8% of my study population reported CKD, a much lower figure than the national prevalence.

In addition, similar to the limitation previously mentioned in section 8.4.3, studies using EHR assume that individuals have a disease if they have the corresponding diagnostic codes. Conversely, people without specific diagnostic codes are assumed not to have a disease. It is possible that some participants with relevant clinical conditions did not visit their GP or did not have a confirmed diagnosis, so their disease statuses may remain unrecorded. The underestimation of clinical covariates would lead to residual confounding in the HZ and COVID-19 studies.

8.5. Implication for public health policy

8.5.1. Vitamin D supplementation recommendation from Public Health England should be followed and extended

Chapter 5 indicated that the distribution of vitamin D deficiency varied geographically and seasonally in the UK, and some demographic factors, including ethnicity, were associated with vitamin D deficiency. These findings support the recommendation of Public Health England, suggesting that people take vitamin D daily in the winter and recommending that people with black or south Asian ethnic backgrounds take supplements regularly. In addition, several factors such as obesity, smoking, and socioeconomic deprivation, were also associated with vitamin D deficiency (117). Consequently, in addition to promoting the recommendation by Public Health England, public health policy could also address vitamin D deficiency issues among people with these additional risk factors pending further supportive evidence.

8.5.2. Other herpes zoster preventive measures are needed for people under 70 years old

Currently, vaccination is regarded as the best preventive strategy for HZ. However, this vaccination program is currently only available for people aged 70 to 79 years in the UK and is still unavailable for those under 70 years old, even for individuals with immunosuppressive conditions (178). In addition, among people under 70 years old, a previous study found that people with specific comorbidities had an increased risk of HZ (39). In chapter 6, no evidence of any association between vitamin D deficiency and the HZ risk

was found. Therefore, vitamin D supplements cannot be recommended as a preventive strategy for HZ, although other benefits have been established. Vaccination remains the most effective preventive measure. In the US, the ACIP recommends recombinant shingles vaccines for immunocompetent adults over 50 years old (42), and recently, the US Centers for Disease Control and Prevention (US–CDC) and ACIP have planned to recommend vaccinations for immunocompromised adults (179). A more extensive vaccination programme should also be considered for people at risk of developing HZ who are under 70 years old.

8.5.3. Vitamin D supplements should be recommended for bone health, and vitamin D should not be prioritised as a preventive strategy for COVID-19

Vitamin D supplementation has been hypothesised as a potential preventive measure for COVID-19 because of its immunomodulatory effects (140). Before introducing COVID-19 vaccines, free vitamin D supplementation was even distributed by the NHS to people at high risk for COVID-19 (180). The precautionary principle of taking action to reduce risk without solid evidence was reasonably applied in this early intervention (181). Nevertheless, chapter 7 showed an inconsistent association between vitamin D status and the risk of COVID-19, and there was no evidence to suggest an association between vitamin D status and hospitalisation or mortality due to COVID-19.

As introduced in Chapter 1, vitamin D is essential for maintaining bone health. Currently, taking daily vitamin D supplements (400 U) is recommended by Public Health England (3). Evidence from systematic

review and meta-analysis of trials indicated that the protective effects of vitamin D supplements were only seen among subgroups taking daily doses between 400-1000 units and among children younger than 16 years old (24). Therefore, the NICE guideline only recommends that people take vitamin D to follow current the SACN recommendations only for bone health instead of COVID-19 prevention or treatment (182, 183). Furthermore, effective vaccines that protect against COVID-19 have been introduced. The current evidence indicates that vitamin D supplementation should not be recommended over other preventive strategies for COVID-19, such as vaccination.

8.6. Implication for research

8.6.1. Using repeated vitamin D measurements for exposure

As mentioned in Chapter 5, vitamin D status changes over time. Assuming that vitamin D level is constant may introduce information bias in the following analysis, as discussed in Chapter 7. Therefore, future longitudinal studies should consider using repeated vitamin D measurement as an exposure; in this way, researchers can more accurately evaluate the long-term accumulated effect of vitamin D status on health outcomes.

8.6.2. More accurate vitamin D consumption estimation

In chapters 6 and chapters 7, information on vitamin D supplementation could only be obtained from self-reported questionnaires; the intakes from food or fortification products could not be ascertained because of data availability. By contrast, the National Diet and Nutrition Survey analysed vitamin D intake from food in detail, providing a more accurate estimation

(184). Future studies exploring the effect of vitamin D supplementation can also consider other sources of vitamin D consumption.

8.6.3. Confounding by indication for vitamin D prescription

Chapter 6 revealed a trend of increasing HZ risk among people who received vitamin D prescriptions; this trend is likely to be confounded by indication because the indications for receiving the prescription were not adjusted for in the analysis. In a cohort study analysing vitamin D prescription patterns, older children and those with non-white ethnicities and socioeconomic deprivation had a higher chance of receiving vitamin D prescriptions (185). Although this study focused on the prescription patterns in children, it implied that the indications and other factors are associated with the probability of receiving vitamin D prescriptions. Consequently, approaches to minimise confounding by indications, such as propensity scores, should be considered in future studies on vitamin D prescriptions.

8.6.4. Vitamin D deficiency might be an outcome of viral infections

In this thesis, two cohort studies were conducted to explore the association between vitamin D status and the risk of two infectious diseases, HZ and COVID-19. Although the cohort design is less likely to be biased by reverse causation, vitamin D deficiency induced by viral infection is still theoretically possible. Previously, an in vitro study demonstrated that the expression of vitamin D dependent receptors (VDR) was downregulated in cells infected with CMV (186), and such a phenomenon was also observed among people receiving hematopoietic stem cell transplantation with CMV infections (187).

Therefore, future studies should consider exploring the possibility of a bidirectional relationship between vitamin D and viral infections, and other approaches such as Mendelian randomisation can be considered to reduce the risk of reverse causation.

8.6.5. Prospective study on vitamin D and COVID-19

Although the possibility of misclassification bias caused by historical vitamin D levels and changing testing strategies cannot be excluded in my research, currently available evidence does not support an important association between vitamin D and COVID-19. As discussed earlier, evidence from a systematic review and meta-analysis of clinical trials indicated that taking vitamin D supplements has only modest effects on reducing respiratory tracts infections (18). By contrast, large observational studies using UK Biobank data, including my analysis presented in this thesis, have not observed a strong association between vitamin D and COVID-19. Recently, a preprint of a randomized-controlled trial reported that among 2,690 participants with serum vitamin D less than 75 nmol/L, no evidence was found that taking vitamin D supplements reduced the risk of incident COVID-19 (high-dose vs not taking vitamin D supplement: OR=1.13, 95% CI=0.78–1.63; lower-dose vs not taking vitamin D supplement: OR=1.39, 95% CI=0.98–1.97) (188). Therefore, instead of focusing on vitamin D, researchers may consider focusing on investigating other risk factors and intervention strategies against COVID-19.

Chapter 9. Conclusion

In summary, I found that vitamin D deficiency was more common in the winter and spring and more prevalent in the northern UK than in the south. Some demographic factors, such as non-white ethnic backgrounds, are associated with vitamin D deficiency. Among UK Biobank participants with linked medical records, no evidence was found that vitamin D deficiency was associated with a higher risk of HZ or COVID-19 diagnosis. In addition, no evidence indicated that taking vitamin D supplements or receiving vitamin D prescriptions was associated with decreased HZ or COVID-19. According to the current evidence derived from this thesis, extra vitamin D supplementation cannot be recommended as a preventive strategy for HZ or COVID-19.

COVID-19 impact statement

Change of research plans due to travel restrictions

Before the COVID-19 pandemic, my original PhD plan included using an EHR database in Taiwan (189). However, Taiwan closed its borders at the beginning of the pandemic, and later the UK issued similar travel restrictions. These measures made my planned research in Taiwan very difficult. Because of the disruption caused by the pandemic, I decided to cancel my plan of researching in Taiwan and add a study on vitamin D and COVID-19 using UK Biobank.

Working from home challenge

During the lockdown, the UK government suggested that people work from home, but the workspace available at home became a problem. Because we have only one desk in our flat in London, my wife and I had to take turns using it. Alternatively, I used a stove in our kitchen as a low-cost standing desk.

Another issue was the hardware needed for my research. My PhD project used UK Biobank and linked EHRs. The sizes of these clinical datasets were enormous and could not be loaded using my outdated desktop with insufficient memory. I planned to upgrade my computer, but this became very difficult during the school closure. Instead of waiting, I used an online platform powered by a workstation of LSHTM to analyse my data. However, this online platform did not fully support Stata, so I learned to analyse my data using R.

New strategies for digital work

The pandemic required everyone to explore new ways of working. Although the remote desktop of LSHTM can handle the data, it runs very slowly during the regular working hours on weekdays. Therefore, I needed to conduct computationally demanding analyses outside of working hours to avoid digital traffic jams. In addition, I learned to use other digital tools to facilitate communication with other collaborators. For instance, I used GitHub as a version control tool for my analysis and a convenient tool to share and discuss my study with others. Another example is learning to write in Markdown language, which allowed me to annotate my projects better. These digital tools helped me overcome the difficulties of remote working.

Stress and mental health

The national lockdown has had a considerable impact on people's lives, and the stay-at-home order created severe stress. When working from home, the boundary between working and living was blurred, and the low efficiency made the work appear endless. As an international student, I was worried about my family in Taiwan. Although Taiwan managed the pandemic relatively well, the travel restrictions and quarantine rules made it difficult to go home. I am grateful for the support from my wife and friends during this challenging period.

References

1. Deluca HF. Chapter 1 - Historical Overview of Vitamin D. In: Feldman D, Pike JW, Adams JS, editors. Vitamin D. San Diego: Academic Press; 2011. p. 3-12.
2. Holick MF. Vitamin D deficiency. New England Journal of Medicine. 2007;357(3):266-81.
3. The Scientific Advisory Committee on Nutrition. Vitamin D and Health. London: Public Health England 2016 21 July 2016.
4. Royal Osteoporosis Society. Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management. Royal Osteoporosis Society,; 2018 December 2018.
5. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.
6. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr. 2007;85(3):860-8.
7. Pan WH. 2013-2016 Nutrition And Health Survey in Taiwan (Chinese, Author's translation). Health Promotion Administration, Ministry of Health and Welfare; 2019.
8. Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ. 2010;340:b5664.
9. Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. A Catharine RCL, Taylor; Ann L, Yaktine; Heather B, Del Valle;, editor. Washington (DC): National Academies Press (US); 2011.
10. Hanley DA, Cranney A, Jones G, Whiting SJ, Leslie WD, Guidelines Committee of the Scientific Advisory Council of Osteoporosis C. Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada (summary). CMAJ. 2010;182(12):1315-9.
11. Department of Health. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. 1991. Report No.: 0300-8045
12. Public Health England. PHE publishes new advice on vitamin D 2016 [Available from: https://www.gov.uk/government/news/phe-publishes-new-advice-on-vitamin-d.
13. Black LJ, Jacoby P, Nowson CA, Daly RM, Lucas RM. Predictors of Vitamin D-Containing Supplement Use in the Australian Population and Associations between Dose and Serum 25-Hydroxyvitamin D Concentrations. Nutrients. 2016;8(6).

14. Hill TR, Aspray TJ. Vitamin D prescribing in older people in the UK depends on postcode. Maturitas. 2017;99:109-13.
15. NICE. Vitamin D: supplement use in specific population groups [PH56] 2014.
16. Carmeliet G. Chapter 25 - Vitamin D and Bone: An Integrated Approach. In: Feldman D, editor. Vitamin D (Fourth Edition): Academic Press; 2018. p. 419-33.
17. Yao P, Bennett D, Mafham M, Lin X, Chen Z, Armitage J, et al. Vitamin D and Calcium for the Prevention of Fracture: A Systematic Review and Meta-analysis. JAMA Network Open. 2019;2(12):e1917789-e.
18. Avenell A, Mak JC, O'Connell D. Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. Cochrane Database Syst Rev. 2014;2014(4):Cd000227.
19. Hiligsmann M, Ben Sedrine W, Bruyère O, Evers SM, Rabenda V, Reginster J-Y. Cost-effectiveness of vitamin D and calcium supplementation in the treatment of elderly women and men with osteoporosis. Eur J Public Health. 2014;25(1):20-5.
20. Weaver CM, Bischoff–Ferrari HA, Shanahan CJ. Cost-benefit analysis of calcium and vitamin D supplements. Archives of Osteoporosis. 2019;14(1):50.
21. Bolland MJ, Avenell A, Smith K, Witham MD, Grey A. Vitamin D supplementation and testing in the UK: costly but ineffective? BMJ. 2021;372:n484.
22. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770-3.
23. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsjo A, Torma H, Stahle M. Vitamin D induces the antimicrobial protein hCAP18 in human skin. J Invest Dermatol. 2005;124(5):1080-2.
24. Jolliffe DA, Camargo CA, Jr., Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. Lancet Diabetes Endocrinol. 2021.
25. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature. 2001;414(6862):454-7.
26. Chromek M, Arvidsson I, Karpman D. The Antimicrobial Peptide Cathelicidin Protects Mice from Escherichia coli O157:H7-Mediated Disease. PLoS One. 2012;7(10):e46476.
27. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J. 2005;19(9):1067-77.

28. Lowry MB, Guo C, Zhang Y, Fantacone ML, Logan IE, Campbell Y, et al. A mouse model for vitamin D-induced human cathelicidin antimicrobial peptide gene expression. J Steroid Biochem Mol Biol. 2020;198:105552.
29. Akiko Iwasaki, Ruslan Medzhitov. Innate Responses to Viral Infections. In: Bernard N Fields, David M Knipe, Howley PM, editors. Fields Virology 6th ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2013. p. 189-213.
30. Heininger U, Seward JF. Varicella. Lancet. 2006;368(9544):1365-76.
31. Robert Johnson. Herpes Zoster Vaccination: A Vaccine to Prevent Pain. In: Jean-Pierre Michel, Stefania Maggi, editors. Adult Vaccinations: Springer International Publishing; 2019. p. 91-5.
32. Whitley RJ. Varicella-Zoster Virus Infections. In: J. Larry Jameson, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, Joseph Loscalzo, editors. Harrison's Principles of Internal Medicine. 20e ed: McGraw-Hill Education; 2018. p. 1355.
33. Gnann JW, Jr., Whitley RJ. Clinical practice. Herpes zoster. New England Journal of Medicine. 2002;347(5):340-6.
34. Kenneth E. Schmader, Robert H. Dworkin. The Epidemiology and Natural History of Herpes Zoster and Postherpetic Neuralgia. 2017. In: Herpes Zoster: Postherpetic Neuralgia and Other Complications [Internet]. ADIS. 1. [34].
35. Cohen JI. Clinical practice: Herpes zoster. New England Journal of Medicine. 2013;369(3):255-63.
36. Gershon AA. Antiviral Therapy and Local Treatment for Herpes Zoster. In: Watson CPN, Gershon AA, Oxman MN, editors. Herpes Zoster: Postherpetic Neuralgia and Other Complications: Focus on Treatment and Prevention. Cham: Springer International Publishing; 2017. p. 97-102.
37. Dworkin RH, Johnson RW, Breuer J, Gnann JW, Levin MJ, Backonja M, et al. Recommendations for the management of herpes zoster. Clin Infect Dis. 2007;44 Suppl 1(Supplement_1):S1-26.
38. Gauthier A, Breuer J, Carrington D, Martin M, Remy V. Epidemiology and cost of herpes zoster and post-herpetic neuralgia in the United Kingdom. Epidemiol Infect. 2009;137(1):38-47.
39. Forbes HJ, Bhaskaran K, Thomas SL, Smeeth L, Clayton T, Langan SM. Quantification of risk factors for herpes zoster: population based case-control study. BMJ. 2014;348:g2911.
40. Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. New England Journal of Medicine. 2005;352(22):2271-84.
41. Cunningham AL, Lal H, Kovac M, Chlibek R, Hwang SJ, Diez-Domingo J, et al. Efficacy of the Herpes Zoster Subunit Vaccine in Adults 70 Years of Age or Older. New England Journal of Medicine. 2016;375(11):1019-32.

42. Dooling KL, Guo A, Patel M, Lee GM, Moore K, Belongia EA, et al. Recommendations of the Advisory Committee on Immunization Practices for Use of Herpes Zoster Vaccines. Morbidity and Mortality Weekly Report (MMWR). 2018;67(3):103–8.
43. Public Health England. Herpes zoster (shingles) immunisation programme 2013/2014: Report for England. London: Public Health England; 2014 December 2014 Contract No.: 2014563
44. Public Health England. Herpes zoster (shingles) immunisation programme 2014/2015: Report for England. London: Public Health England; 2015.
45. Public Health England. Herpes zoster (shingles) immunisation programme 2016 to 2017: evaluation report. London: Public Health England; 2017. Available from: https://www.gov.uk/government/publications/herpes-zoster-shingles-immunisation-programme-2016-to-2017-evaluation-report.
46. NHS. Shingles vaccination - NHS 2018 [updated 6 July 2018 Available from: https://www.nhs.uk/conditions/vaccinations/shingles-vaccination/.
47. Public Health England. Herpes zoster (shingles) immunisation programme September 2015 to August 2016: Report for England. 2016.
48. Public Health England. Herpes zoster (shingles) immunisation programme 2017 to 2018: evaluation report. London: Public Health England; 2018 23 November 2018. Contract No.: 42.
49. Public Health England. Herpes zoster (shingles) immunisation programme 2018 to 2019: evaluation reports. Public Health England; 2020.
50. Public Health England. Herpes zoster (shingles) immunisation programme 2019 to 2020: evaluation reports. 2020 10 January 2020.
51. World Health Organization. Listings of WHO's response to COVID-19 2020 [Available from: https://www.who.int/news/item/29-06-2020-covidtimeline.
52. World Health Organization. WHO Coronavirus (COVID-19) Dashboard: World Health Organization; 2021 [updated 20 October 2021. Available from: https://covid19.who.int/.
53. GOV.UK. Coronavirus (COVID-19) in the UK 2021 [Available from: https://coronavirus.data.gov.uk/.
54. Office for National Statistics. Coronavirus and the social impacts on behaviours during different lockdown periods, Great Britain up to February 2021 2021 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandwellbeing/articles/coronavirusandthesocialimpactsonbehavioursduringdifferentlockdownperiodsgreatbritain/uptofebruary2021#optimism-about-when-life-will-return-to-normal.
55. Mansfield KE, Mathur R, Tazare J, Henderson AD, Mulick AR, Carreira H, et al. Indirect acute effects of the COVID-19 pandemic on

physical and mental health in the UK: a population-based study. The Lancet Digital health. 2021;3(4):e217-e30.
56. Meyerowitz EA, Richterman A, Gandhi RT, Sax PE. Transmission of SARS-CoV-2: A Review of Viral, Host, and Environmental Factors. Ann Intern Med. 2020;174(1):69-79.
57. Xin H, Wong JY, Murphy C, Yeung A, Taslim Ali S, Wu P, et al. The Incubation Period Distribution of Coronavirus Disease 2019: A Systematic Review and Meta-analysis. Clin Infect Dis. 2021.
58. Oran DP, Topol EJ. The Proportion of SARS-CoV-2 Infections That Are Asymptomatic. Ann Intern Med. 2021;0(0).
59. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020.
60. Beeching NJ, Fletcher TE, Fowler R. Coronavirus disease 2019 (COVID-19): BMJ Best Practice; 2021 [updated 14 Oct 2021. Available from: https://bestpractice.bmj.com/topics/en-gb/3000201.
61. NICE. COVID-19 rapid guideline: managing the long-term effects of COVID-19 2020. Available from: https://www.nice.org.uk/guidance/ng188.
62. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020;584(7821):430-6.
63. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021(3).
64. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020;324(8):782-93.
65. Mallett S, Allen AJ, Graziadio S, Taylor SA, Sakai NS, Green K, et al. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. BMC Med. 2020;18(1):346.
66. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020;323(18):1843-4.
67. Higgins TS, Wu AW, Ting JY. SARS-CoV-2 Nasopharyngeal Swab Testing—False-Negative Results From a Pervasive Anatomical Misconception. JAMA Otolaryngology–Head & Neck Surgery. 2020;146(11):993-4.
68. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine. 2020;384(5):403-16.

69. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine. 2020;383(27):2603-15.
70. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. The Lancet. 2021;397(10269):99-111.
71. Pouwels KB, Pritchard E, Matthews PC, Stoesser N, Eyre DW, Vihta K-D, et al. Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. Nat Med. 2021.
72. Gandhi RT, Lynch JB, Del Rio C. Mild or Moderate Covid-19. New England Journal of Medicine. 2020;383(18):1757-66.
73. Mahase E. Covid-19: Molnupiravir reduces risk of hospital admission or death by 50% in patients at risk, MSD reports. BMJ. 2021;375:n2422.
74. First oral antiviral for COVID-19, Lagevrio (molnupiravir), approved by MHRA [press release]. 4 November 2021 2021.
75. The RECOVERY Collaborative Group. Dexamethasone in Hospitalized Patients with Covid-19. New England Journal of Medicine. 2020;384(8):693-704.
76. NICE. COVID-19 rapid guideline: managing COVID-19 2021. Available from: https://www.nice.org.uk/guidance/ng191.
77. Lee S, Lee JE, Lee SO, Sim YK, Lee SH. Influence of Vitamin D Deficiency on the Development of Opportunistic Infection in People Living with HIV/AIDS (PWHAs). J Am Coll Nutr. 2021;40(6):545-50.
78. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. Am J Epidemiol. 2017;186(9):1026-34.
79. Allen NE, Sudlow C, Peakman T, Collins R, Biobank UK. UK biobank data: come and get it. Sci Transl Med. 2014;6(224):224ed4.
80. Department for Communities and Local Government. The English Indices of Deprivation 2010 Department for Communities and Local Government; 2011. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/6871/1871208.pdf.
81. Office for National Statistics. Socioeconomic inequalities in avoidable mortality in England: 2019 2021 [updated 11 March 2021. Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/socioeconomicinequalitiesinavoidablemortalityinenglandd/2019.
82. UK Biobank. Sources and cleaning of IMD scores 2018 [Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=6810.
83. UK Biobank. Verbal Interview stage 2012 [1.1:[Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=100235.

84. UK Biobank. Non-cancer illness code, self-reported 2020 [updated 30 Oct 2020. Available from: https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20002.
85. UK Biobank. UK Biobank Touch Screen Questionnaire 2011 [Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=100247.
86. UK Biobank. UK Biobank Biomarker Panel 2015 [updated 12 Aug 2015. 1.0:[Available from: https://www.ukbiobank.ac.uk/media/oiudpjqa/bcm023_ukb_biomarker_panel_website_v1-0-aug-2015-edit-2018.pdf.
87. Elliott P, Peakman TC, Biobank UK. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol. 2008;37(2):234-44.
88. Herrett E, Gallagher AM, Bhaskaran K, Forbes H, Mathur R, van Staa T, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). Int J Epidemiol. 2015;44(3):827-36.
89. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.
90. UK Biobank. Hospital inpatient data 2020 [updated August 2020. 3.0:[Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=138483.
91. UK Biobank. Data-Field 41259 : Records in HES inpatient main dataset 2021 [updated 25 Jul 2021. Available from: https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=41259.
92. UK Biobank. Timelines for data availability 2021 [updated July 2021. Available from: https://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=timelines_all.
93. UK Biobank. Primary Care Linked Data 2019 [Available from: https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=591.
94. UK Biobank. Integrating electronic health records into the UK Biobank Resource. 2014.
95. UK Biobank. Summary de-identification protocol 2021 [updated 6 July 2021. 2:[Available from: https://www.ukbiobank.ac.uk/media/5bvp0vqw/de-identification-protocol.pdf.
96. UK Biobank. Data providers and dates of data availability 2021 [updated Sep 6 2021. Available from: https://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=Data_providers_and_dates.
97. GOV.UK. Coronavirus (COVID-19): notice under regulation 3(4) of the Health Service (Control of Patient Information) Regulations 2002 – general 2021 [updated 10 September 2021. Available from: https://www.gov.uk/government/publications/coronavirus-covid-19-

notification-of-data-controllers-to-share-information/coronavirus-covid-19-notice-under-regulation-34-of-the-health-service-control-of-patient-information-regulations-2002-general--2#contents.
98. Armstrong J, Rudkin JK, Allen N, Crook DW, Wilson DJ, Wyllie DH, et al. Dynamic linkage of COVID-19 test results between Public Health England's Second Generation Surveillance System and UK Biobank. Microbial Genomics. 2020;6(7).
99. Public Health England. PHE data series on deaths in people with COVID-19: technical summary - 12 August update. 2020 12 August 2020.
100. UK Biobank. COVID-19 test results data 2021 [Available from: https://biobank.ctsu.ox.ac.uk/crystal/exinfo.cgi?src=COVID19_tests.
101. UK Biobank. Data for COVID-19 research 2021 [Available from: https://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=COVID19.
102. UK Biobank. Primary Care (GP) Data for COVID-19 Research 2021 [updated April 2021. 3.0:[Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=3151.
103. UK Biobank. Mortality data: linkage to death registries 2020 [updated June 2020. Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=115559.
104. Jackson C, Best N, Elliott P. UK Biobank Pilot Study: stability of haematological and clinical chemistry analytes. Int J Epidemiol. 2008;37 Suppl 1:i16-22.
105. UK Biobank. Biomarker assay quality procedures: approaches used to minimise systematic and random errors (and the wider epidemiological implications) 2019 [updated 02 April 2019. 1.2:[Available from: https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/biomarker_issues.pdf.
106. UK Biobank. Companion document for serum biomarker data. 2019 11/03/2019.
107. Collins R. What makes UK Biobank special? The Lancet. 2012;379(9822):1173-4.
108. Farmer R, Mathur R, Bhaskaran K, Eastwood SV, Chaturvedi N, Smeeth L. Promises and pitfalls of electronic health record analysis. Diabetologia. 2018;61(6):1241-8.
109. Keyes KM, Westreich D. UK Biobank, big data, and the consequences of non-representativeness. Lancet. 2019;393(10178):1297.
110. UK Biobank. Access matter: representativeness of the UK Biobank resource 2017 [Available from: http://www.ukbiobank.ac.uk/wp-content/uploads/2017/03/access-matters-representativeness-1.pdf.
111. Batty GD, Gale CR, Kivimäki M, Deary IJ, Bell S. Comparison of risk factor associations in UK Biobank against representative, general population based studies with conventional response rates: prospective cohort study and individual participant meta-analysis. BMJ. 2020;368:m131.
112. UK Biobank. Repeat Assessment: Participant Characteristics of responders vs. non-responders 2014 [1.1:[Available from:

https://biobank.ndph.ox.ac.uk/~bbdatan/repeat_assessment_characteristics_v1.pdf.
113. Roy AS, Matson M, Herlekar R. Response to 'Vitamin D concentrations and COVID-19 infection in UK Biobank'. Diabetes Metab Syndr. 2020;14(5):777-.
114. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr. 2020;14(4):561-5.
115. McDonald HI, Shaw C, Thomas SL, Mansfield KE, Tomlinson LA, Nitsch D. Methodological challenges when carrying out research on CKD and AKI using routine electronic health records. Kidney Int. 2016;90(5):943-9.
116. Wilkinson T, Schnier C, Bush K, Rannikmäe K, Henshall DE, Lerpiniere C, et al. Identifying dementia outcomes in UK Biobank: a validation study of primary care, hospital admissions and mortality data. Eur J Epidemiol. 2019;34(6):557-65.
117. Lin L-Y, Smeeth L, Langan S, Warren-Gash C. Distribution of vitamin D status in the UK: a cross-sectional analysis of UK Biobank. BMJ Open. 2021;11(1):e038503.
118. Aspell N, Laird E, Healy M, Shannon T, Lawlor B, O'Sullivan M. The Prevalence and Determinants of Vitamin D Status in Community-Dwelling Older Adults: Results from the English Longitudinal Study of Ageing (ELSA). Nutrients. 2019;11(6):1253.
119. Lin L-Y, Mulick A, Mathur R, Smeeth L, Langan S, Warren-Gash C. The Association between Vitamin D and Incident Herpes Zoster: A UK Biobank Study. In: Medicine LSoHT, editor. 2021.
120. Brisson M, Edmunds WJ, Law B, Gay NJ, Walld R, Brownell M, et al. Epidemiology of varicella zoster virus infection in Canada and the United Kingdom. Epidemiol Infect. 2001;127(2):305-14.
121. Forbes HJ, Bhaskaran K, Grint D, Hu VH, Langan SM, McDonald HI, et al. Incidence of acute complications of herpes zoster among immunocompetent adults in England: a matched cohort study using routine health data*. Br J Dermatol. 2021;184(6):1077-84.
122. Amirthalingam G, Andrews N, Keel P, Mullett D, Correa A, de Lusignan S, et al. Evaluation of the effect of the herpes zoster vaccination programme 3 years after its introduction in England: a population-based study. The Lancet Public Health. 2017;2667:1-9.
123. Public Health England. Shingles: guidance and vaccination programme 2013 [updated 13 March 2020. Available from: https://www.gov.uk/government/collections/shingles-vaccination-programme.
124. Lin L-Y, Bhate K, Forbes H, Smeeth L, Warren-Gash C, Langan SM. Vitamin D Deficiency or Supplementation and the Risk of Human Herpesvirus Infections or Reactivation: A Systematic Review and Meta-analysis. Open Forum Infect Dis. 2020;8(1).

125. Chao CT, Lai CF, Huang JW. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. Eur J Intern Med. 2012;23(8):711-5.
126. UK Biobank. Primary Care Linked Data 2019 [cited 2021 10 August]. Available from: https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=591.
127. UK Biobank. Hospital inpatient data 2020 [Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=138483.
128. UK Biobank. Health-related outcomes data 2021 [updated April 1st 2021. Available from: https://www.ukbiobank.ac.uk/enable-your-research/about-our-data/health-related-outcomes-data.
129. Hughes RA, Heron J, Sterne JAC, Tilling K. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. Int J Epidemiol. 2019;48(4):1294-304.
130. Meng JE, Hovey KM, Wactawski-Wende J, Andrews CA, LaMonte MJ, Horst RL, et al. Intraindividual Variation in Plasma 25-Hydroxyvitamin D Measures 5 Years Apart among Postmenopausal Women. Cancer Epidemiology Biomarkers & Prevention. 2012;21(6):916.
131. Batram M, Witte J, Schwarz M, Hain J, Ultsch B, Steinmann M, et al. Burden of Herpes Zoster in Adult Patients with Underlying Conditions: Analysis of German Claims Data, 2007-2018. Dermatol Ther (Heidelb). 2021;11(3):1009-26.
132. Public Health England. Chronic kidney disease (CKD) prevalence model: Public Health England,; 2014 [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/612303/ChronickidneydiseaseCKDprevalencemodelbriefing.pdf.
133. Wan M, Patel A, Patel JP, Rait G, Jones SA, Shroff R. Quality and use of unlicensed vitamin D preparations in primary care in England: Retrospective review of national prescription data and laboratory analysis. Br J Clin Pharmacol. 2021;87(3):1338-46.
134. Lin L-Y, Mulick A, Mathur R, Smeeth L, Warren-Gash C, Langan S. The Association between Vitamin D Status and COVID-19 in England: a Cohort Study using UK Biobank. 2021.
135. GOV.UK. Daily summary: Coronavirus in the UK 2021 [updated 25 March 2021. Available from: https://coronavirus.data.gov.uk/.
136. Iacobucci G. How is the pandemic affecting non-covid services? BMJ. 2021;372:n215.
137. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004;173(5):2909-12.
138. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, Hormone, and Immunomodulator. Nutrients. 2018;10(11).

139. Wang MX, Koh J, Pang J. Association between micronutrient deficiency and acute respiratory infections in healthy adults: a systematic review of observational studies. Nutr J. 2019;18(1):80-.
140. Martineau AR, Forouhi NG. Vitamin D for COVID-19: a case to answer? The Lancet Diabetes & Endocrinology. 2020;8(9):735-6.
141. UK Biobank. Biomarker assay quality procedures: approaches used to minimise systematic and random errors. 2019.
142. NHS Business Services Authority. BNF SNOMED mapping 2021 [Available from: https://www.nhsbsa.nhs.uk/prescription-data/understanding-our-data/bnf-snomed-mapping.
143. National Institute for Health and Care Excellence (NICE). BMI: preventing ill health and premature death in black, Asian and other minority ethnic groups: National Institute for Health and Clinical Excellence; 2013 3 July 2013. 13-4 p.
144. Public Health England. Guidance on shielding and protecting people who are clinically extremely vulnerable from COVID-19 2020 [Available from: https://www.gov.uk/government/publications/guidance-on-shielding-and-protecting-extremely-vulnerable-persons-from-covid-19/guidance-on-shielding-and-protecting-extremely-vulnerable-persons-from-covid-19#who-is-clinically-extremely-vulnerable.
145. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
146. Amin HA, Drenos F. No evidence that vitamin D is able to prevent or affect the severity of COVID-19 in individuals with European ancestry: a Mendelian randomisation study of open data. BMJ Nutrition, Prevention & Health. 2021:bmjnph-2020-000151.
147. Hastie CE, Pell JP, Sattar N. Vitamin D and COVID-19 infection and mortality in UK Biobank. Eur J Nutr. 2021;60(1):545-8.
148. Raisi-Estabragh Z, McCracken C, Bethell MS, Cooper J, Cooper C, Caulfield MJ, et al. Greater risk of severe COVID-19 in Black, Asian and Minority Ethnic populations is not explained by cardiometabolic, socioeconomic or behavioural factors, or by 25(OH)-vitamin D status: study of 1326 cases from the UK Biobank. Journal of Public Health. 2020;42(3):451-60.
149. Elliott J, Bodinier B, Whitaker M, Delpierre C, Vermeulen R, Tzoulaki I, et al. COVID-19 mortality in the UK Biobank cohort: revisiting and evaluating risk factors. Eur J Epidemiol. 2021:1-11.
150. Public Health England. New guidance for households with possible COVID-19 infection 2020 [Available from: https://www.gov.uk/government/news/new-guidance-for-households-with-possible-covid-19-infection.
151. Infante M, Buoso A, Pieri M, Lupisella S, Nuccetelli M, Bernardini S, et al. Low Vitamin D Status at Admission as a Risk Factor for Poor Survival in

Hospitalized Patients With COVID-19: An Italian Retrospective Study. J Am Coll Nutr. 2021:1-16.
152. Angelidi AM, Belanger MJ, Lorinsky MK, Karamanis D, Chamorro-Pareja N, Ognibene J, et al. Vitamin D Status Is Associated With In-Hospital Mortality and Mechanical Ventilation: A Cohort of COVID-19 Hospitalized Patients. Mayo Clin Proc. 2021.
153. Cereda E, Bogliolo L, Lobascio F, Barichella M, Zecchinelli AL, Pezzoli G, et al. Vitamin D supplementation and outcomes in coronavirus disease 2019 (COVID-19) patients from the outbreak area of Lombardy, Italy. Nutrition. 2021;82:111055.
154. Gavioli EM, Miyashita H, Hassaneen O, Siau E. An Evaluation of Serum 25-Hydroxy Vitamin D Levels in Patients with COVID-19 in New York City. J Am Coll Nutr. 2021:1-6.
155. Lohia P, Nguyen P, Patel N, Kapur S. Exploring the link between vitamin D and clinical outcomes in COVID-19. Am J Physiol Endocrinol Metab. 2021;320(3):E520-e6.
156. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. JAMA Network Open. 2020;3(9):e2019722-e.
157. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, et al. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. The FEBS Journal. 2020;287(17):3693-702.
158. Shah K, Saxena D, Mavalankar D. Vitamin D supplementation, COVID-19 & Disease Severity: A meta-analysis. QJM. 2021.
159. Li S, Cao Z, Yang H, Zhang Y, Xu F, Wang Y. Metabolic Healthy Obesity, Vitamin D Status, and Risk of COVID-19. Aging Dis. 2021;12(1):61-71.
160. Li X, van Geffen J, van Weele M, Zhang X, He Y, Meng X, et al. An observational and Mendelian randomisation study on vitamin D and COVID-19 risk in UK Biobank. Sci Rep. 2021;11(1):18262-.
161. Ma H, Zhou T, Heianza Y, Qi L. Habitual use of vitamin D supplements and risk of coronavirus disease 2019 (COVID-19) infection: a prospective study in UK Biobank. The American Journal of Clinical Nutrition. 2021;113(5):1275-81.
162. Bugbank News. Identifying inpatients: comparison to Hospital Episode Statistics 2020 [updated 19 August, 2020 Available from: https://news.bugbank.uk/2020/08/identifying-inpatients-comparison-to.html.
163. Darling AL, Blackbourn DJ, Ahmadi KR, Lanham-New SA. Vitamin D supplement use and associated demographic, dietary and lifestyle factors in 8024 South Asians aged 40–69 years: analysis of the UK Biobank cohort. Public Health Nutr. 2018;21(14):2678-88.

164. Liu X, Baylin A, Levy PD. Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. Br J Nutr. 2018;119(8):928-36.
165. Santos A, Amaral TF, Guerra RS, Sousa AS, Álvares L, Moreira P, et al. Vitamin D status and associated factors among Portuguese older adults: results from the Nutrition UP 65 cross-sectional study. BMJ Open. 2017;7(6):e016123.
166. Chen J-Y, Lin Y-T, Wang L-K, Hung K-C, Lan K-M, Ho C-H, et al. Hypovitaminosis D in Postherpetic Neuralgia-High Prevalence and Inverse Association with Pain: A Retrospective Study. Nutrients. 2019;11(11):2787.
167. Ban TH, Kim JH, Jang HB, Lee YS, Choi BS, Park CW, et al. Clinical effects of pre-transplant serum 25-hydroxyvitamin D level on post-transplant immunologic and non-immunologic outcomes in kidney transplant recipients. Transplant Immunology. 2017;40:51-6.
168. Chao CT, Lai CF, Huang JW. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. Eur J Intern Med. 2012;23(8):711-5.
169. Hernández JL, Nan D, Fernandez-Ayala M, García-Unzueta M, Hernández-Hernández MA, López-Hoyos M, et al. Vitamin D Status in Hospitalized Patients with SARS-CoV-2 Infection. J Clin Endocrinol Metab. 2021;106(3):e1343-e53.
170. Kaufman HW, Niles JK, Kroll MH, Bi C, Holick MF. SARS-CoV-2 positivity rates associated with circulating 25-hydroxyvitamin D levels. PLoS One. 2020;15(9):e0239252.
171. Li Y, Tong CH, Bare LA, Devlin JJ. Assessment of the Association of Vitamin D Level With SARS-CoV-2 Seropositivity Among Working-Age Adults. JAMA Netw Open. 2021;4(5):e2111634.
172. Wang Z, Joshi A, Leopold K, Jackson S, Christensen S, Nayfeh T, et al. Association of vitamin D deficiency with COVID-19 infection severity: Systematic review and meta-analysis. Clin Endocrinol (Oxf). 2021.
173. Annweiler G, Corvaisier M, Gautier J, Dubée V, Legrand E, Sacco G, et al. Vitamin D Supplementation Associated to Better Survival in Hospitalized Frail Elderly COVID-19 Patients: The GERIA-COVID Quasi-Experimental Study. Nutrients. 2020;12(11).
174. Murai IH, Fernandes AL, Sales LP, Pinto AJ, Goessler KF, Duran CSC, et al. Effect of a Single High Dose of Vitamin D3 on Hospital Length of Stay in Patients With Moderate to Severe COVID-19: A Randomized Clinical Trial. JAMA. 2021;325(11):1053-60.
175. Munafò MR, Tilling K, Taylor AE, Evans DM, Davey Smith G. Collider scope: when selection bias can substantially influence observed associations. Int J Epidemiol. 2017;47(1):226-35.
176. Holick MF. Chapter 4 - Photobiology of Vitamin D. In: Feldman D, editor. Vitamin D (Fourth Edition): Academic Press; 2018. p. 49.

177. Klompas M, Kulldorff M, Vilk Y, Bialek SR, Harpaz R. Herpes zoster and postherpetic neuralgia surveillance using structured electronic data. Mayo Clin Proc. 2011;86(12):1146-53.
178. UK Health Security Agency. Shingles (herpes zoster): the green book, chapter 28a 2013. Available from: https://www.gov.uk/government/publications/shingles-herpes-zoster-the-green-book-chapter-28a.
179. US Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices votes unanimously to recommend Shingrix for immunocompromised adults aged 19 and up [press release]. 20 October 2021 2021.
180. NHS. Free vitamin D supplements for people at high risk from coronavirus (COVID-19) 2020 [updated 25 November 2021. Available from: https://www.nhs.uk/conditions/coronavirus-covid-19/people-at-higher-risk/free-vitamin-d-supplements-for-people-at-high-risk/.
181. Porta MS. A dictionary of epidemiology. ed, editor: Oxford: Oxford University Press; 2014. 255 p.
182. NICE. COVID-19 rapid guideline: vitamin D [NG187]. London: National Institute for Health and Care Excellence (NICE); 2020. Available from: https://www.nice.org.uk/guidance/ng187.
183. The Scientific Advisory Committee on Nutrition. SACN rapid review: Vitamin D and acute respiratory tract infections. 2021 17 December 2020.
184. Public Health England. National Diet and Nutrition Survey Rolling programme Years 9 to 11 (2016/2017 to 2018/2019) 2020 December 2020.
185. Wan M, Horsfall LJ, Basatemur E, Patel JP, Shroff R, Rait G. Vitamin D prescribing in children in UK primary care practices: a population-based cohort study. BMJ Open. 2019;9(12):e031870.
186. Rieder FJJ, Gröschel C, Kastner MT, Kosulin K, Laengle J, Zadnikar R, et al. Human cytomegalovirus infection downregulates vitamin-D receptor in mammalian cells. J Steroid Biochem Mol Biol. 2017;165(Pt B):356-62.
187. Robak O, Kastner MT, Stecher C, Schneider M, Andreas M, Greinix H, et al. Cytomegalovirus Infection Downregulates Vitamin D Receptor in Patients Undergoing Hematopoietic Stem Cell Transplantation. Transplantation. 2021;105(7):1595-602.
188. Jolliffe DA, Holt H, Greenig M, Talaei M, Perdek N, Pfeffer P, et al. Vitamin D Supplements for Prevention of Covid-19 or other Acute Respiratory Infections: a Phase 3 Randomized Controlled Trial (CORONAVIT). medRxiv. 2022:2022.03.22.22271707.
189. Lin L-Y, Warren-Gash C, Smeeth L, Chen P-C. Data Profile: The National Health Insurance Research Database (NHIRD) of Taiwan. Epidemiology and Health. 2018;0(0):e2018062-0.

Appendix

Appendix 1. Chapter 3: PROSPERO Registration

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

Vitamin D and human herpesvirus infections

Liang-yu Lin, Charlotte Warren-Gash, Sinéad Langan, Ketaki Bhate, Harriet Forbes, Liam Smeeth

Citation

Liang-yu Lin, Charlotte Warren-Gash, Sinéad Langan, Ketaki Bhate, Harriet Forbes, Liam Smeeth. Vitamin D and human herpesvirus infections. PROSPERO 2019 CRD42019130153 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42019130153

Review question

Is serum vitamin D deficiency/insufficiency associated with increased risk of infection or reactivation of human herpesviruses?

Does oral vitamin D supplementation protect against infection or reactivation of human herpesviruses?

Searches

MEDLINE, EMBASE, Web of Science, the Cochrane Library, Global Health, IndMED, OpenGrey, the Prevention Information & Evidence eLibrary, dissertations and theses, and EThOS will be searched for relevant literature.

The references of any relevant reviews will also be screened to ensure the completeness of our literature search.

Additional search strategy information is available in the attached PDF document (link provided below).

Search strategy

https://www.crd.york.ac.uk/PROSPEROFILES/130153_STRATEGY_20190327.pdf

Types of study to be included

We will include observational studies, including case-control studies, cohort studies as well as randomized or non-randomized controlled trials.

Ecological studies, cross-sectional studies, case-report, case-series or literature review will not be included (we will, however, screen the references included in any reviews to ensure the completeness of our literature search).

Studies reporting only serum antibodies against herpesviruses will also be excluded.

Condition or domain being studied

Vitamin D; human herpesviruses.

Participants/population

Human studies on immunocompetent and immunocompromised adults or children.

Intervention(s), exposure(s)

Exposure 1: deficient or insufficient serum vitamin D levels.

Exposure 2: oral vitamin D supplementation or oral vitamin D analogue treatment.

Comparator(s)/control

Comparator 1: sufficient serum vitamin D levels.

Comparator 2: no oral vitamin D supplementation, or oral vitamin D analogue treatment.

Context

Main outcome(s)
Infection with, or reactivation of, the eight human herpesviruses: herpes simplex virus types 1 and 2, varicella zoster virus, Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, human herpesvirus 7, and human herpesvirus 8, defined either clinically e.g. presenting with classical symptoms such as herpes zoster, or using laboratory criteria e.g. PCR detection of virus.

Studies reporting only serum antibodies against herpesviruses will be excluded.

Additional outcome(s)
None.

Data extraction (selection and coding)
Two researchers will independently screen the titles and the abstracts of all identified studies.

We will obtain the full-texts of all studies fulfilling reviewing criteria, and the two authors will further decide their eligibilities.

Any discrepancies in the reviewing process will be assessed by a third researcher who will make an overall judgement on the study in question.

Data will then be extracted from the studies selected for inclusion. Data extraction for the first three studies will be done by two independent researchers to ensure the integrity of the process, and then one researcher will extract data from the remaining studies.
We will summarize and extract data from the included studies using the Population, Exposure, Comparator, Outcomes, and Study characteristics (PECOS) framework:
Population: sample sizes of study population, inclusion or exclusion criteria of participants, and demographic characteristics such as sex, age, or immune status.

Exposure 1: people with insufficient serum vitamin D levels.

Exposure 2: people receive vitamin D supplementation or a vitamin D analogue.

Comparator 1: people with sufficient serum vitamin D levels.

Comparator 2: people without vitamin D supplementation or who have not used vitamin D analogue.

Outcomes: clinically diagnosed herpesvirus infection, laboratory-confirmed herpes viral infection.

Study characteristics: publication details (authors, publication year, and journal); study design.

Risk of bias (quality) assessment
We will assess the risk of bias in included studies.

The assessment will be based on the Cochrane approach to trials and observational studies and the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework.
To ensure the quality and consistency of risk of bias assessment, the first three studies will be evaluated by two researchers. One researcher will then complete the evaluation of the remaining included studies. Any discrepancies will be further examined by the third researcher.

Strategy for data synthesis
We will use a narrative synthesis to summarize the data and results from the included studies, by the criteria of exposures and outcomes.

If there are adequate numbers and acceptable homogeneity of results, a meta-analysis will be performed to integrate the studying results. The choice of a fixed or random effects model will be guided by the level of statistical heterogeneity (assessed using the I^2 statistic).

Analysis of subgroups or subsets
If the number of included studies are sufficient, we will analyse the risk of herpesvirus infection in subjects with different immune statuses.

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01 April 2019

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01 April 2020

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KB is supported by a National Institute of Health Research (NIHR) Doctoral Research Fellowship (DRF-2018-11-ST2-066)

Conflicts of interest
None known

Language
English

Country
England

Stage of review
Review_Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Administration, Oral; Adult; Child; Cholestanes; Dietary Supplements; Herpesviridae Infections; Humans; Primary Prevention; Risk Factors; Vitamin D; Vitamin D Deficiency

Date of registration in PROSPERO
27 March 2019

Date of publication of this version
27 March 2019

Details of any existing review of the same topic by the same authors

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Versions
27 March 2019

Appendix 2. Chapter 4: UK Biobank project application

Project title (200 characters):

Association between serum vitamin D deficiency and the risk of herpes zoster: a longitudinal UK Biobank study

A2. Research question(s) and aim(s) (up to 5000 characters or 200 words):

Research Questions:

1. What is the distribution of serum vitamin D levels among the UK Biobank participants?
2. Does vitamin D deficiency increase the risk of herpes zoster?
3. Is there any valid proxy for vitamin D deficiency in electronic health records, e.g. diseases such as osteomalacia, which could be used in future epidemiological studies?

Aim:

- To explore the association between serum vitamin D levels and the risk of herpes zoster
- To determine whether there are valid disease proxies for vitamin D deficiency in the UK population.

A3. The background and scientific rationale of the proposed research project in general (up to 5000 characters or 300 words):

Underlying diseases, stress, nutrition status, or medical treatments can affect the human immune system. Reduced immune system function due to immunosenescence or immunosuppression may increase the risk of infections or reactivation of viruses, such as varicella-zoster viruses (VZV). VZV is a double-stranded DNA virus. Infection with VZV will result in chickenpox among young patients, and its reactivation from latency may lead to herpes zoster (HZ) in adults. HZ is characterized by a rash consisting of painful erythematous vesicles typically occurring in a dermatomal distribution. Furthermore, some patients may develop post-herpetic neuralgia (PHN), leading to severe neuropathic pain. These symptoms of HZ have been shown to significantly reduce the quality of life of patients, push up the costs from absenteeism, and increase the financial burden of health care (Scott et al., 2006). The most important risk factor for HZ is age, while severe immunosuppression is also a strong risk factor (Forbes et al., 2014). Recently, some studies indicated that vitamin D, an essential element in bone formation, may have potential immunomodulatory effects. In response to the exposure to pathogens, immune cells such as monocytes or macrophages would upregulate the vitamin D receptors and enzymes and synthesize anti-pathogenic peptides (Holick, 2007). However, in previous epidemiological studies, there is no consistent effect of vitamin D supplementation on protecting against viral infections. Some studies reported that vitamin D supplementation would prevent acute respiratory tract infections (Martineau et al., 2017), while other studies showed no protective effect (Aglipay et al., 2017). Furthermore, there is limited evidence showing the possible association between vitamin D and VZV infection and

reactivation. In addition, vitamin D is not routinely measured and recorded in regular medical practice, so using electronic records to study the health outcomes associated with vitamin D deficiency is challenging. Consequently, by using UK Biobank data, we aim to investigate the effect of vitamin D status on the risk of HZ and explore whether proxies for vitamin D deficiency can be identified in linked data from primary care records.

A4. A brief description of the method(s) to be used (up to 5000 characters or 300 words):

We will include all participants from the UK Biobank as our study population. The first part of our study is to describe the distribution of serum vitamin D levels in the UK Biobank population, analysing vitamin D levels against age, sex, geographical area and different seasons. Our second part is to investigate the association between the serum vitamin D levels and the risk of developing herpes zoster. Vitamin D deficiency will be defined as serum 25(OH) D levels less than 25 nmol/L, which is recommended by Public Health England. The outcome will be the diagnosis of herpes zoster from linked primary care (GP) data, available for half of the UK Biobank participants. In addition, a routine herpes zoster vaccination program was initiated in 2013 in the UK, so we will include vaccination status in our analysis. Covariates such as age, sex and immunosuppression and vaccination status will be assessed using Poisson regression models. The last part of our study aims to find proxies for vitamin D deficiency using UK Biobank data. We will identify some vitamin D deficiency-associated diseases from the literature, develop a code list from the primary care

records, and evaluate their association with vitamin D deficiency in the UK Biobank database.

A5. The type and size of dataset required (e.g., case-control subset, men only, imaging data only, whole cohort, etc.) (up to 5000 characters or 100 words):

To analyse the distribution of vitamin D deficiency, we will need to use data from the 449,978 participants who had their serum vitamin D levels measured. In addition, the baseline characteristics, including age, sex, ethnicity, home location, vitamin D assay date, and vitamin D supplementation, are also required. Participants with linked primary care records and self-reported medical conditions, about 250,000 people, will then be included in the analysis of vitamin D and herpes zoster.

A6. The expected value of the research (taking into account the public interest requirement) (up to 5000 characters or 100 words):

Our study will describe the distribution of vitamin D levels in an older UK population that may inform nutritional and public health guidance. In addition, we will explore whether there is an association between vitamin D levels and herpes zoster. This will improve understanding of a novel, potentially modifiable risk factor for herpes zoster and, depending on results, may inform the development of further intervention studies. Furthermore, identifying a valid proxy for vitamin D deficiency in electronic health records will inform the design of future epidemiological studies.

A7. Please provide up to 6 keywords which best summarise your proposed research project:

Vitamin D, Herpes zoster, electronic health records, UK Biobank

A8. Please provide a lay summary of your research project in plain English, stating the aims, scientific rationale, rationale, project duration and public health impact (up to 5000 characters or 400 words):

When our immune system does not work well, we are more vulnerable to getting infections, such as chickenpox and shingles. This virus that causes chickenpox causes lifelong infections, and it cannot be removed. When the virus that causes chickenpox reactivates in adults, shingles develops. A common symptom of shingles is a painful skin rash. Some shingles patients may suffer from long-term nerve pain, which will significantly decrease their quality of life. The treatment for pain symptoms is not very effective, and it increases health spending. Therefore, it is important to study what cause shingles, and to find new ways to prevent it.

Vitamin D is produced by the skin after sun exposure, and it is regarded to be an essential element to bone health. Public Health England advises taking vitamin D supplements every day. Recent studies suggest vitamin D has some effect on immunity, and it might help to prevent viral infections. However, we do not know whether vitamin D levels affect the chance of getting shingles. Furthermore, vitamin D levels are not routinely measured and recorded in patients' GP records. It is very difficult to study vitamin D by using GP records unless we can find another way of findings low vitamin D levels. We aim to 1. To describe how many people in the UK are deficient in vitamin D; 2. To investigate whether vitamin D deficiency increases the risk

of shingles; 3. To find ways of identifying vitamin D deficiency in GP records using UK Biobank data linked to GP records.

Understanding the proportion of people with vitamin D deficiency in the UK population will help the public health department to develop guidance about vitamin supplementation. If we find that vitamin D deficiencies increase the risk of shingles, this will inform future research into shingles prevention. Furthermore, our work will also help other researchers to use electronic medical records to study vitamin D.

A10. What is the estimated duration of your project, in months? If you consider (because for example (because for example the project is one involving the generation of hypotheses) that it would be difficult to set a fixed end point, we are prepared to consider a rolling 3-year period (during which annual updates are required):

18 months

B. Selection of data-fields

Use the **data showcase**

Reference:

AGLIPAY, M., BIRKEN, C. S., PARKIN, P. C., LOEB, M. B., THORPE, K., CHEN, Y., LAUPACIS, A., MAMDANI, M., MACARTHUR, C., HOCH, J. S., MAZZULLI, T., MAGUIRE, J. L. & COLLABORATION, T. A. K. 2017. Effect of High-Dose vs Standard-Dose Wintertime Vitamin D Supplementation on Viral Upper Respiratory Tract Infections in Young Healthy Children. *JAMA,* 318**,** 245-254.

FORBES, H. J., BHASKARAN, K., THOMAS, S. L., SMEETH, L., CLAYTON, T. & LANGAN, S. M. 2014. Quantification of risk factors for herpes zoster: population based case-control study. *Bmj,* 2911**,** g2911.

HOLICK, M. F. 2007. Vitamin D deficiency. *N Engl J Med,* 357**,** 266-81.

MARTINEAU, A. R., JOLLIFFE, D. A., HOOPER, R. L., GREENBERG, L., ALOIA, J. F., BERGMAN, P., DUBNOV-RAZ, G., ESPOSITO, S.,

GANMAA, D., GINDE, A. A., GOODALL, E. C., GRANT, C. C., GRIFFITHS, C. J., JANSSENS, W., LAAKSI, I., MANASEKI-HOLLAND, S., MAUGER, D., MURDOCH, D. R., NEALE, R., REES, J. R., SIMPSON, S., JR., STELMACH, I., KUMAR, G. T., URASHIMA, M. & CAMARGO, C. A., JR. 2017. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ,* 356**,** i6583.

SCOTT, F. T., JOHNSON, R. W., LEEDHAM-GREEN, M., DAVIES, E., EDMUNDS, W. J. & BREUER, J. 2006. The burden of Herpes Zoster: a prospective population based study. *Vaccine,* 24**,** 1308-14.

Appendix 3. Chapter 4: Study Protocol for LSHTM Ethical approval

Study title: Association between serum vitamin D deficiency and the risk of herpes zoster: a longitudinal UK Biobank study

Principal investigator

- Liang-Yu Lin, Faculty of Epidemiology & Population Health (EPH), London School of Hygiene & Tropical Medicine (LSHTM)

Co-investigators

- Sinéad Langan, EPH, LSHTM
- Charlotte Warren-Gash, EPH, LSHTM
- Liam Smeeth, EPH, LSHTM
- Rohini Mathur, EPH, LSHTM
- Amy Mulick, EPH, LSHTM

Funding

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Background

Herpes zoster (HZ), commonly called shingles, is caused by reactivation of clinically latent varicella-zoster virus (VZV). VZV is a member of a herpesviridae, a family of double-stranded DNA viruses widely prevalent in nature (1). Infection with VZV will usually result in chickenpox, usually among

young children but less commonly in adolescents and adults, which is characterised as pruritic vesicular and papular rashes all over the body that will become crusted within days (2). After recovering from varicella, instead of being eradicated, VZV will lead to lifelong latent infection. This virus will remain in cranial nerves or dorsal root ganglia, and its reactivation from latency may lead to HZ, which is most commonly seen in adults. HZ is characterised by a rash consisting of painful erythematous vesicles. The rashes usually progress to pustules before forming scabs, and typically occur in a unilateral dermatomal distribution. HZ is usually self-limited, and the rash resolves after 10-20 days (3). However, some patients may experience persisting neuropathic pain called post-herpetic neuralgia (PHN), which is traditionally defined as any persisting pain at least 90 days after the rash appearance (4). In some patients with trigeminal nerve involvement, VZV may cause zoster ophthalmicus, which may result in blindness without proper treatment (5).

Among the risk factors for HZ, the most important one is age, especially for those older than 50 years. This is due to immunosenescence associated with decreased T-cell immunity against VZV. Another main risk factor for HZ is severe immunocompromised status, which results from underlying diseases or immunosuppressive treatments. Furthermore, some diseases, such as HIV infection, lymphoma, leukaemia, myeloma, rheumatoid arthritis, inflammatory bowel disease, chronic obstructive pulmonary disease, asthma, chronic kidney disease, depression and diabetes, are found to be associated with higher HZ risk; some medical treatments, such as chemotherapy or

immunosuppressant use after organ transplantation, also increase the risk of HZ (6, 7).

Among the general population, HZ is relatively common. Through retrospective analysis of electronic health records (EHRs), the incidence of HZ is between 2 to 5 per 1000 person-years in many countries (4). In the UK, in 2009, the annual average incidence of HZ was 5.23 per 1000 people. HZ incidence increased as ageing, and female had a higher incidence than male (8). In addition, HZ and PHN increase financial burdens and social impact, especially for people older than aged 65 years (9). Consequently, investigating immunomodulatory factors associated with infection or reactivation of this viral disease is important.

Vitamin D is mainly endogenously synthesized by the skin after sun exposure and can be supplied through dietary intake and supplementation. It plays an important role in absorbing calcium and phosphate, which are essential for bone health (10). Recently, some studies have indicated that vitamin D may have potential immunomodulatory effects associated with the regulation of antimicrobial peptides (AMPs) (11). In previous cell studies, vitamin D induced gene expression of an AMP named Cathelicidin. In response to pathogen exposure, immune cells such as monocytes or macrophages, upregulate vitamin D receptors and enzymes to increase the production of Cathelicidin (12-14). A cell study further showed that vitamin D supplementation can reduce herpes simplex 1 viral load, which is the same virus family of VZV (15).

Vitamin D also shows some anti-infective potential in epidemiological studies. A meta-analysis using original patient data from 25 randomized controlled trials showed that among the general population, vitamin D supplementation reduced the risk of acute respiratory infections (16). Furthermore, there is some evidence to suggest an anti-infective effect of vitamin D in specific patient groups, such as patients with chronic kidney disease (CKD). A case-control study indicated that the risk of herpes zoster was significantly lower in those who received vitamin D supplementation (17). Nevertheless, there is little evidence about vitamin D deficiency and the risk of herpes zoster, especially among general population. In addition, because vitamin D is not routinely measured and recorded in regular medical practice, it is very difficult to study vitamin D deficiency using EHRs unless there are some reliable proxies for vitamin D deficiency. Consequently, by using UK Biobank data, we aim to investigate the effect of vitamin D status on the risk of HZ and explore whether proxies for vitamin D deficiency can be identified.

The rapid global spread of the COVID-19 pandemic has led to an unprecedented burden on health, healthcare systems and economies. In the absence of a vaccine or any specific proven treatments, identifying factors that modulate the risk of severe COVID-19 is critical to understanding the epidemiology of this novel coronavirus and informing global prevention strategies. A previous meta-analysis of 25 randomized trials showed that vitamin D supplementation can prevent acute respiratory infections (16). While untested in COVID-19, vitamin D supplementation is a simple low-cost

public health intervention which already shows benefits for preventing other respiratory infections among populations vulnerable to vitamin D deficiency. Therefore, we aimed to investigate the effects of vitamin D deficiency and vitamin D supplementation on the incidence of severe COVID-19 infection and complications in a large UK cohort.

Aims

1. To explore the association between vitamin D deficiency and the risk of herpes zoster and identify valid proxies for vitamin D deficiency that can be used in further studies.
2. To investigate the effects of vitamin D deficiency and vitamin D supplementation on the incidence of severe COVID-19 infection and complications.

Study operational plan and rationale

We plan to use UK Biobank data to analyse the association between serum vitamin D levels and HZ and COVID-19 infection and determine whether there are valid surrogate exposures for vitamin D deficiency. UK Biobank is a longitudinal cohort containing information about serum vitamin D levels and vitamin D supplementation, and this database will be linked to primary care and a databased of inpatient admissions. Therefore, we can explore the association between vitamin D and the risk of herpes zoster and COVID-19.

Specific objectives

Objective 1: To explore the association between serum vitamin D levels and the risk of herpes zoster using the UK Biobank.

Objective 2: To determine whether there are valid disease proxies for vitamin D deficiency using UK Biobank data.

Objective 3: To explore the association between serum vitamin D levels or vitamin D supplementation and the risk of COVID-19 infection using the UK Biobank.

Objective 4: To explore the association between serum vitamin D levels or vitamin D supplementation and severe outcomes of COVID-19.

Data

UK Biobank is a prospective cohort aimed to investigate a wide range of risk factors for major diseases in middle and old age. This national cohort was compiled from 2006 to 2010, recruiting people throughout England, Wales, and Scotland. The participants were aged 40-69 years and lived within 40 km of one of the 22 UK Biobank assessment centres. Over 9,200,000 individuals who were registered with the NHS were invited to join the cohort, and 500,000 volunteers were finally recruited (18). These participants received physical examinations, questionnaires, image studies, and blood, urine, and saliva samples for assays. Biochemical assays of blood samples were analysed for various biomarkers, including serum vitamin D levels, which were measured for disease diagnosis or characterisation (19). In addition, the participants' data can be linked to medical records in various electronic health records databases, such as primary care database, hospitalisation records, cancer or death registries (20).

By using a prospective cohort, researchers can investigate the possible temporality between risk factors and outcomes. UK Biobank measured numerous exposures in detail and provides researchers with the opportunity to study the effects of many exposures on a range of outcomes. Furthermore, the linkage of the UK Biobank database and clinical databases can further extend the range of disease outcomes (21). However, some people argue that UK Biobank is not generalizable (external validity). The response rate of this cohort is only approximately 5.45%. Comparing to nonparticipating invitees, the UK Biobank has more female, older participants, and more people living in less socioeconomically deprived areas. Furthermore, if we compare these participants to other nationally representative surveys, the UK Biobank participants are less obese, are less likely to be smokers, and they have fewer self-reported health issues and cancer incidence rates (18). Although UK Biobank may not provide a valid estimation of the prevalence or incidence of diseases in the UK population, nevertheless, this large database is still useful in assessing the association between exposure and diseases (22).

In response to COVID-19, Public Health England have provided COVID-19 tests results of the UK Biobank participants resident in England, and these data have been updated and released on a weekly basis. In addition to test results, primary care data, hospital inpatient data, death data, and critical care data will be released by the UK Biobank (23).

Data management

The UK Biobank data will always be stored on the encrypted LSHTM secure server. The data will only be accessed on LSHTM password-protected networks. Datasets received from the UK Biobank will be deleted within 12 months after the completion date, according to the agreement terms and conditions of the UK Biobank.

Statistical and mathematical analysis

To analyse the distribution of vitamin D deficiency, we will need to use data from the 449,978 participants who had their serum vitamin D levels measured. These participants are between 40 and 69 years old, and about half of the participants, approximately 225,000 people, have their data linked to primary care records.

There will be four parts in our study. The first part will describe the distribution of serum vitamin D levels in the UK Biobank population. There are 448,376 participants received serum vitamin D levels measurement, and 17,039 participants received a repeated assessment. Vitamin D deficiency will be defined as serum 25(OH) D levels less than 25 nmol/L, which is recommended by Public Health England. We will compare the distribution of age, sex, geographical area, and different seasons among vitamin D status using a Chi-square test.

The second part will investigate the association between serum vitamin D levels and the risk of developing HZ. The outcome will be the diagnosis of HZ from the linked primary care (GP) data, available for half of the UK

Biobank participants, about 225,000 people. Using parameters obtained from previous studies and the preliminary data from the UK Biobank, the power will over 70% when the detectable risk ratio is 1.3 (Table 1). In addition, a routine HZ vaccination program was initiated in 2013 in the UK, so we will include vaccination status in our analysis. Covariates such as age, sex and immunosuppression and vaccination status will be assessed using Poisson regression models.

The third part of the study will explore the association between vitamin D and the risk of COVID-19 infection or severe outcomes related to COVID-19. The first exposure is vitamin D deficiency, which will be defined as serum 25(OH) D levels less than 25 nmol/L, and the other exposure will be vitamin D supplementation, which was from previously answered questionnaires. The first outcome will be COVID-19 testing results, and the second outcomes will be severe health consequences of COVID-19, including mortality rate and ICU admission. These clinical data will be provided by Public Health England and released by the UK Biobank. Because the risk of infecting with COVID-19 increased drastically after January 2020, we will assess the hazards of COVID-19 infection using Cox proportional hazard models to adjust for covariates age, sex, immunosuppression, and underlying comorbidities. We will also carry out post hoc sensitivity analysis to test the proportionality assumption and effect modification.

The last part of our study aims to find proxies for vitamin D deficiency using UK Biobank data. We will identify vitamin D deficiency-associated diseases from the literature, develop a code list from the primary care records. These

diseases will be identified from linked primary care records prior to the measurement of vitamin D between 2007 to 2010. We will evaluate the association between these proxies and vitamin D deficiency by applying logistic regression, and further evaluate the validity of these proxies with positive predictive value (PPV), sensitivity and specificity (24).

Table 1 Power Estimation using the UK Biobank data showcase

Incidence of herpes zoster		0.52%[2]	0.8%[3]
Number of vitamin D deficiency		N=22,400[4]	
Minimum risk ratio detectable	1.1	0.15	0.23
	1.2	0.47	0.7
	1.3	0.82	0.96
	1.5	1.00	1.00

1.Given alpha=0.05, number of vitamin D sufficiency=210600. Results from OpenEpi, Version 3, open source calculator—Power Cohort; 2. estimated from Gauthier, A., et al. (2009); 3. estimated from Matthews I, et al. (2018); 3. estimated using the UK Biobank data showcase.

Expected outcomes

Our project expects to find:

1. The distribution of vitamin D deficiency in the UK Biobank by different age, sex, geographical area, and different seasons.
2. The association of vitamin D deficiency and the risk of developing herpes zoster among UK Biobank participants.
3. Identifying proxies for vitamin D deficiency that can be used in electronic health record studies.

4. The association between vitamin D deficiency or vitamin D supplementation and the risk of COVID-19 infection or severe outcomes.

Reporting plan

We will submit our results to open access journals to follow the Open Access Publishing Policy of LSHTM. This project will also be published on the UK Biobank website.

Duration

The duration of the project will be 18 months (finishing in 2021).

Problems anticipated

The UK Biobank population is healthier and older than the general population. Therefore, we must be cautious when interpreting the generalizability of the association between the herpes virus and vitamin D deficiency found in our study. Another limitation is the selection of vitamin D deficiency surrogate exposures. The specificity of these exposure proxies may be relatively high, but the sensitivity may be low, leading to misclassification. Finally, it would be possible that there is no useful proxy for vitamin D deficiency.

Because of the testing policy of the UK government, only participants with severe COVID-19 symptoms were able to receive laboratory tests. Therefore, our outcome will be limited to severe COVID-19 infections and milder or asymptomatic cases will be misclassified. In addition, most

participants had their serum vitamin D levels measured between 2006 to 2010. These old data may not reflect their real vitamin D status in 2020, which may introduce non-differential misclassification in exposures. Considering this issue, we will use repeated measured serum vitamin D levels among 15,000 participants to investigate the stability of serum vitamin D levels over time.

Ethical issues

UK Biobank already has its Research Tissue Bank (RTB) approval from its Research Ethics Committee (REC). This approval covers most usage of the database. Although additional ethical approval is not required, my project will be reviewed and approved by the UK Biobank coordinating centre.

For COVID-19 data, an approved UK Biobank project will be automatically authorised to conduct COVID-19 related research after registering to access COVID-19 data (25).

Role of team members

Liang-Yu Lin will lead the study design, data analysis and reporting. Sinéad, Charlotte, Liam, Rohini and Amy will provide support with study design, analysis, and interpretation.

References

1. Knipe DM, Howley P. Fields Virology: Wolters Kluwer Health; 2013.
2. Heininger U, Seward JF. Varicella. Lancet. 2006;368(9544):1365-76.
3. Robert Johnson. Herpes Zoster Vaccination: A Vaccine to Prevent Pain. In: Jean-Pierre Michel, Stefania Maggi, editors. Adult Vaccinations: Springer International Publishing; 2019. p. 91-5.

4. Kenneth E. Schmader, Robert H. Dworkin. The Epidemiology and Natural History of Herpes Zoster and Postherpetic Neuralgia. 2017. In: Herpes Zoster: Postherpetic Neuralgia and Other Complications [Internet]. ADIS. 1. [34].
5. Whitley RJ. Varicella-Zoster Virus Infections. In: J. Larry Jameson, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, Joseph Loscalzo, editors. Harrison's Principles of Internal Medicine. 20e ed: McGraw-Hill Education; 2018. p. 1355.
6. Cohen JI. Herpes Zoster. New England Journal of Medicine. 2013;369(3):255-63.
7. Forbes HJ, Bhaskaran K, Thomas SL, Smeeth L, Clayton T, Langan SM. Quantification of risk factors for herpes zoster: population based case-control study. Bmj. 2014;2911:g2911.
8. Gauthier A, Breuer J, Carrington D, Martin M, Remy V. Epidemiology and cost of herpes zoster and post-herpetic neuralgia in the United Kingdom. Epidemiology and infection. 2009;137(1):38-47.
9. Scott FT, Johnson RW, Leedham-Green M, Davies E, Edmunds WJ, Breuer J. The burden of Herpes Zoster: a prospective population based study. Vaccine. 2006;24(9):1308-14.
10. Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.
11. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389-95.
12. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. Faseb J. 2005;19(9):1067-77.
13. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004;173(5):2909-12.
14. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsjo A, Torma H, Stahle M. Vitamin D induces the antimicrobial protein hCAP18 in human skin. The Journal of investigative dermatology. 2005;124(5):1080-2.
15. Kumar A, Singh MP, Kumar RS, Ratho RK. 25-Hydroxyvitamin D3 and 1,25 Dihydroxyvitamin D3 as an Antiviral and Immunomodulator Against Herpes Simplex Virus-1 Infection in HeLa Cells. Viral Immunol. 2018;31(8):589-93.
16. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. Bmj. 2017;356:i6583.
17. Chao CT, Lai CF, Huang JW. Risk factors for herpes zoster reactivation in maintenance hemodialysis patients. Eur. 2012;23(8):711-5.
18. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. American journal of epidemiology. 2017;186(9):1026-34.
19. UK Biobank. UK Biobank Biomarker Panel. 2015.

20. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.
21. Collins R. What makes UK Biobank special? The Lancet. 2012;379(9822):1173-4.
22. UK Biobank. Access matter: representativeness of the UK Biobank resource 2017 [Available from: http://www.ukbiobank.ac.uk/wp-content/uploads/2017/03/access-matters-representativeness-1.pdf.
23. UK Biobank makes health data available to tackle COVID-19 2020 [updated Apr 14, 2020. Available from: https://www.ukbiobank.ac.uk/2020/04/covid/.
24. Newman TB, Browner WS, Hulley SB, Cummings SR. Designing Studies of Medical Tests. Designing Clinical Research. 4th ed: Lippincott Williams & Wilkins; 2013. p. 177.
25. UK Biobank. UK Biobank - COVID-19 Data Release FAQs 2020 [Available from: https://www.ukbiobank.ac.uk/wp-content/uploads/2020/05/ACCESS_066V1.1.A.COVID-19-FAQs_v1.1_DRAFT.docxAMENDEDBH.pdf.

Additional information for protocol amendment :

Summary of the objectives, methods and other main features of the original study.

***Please ensure that you do not exceed a maximum of 300 words.**

The deterioration of the human immune system may increase the risk of viral infections, such as Varicella-zoster virus. The reactivation of this virus in adults may lead to herpes zoster (HZ), which is also known as shingles. A common symptom of HZ is a painful skin rash, and some patients may suffer from long-term nerve pain. Vitamin D is essential to our bone formation. Recently, some studies have indicated that vitamin D may have immunomodulatory effects. Studies reported that vitamin D deficiency is associated with an increased risk of viral infections. However, there is limited evidence showing the possible association between vitamin D and HZ. In addition, vitamin D levels are not routinely measured and recorded in patients' GP records. It is very difficult to study vitamin D by using electronic health records.

Our project aims to investigate the effect of vitamin D status on the risk of HZ, and to explore whether proxies for vitamin D deficiency can be identified by using the UK Biobank, a large national cohort of 500,000 participants aged 40 to 69. The first part of our study is a cross-sectional study describing the distribution of vitamin D deficiency in the UK Biobank. We analysed serum vitamin D levels by demographic characteristics, geographical areas, and different seasons. Our second study is to investigate the association

between the serum vitamin D levels and the risk of developing HZ. The outcome of HZ diagnosis will be obtained from linked primary care (GP) data, available for half of the UK Biobank participants. The last part of our study is to find proxies for vitamin D deficiency. We will identify some vitamin D deficiency-associated diseases from the literature and evaluate their association with vitamin D deficiency by using the linked GP data.

(Word count: 293 words)

Summary of the specific amendment/extension requested.
(Sufficient detail must be given to allow the Committee to make an informed decision. Please list the pages on which changes to the main protocol have occurred due to the proposed amendment. Please also ensure, where relevant, details on changes to taking/storage of human tissue are provided)

In this amendment we propose to undertake an additional study using our existing UK Biobank dataset to investigate the effect of vitamin D status on an alternative outcome: COVID-19.

The rapid global spread of the COVID-19 pandemic has led to an unprecedented burden on health, healthcare systems and economies. In the absence of a vaccine or any specific proven treatments, identifying factors that modulate the risk of severe COVID-19 is critical to understanding the epidemiology of this novel coronavirus and informing global prevention strategies. Previous studies showed that vitamin D supplementation can prevent acute respiratory infections.

While untested in COVID-19, vitamin D supplementation is a simple low-cost public health intervention which already shows benefits for preventing other respiratory infections among populations vulnerable to vitamin D deficiency. We therefore aimed to investigate the effects of vitamin D deficiency and vitamin D supplementation on the incidence of severe COVID-19 infection and complications using the UK Biobank cohort.

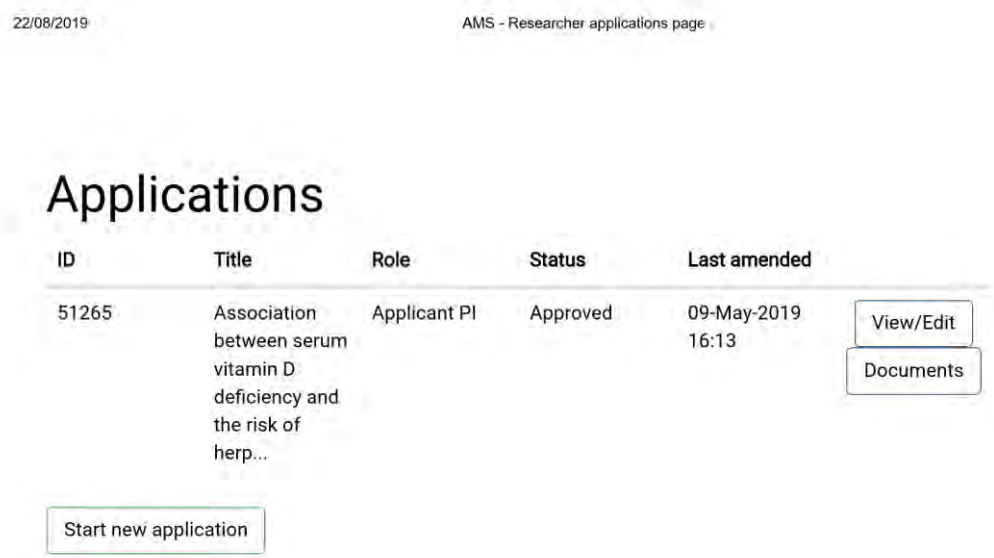
In response to COVID-19 outbreak, Public Health England has provided COVID-19 tests results of, and these data have been updated and released weekly. Therefore, the exposure of our project will be vitamin D deficiency and vitamin D supplementation, and the outcomes will be incident COVID-19 infection among the UK Biobank participants resident in England. We will further adjust for potential confounders such as demographic factors, socioeconomic status, and severe immunosuppression.

The Detailed change to the main protocol was listed as follows:

- Page 1: include two new co-investigators, Rohini Mathur and Amy Mulick
- Page 4:
    - Background: add background on vitamin D and COVID-19
    - Aims: add the second aims to investigate the effects of vitamin D deficiency and supplementation on the incidence of severe COVID-19
    - Update details of study operational plan
- Page 5: add two new study objectives
- Page 6: explain the process of COVID-19 data released by the UK Biobank
- Page 7: explain the statistical analysis plan of COVID-19 data
- Page 9:
    - Outcome: add the expected outcomes of COVID-19 study
    - Possible problems anticipated: add potential issues of COVID-19 project
- Page 10: explain the authorization received from the UK Biobank for conducting COVID-19 research

Appendix 4. Chapter 4: Ethical approval documents of UK Biobank

Appendix 4.1. Original UK Biobank approval message and Material transferring agreement



Annex II: Material Transfer Agreement with the Applicant for data and/or samples

Dear Mr Lin,

UK Biobank is pleased to approve your Application Reference Number 51265 to use the UK Biobank Resource. Execution of this Material Transfer Agreement (MTA) and payment of the Access Charges are the final steps before access is granted. UK Biobank's approval of this Application is valid for 90 days, after which the Applicant Principal Investigator (PI) will need to re-apply for access. The content of UK Biobank's standard MTA, and the conditions contained within it, are non-negotiable.

Parties

This is an agreement between UK Biobank Limited on the one hand and the Applicant Institution London School of Hygiene and Tropical Medicine on the other hand. The Applicant PI is not a party to the MTA, however, UK Biobank requires that the Applicant PI acknowledges that the provisions of this MTA have been "read and understood" by the Applicant PI so that they are fully aware of their Institution's obligations to both UK Biobank and to UK Biobank's participants.

The Applicant Institution will be responsible for the conduct of any and all of the Applicant Researchers involved in this Research Project.

Structure of agreement

The MTA will become effective on receipt by UK Biobank of:

(i) A copy of this MTA Agreement executed by an authorised signatory of the Applicant Institution and confirmed as "read and understood" by the Applicant PI; and
(ii) Cleared funds covering the Access Charges from the Applicant Institution.

UK Biobank will then promptly send a dated confirmatory email.

Provision of samples and/or data

Annex A summarises the data and/or samples that UK Biobank will make available to the Applicant in accordance with their approved Application Reference Number 51265. The timeframe and methodology by which the data and/or samples will be dispatched is also set out in Annex A.

Payment

The Access Charges which are payable are set out in Annex B. This also serves as an invoice on which VAT will be included (as appropriate). The derivation of these Access Charges is also set out in Annex B.

This payment should be submitted in cleared funds to Barclays Bank PLC, Account name: UK Biobank Limited, Account number: 33069427 and Sort code: 20-24-09.

Standard terms and schedules

This Agreement incorporates the attached terms and conditions (including any documents and/or materials that are referred to in them), the Annexes and where applicable the contents of the Preliminary and Main Application Forms with Reference Number 51265.

Yours faithfully

For and on behalf of UK Biobank/Effective Date
(Jonathan Sellors / Company Solicitor)

13/9/2019

Accepted and agreed

For and on behalf of Applicant Institution
(Please sign and print your name and position) Alex Hollander, Head of Research Contracts

Alex Hollander

Read and Understood by the Applicant Principal Investigator
(Please sign and print your name and position) Liang-Yu Lin Principal Investigator, PhD student at LSHTM

Mr Liang-Yu Lin MLin

Appendix 4.2. Approval from the Research Ethics Committee of the London School of Hygiene and Tropical Medicine

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Mr Liang-yu Lin
LSHTM

23 August 2019

Dear Liang-yu

Submission Title: Association between serum vitamin D deficiency and the risk of herpes zoster: a longitudinal UK Biobank study

LSHTM Ethics Ref: 17158

Thank you for responding to the Observational Committee Chair's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved is as follows:

Document Type	File Name	Date	Version
Consent form	UKBB_Consent_form	24/11/2006	1
Consent form	UK BIOBANK ETHICS AND GOVERNANCE FRAMEWORK	01/10/2007	3
Local Approval	Favourable-Ethical-Opinion-and-RTB-Approval-16.NW_0274-200778-May-2016	13/05/2016	1
Investigator CV	Sinead_CVDec2018	15/12/2018	1
Investigator CV	Short cv_CWG_16-07-2019	16/07/2019	1
Investigator CV	Liam Smeeth 2 page CV 2019	19/07/2019	1
Protocol / Proposal	UKB_protocol_v1.5	15/08/2019	1
Investigator CV	2019_08_LiangyuLin_CV_short	15/08/2019	1
Covering Letter	Covering letter	22/08/2019	1
Covering Letter	UK Biobank approval	22/08/2019	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study

At the end of the study, the CI or delegate must notify the committee using the End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk.

Further information is available at: www.lshtm.ac.uk/ethics.

Yours sincerely,

[redacted]

Professor Jimmy Whitworth
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

London School of Hygiene & Tropical Medicine
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United Kingdom
Switchboard: +44 (0)20 7636 8636

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Observational / Interventions Research Ethics Committee

Mr Liang-yu Lin
LSHTM

10 June 2020

Dear Mr Lin

Study Title: Association between serum vitamin D deficiency and the risk of herpes zoster: a longitudinal UK Biobank study

LSHTM Ethics Ref: 17158 - 1

Thank you for your application for the above amendment to the existing ethically approved study and submitting revised documentation The amendment application has been considered by the Observational Committee via Chair's Action.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved is as follows:

Document Type	File Name	Date	Version
Local Approval	ACCESS_066V1.1.A.COVID-19-FAQs_v1.1_DRAFT.docx	17/04/2020	V1.1
Other	UKB_COVID_protocol_amendament_tracked	01/06/2020	V2

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using the End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk.

Further information is available at: www.lshtm.ac.uk/ethics.

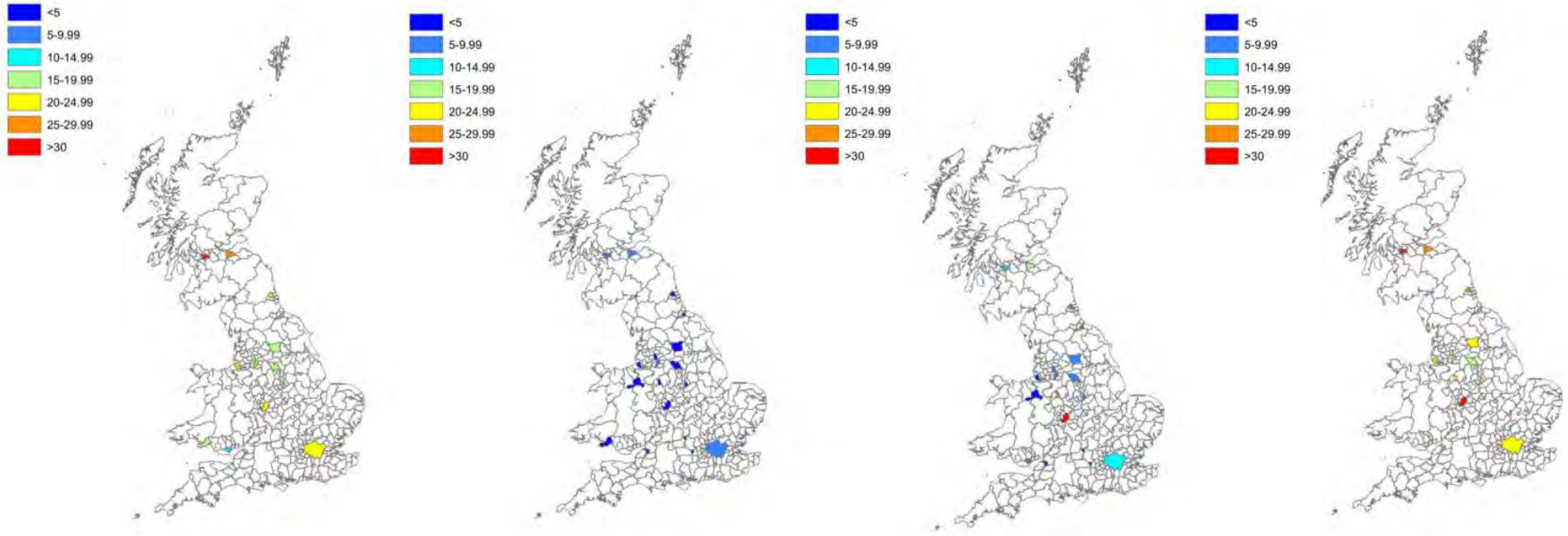
Yours sincerely,

[redacted]

Professor Jimmy Whitworth
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Appendix 5. Chapter 5: Supplementary Figure



a. Spring b. Summer c. Autumn d. Winter

Appendix 6. Chapter 6: Supplementary materials

Appendix 6.1. Box S1.

Box S1. Measurement of serum vitamin D levels

Participants' serum samples were collected at the UK Biobank assessment centres, and these samples were analysed in a central laboratory (1). An automated dispensing system had been used to process the samples, and a chemiluminescence immunoassay (DiaSorin Ltd. LIASON XL, Italy) was performed to measure the hydroxyvitamin D status. The analysis process was examined by quality control samples and an external quality assurance scheme (2, 3).

Appendix 6.2. Box S2.

Box S2. Data management of secondary exposure vitamin D prescriptions

A code list of vitamin D prescriptions was developed by including all medications listed in British National Formula (BNF) section 9.6.4, with BNF codes initiated with "0906040". This vitamin D code list was also converted into read codes version 2 and Dictionary of Medicines and Devices (DM+D) codes using existing mapping tools provided by UK Biobank (4) and NHS Business Service Authority (5). The assessment code lists used for our analysis were available on GitHub. We used these code lists to identify participants every being prescribed for vitamin D from the primary care prescription data, which were coded in BNF codes, DM+D codes and Read 2 codes (6). Participants ever received vitamin D prescription during the

assessment window were labelled as “had vitamin D prescriptions’, and the others were coded as ‘not receiving prescriptions,’ respectively.

Appendix 6.3. Box S3.

Box S3. Data management of demographic covariates

Demographic factors, except age, were coded as categorical variables. Sex was coded into male/female, and BMI was regrouped as categorical variables according to National Institute for Health and Care Excellence guidelines (7). Information about ethnicity, smoking status, drinking frequency was obtained from self-reported questionnaires. Ethnicity was categorized as white, mixed, Asian, black, Chinese, and others. Smoking statuses were grouped as "non-smoker", "ex-smoker", and "current-smoker," and drinking frequency was coded as “daily,” “weekly,” “sometimes,” and “never.” IMD scores were recoded using five quintiles, assigning the fifth quintile as the “most deprived” group. The geographical regions of the UK were used to represent the locations of 22 UK Biobank assessment centres, and the vitamin D testing seasons were defined by the testing dates.

Reference:

1. Elliott P, Peakman TC, Biobank UK. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International journal of epidemiology. 2008;37(2):234-44.
2. UK Biobank. Companion document for serum biomarker data. 2019 11/03/2019.
3. UK Biobank. Biomarker assay quality procedures: approaches used to minimise systematic and random errors. 2019.
4. UK Biobank. Clinical coding classification systems and maps 2021 [Available from: https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=592.
5. NHS Business Services Authority. BNF SNOMED mapping 2020 [Available from: https://www.nhsbsa.nhs.uk/prescription-data/understanding-our-data/bnf-snomed-mapping.

6. UK Biobank. Primary Care Linked Data 2019 [cited 2021 10 August]. Available from: https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=591.
7. National Institute for Health and Care Excellence (NICE). BMI: preventing ill health and premature death in black, Asian and other minority ethnic groups: National Institute for Health and Clinical Excellence; 2013 3 July 2013. 13-4 p.

Appendix 6.5. Table S1.

Table S1. The comparison of inclusion and exclusion participants

	Included (N=177,572)	Excluded (N=324,917)
sex		
- Number of missing	0	1
- Female	96,292 (54.2%)	177,083 (54.5%)
- Male	81,280 (45.8%)	147,833 (45.5%)
Age		
- Mean (SD)	57 (8.1)	56 (8.2)
Ethnicity		
- White	169,186 (95.3%)	303,493 (93.4%)
- Mixed	878 (0.5%)	2,080 (0.6%)
- Asian	3,304 (1.9%)	6,578 (2.0%)
- Black	1,878 (1.1%)	6,183 (1.9%)
- Chinese	402 (0.2%)	1,172 (0.4%)
- Others	1,924 (1.1%)	5,411 (1.7%)
Body mass index (BMI) group		
- Number of missing	765	2,326
- Underweight	845 (0.5%)	1,781 (0.6%)
- Healthy weight	54,333 (30.7%)	105,956 (32.8%)
- Overweight	75,141 (42.5%)	137,099 (42.5%)
- Obese	46,488 (26.3%)	77,755 (24.1%)
Drinking frequency		
- Number of missing	330	1,168
- never	14,791 (8.3%)	25,848 (8.0%)
- sometimes	40,846 (23.0%)	73,016 (22.6%)
- weekly	86,927 (49.0%)	157,796 (48.7%)
- daily	34,678 (19.6%)	67,089 (20.7%)
Drinking status		
- Number of missing	379	1,271

- Never	7,910 (4.5%)	14,475 (4.5%)
- Previous	6,832 (3.9%)	11,270 (3.5%)
- Current	162,451 (91.7%)	297,901 (92.0%)
Smoking status		
- Number of missing	813	2,132
- non-smoker	95,117 (53.8%)	178,400 (55.3%)
- ex-smoker	62,720 (35.5%)	110,330 (34.2%)
- current-smoker	18,922 (10.7%)	34,055 (10.6%)
Index of Multiple Deprivation (IMD)		
- Least deprived	4,570	8,167
- 2nd deprived	31,711 (18.3%)	66,420 (21.0%)
- 3rd deprived	33,707 (19.5%)	64,285 (20.3%)
- 4th deprived	36,033 (20.8%)	61,798 (19.5%)
- Most deprived	36,265 (21.0%)	61,624 (19.5%)
- Least deprived	35,286 (20.4%)	62,623 (19.8%)
Vitamin D and associated mineral supplement		
- Number of missing	96,695	178,460
- No vitamin D supplement	1,804 (2.2%)	3,313 (2.3%)
- With vitamin D and mineral supplement	79,073 (97.8%)	143,115 (97.7%)
Vitamin D status testing seasons		
- Number of missing	0	51,941
- Spring	52,211 (29.4%)	77,880 (28.5%)
- Summer	43,629 (24.6%)	75,470 (27.6%)
- Autumn	42,962 (24.2%)	66,736 (24.4%)
- Winter	38,770 (21.8%)	52,890 (19.4%)
Regions of UK Biobank assessment centres		
- Number of missing	0	51,941
- East Midlands	20,867 (11.8%)	9,469 (3.5%)
- London	15,519 (8.7%)	45,772 (16.8%)
- Northeast	25,650 (14.4%)	26,627 (9.8%)

- Northwest	13,078 (7.4%)	57,184 (20.9%)
- Southeast	3,365 (1.9%)	36,449 (13.4%)
- Southwest	5,516 (3.1%)	33,353 (12.2%)
- West Midlands	11,117 (6.3%)	28,911 (10.6%)
- Yorkshire and The Humber	46,645 (26.3%)	19,453 (7.1%)
- Wales	13,764 (7.8%)	5,390 (2.0%)
- Scotland	22,051 (12.4%)	10,368 (3.8%)

Appendix 6.7. Table S2**.**

Table S2. The distribution of missingness by different variables

Variable	Total number	Missing number	Missing percentage (%)
Sex	177572	0	0.0
Age	177572	0	0.0
Ethnicity	177572	0	0.0
BMI	176807	765	0.4
Drinking frequency	177242	330	0.2
Smoking status	176759	813	0.5
IMD quintile	173002	4570	2.6
Regions of UK Biobank assessment centres	177572	0	0.0
Seasons	177572	0	0.0
Asthma	177572	0	0.0
Chronic obstructive pulmonary disease (COPD)	177572	0	0.0
Chronic kidney disease (CKD)	177572	0	0.0
Depression	177572	0	0.0
Diabetes mellitus (DM)	177572	0	0.0
Inflammatory bowel diseases (IBD)	177572	0	0.0
Rheumatoid arthritis (RA)	177572	0	0.0
Systemic lupus erythematous (SLE)	177572	0	0.0
immunosuppression	177572	0	0.0
Self-reported supplementation	80,877	96,695	54.5
GP prescribed vitamin D supplements	177572	0	0.0

Appendix 6.8. Table S3.

Table S3. The distribution of demographic characteristics by vitamin D status

Variables	Total (N=177,572)	Deficiency (N=25,274)	Insufficiency (N=74,963)	Sufficiency (N=77,335)
Sex				
- Female	96,292 (54.2%)	13,772 (54.5%)	40,599 (54.2%)	41,921 (54.2%)
- Male	81,280 (45.8%)	11,502 (45.5%)	34,364 (45.8%)	35,414 (45.8%)
Age				
- Mean (SD)	56.82 (8.08)	55.08 (8.14)	56.55 (8.10)	57.66 (7.93)
Ethnicity				
- White	169,186 (95.3%)	21,892 (86.6%)	71,233 (95.0%)	76,061 (98.4%)
- Mixed	878 (0.5%)	229 (0.9%)	438 (0.6%)	211 (0.3%)
- Asian	3,304 (1.9%)	1,750 (6.9%)	1,256 (1.7%)	298 (0.4%)
- Black	1,878 (1.1%)	679 (2.7%)	938 (1.3%)	261 (0.3%)
- Chinese	402 (0.2%)	116 (0.5%)	217 (0.3%)	69 (0.1%)
- Others	1,924 (1.1%)	608 (2.4%)	881 (1.2%)	435 (0.6%)
Body mass index (BMI) group				
- Healthy weight	54,333 (30.7%)	6,348 (25.3%)	20,502 (27.5%)	27,483 (35.6%)
- Underweight	845 (0.5%)	155 (0.6%)	313 (0.4%)	377 (0.5%)
- Overweight	75,141 (42.5%)	9,424 (37.6%)	31,710 (42.5%)	34,007 (44.1%)
- Obese	46,488 (26.3%)	9,118 (36.4%)	22,116 (29.6%)	15,254 (19.8%)
Drinking frequency				
- never	14,791 (8.3%)	3,579 (14.2%)	6,363 (8.5%)	4,849 (6.3%)

- sometimes	40,846 (23.0%)	6,947 (27.6%)	18,180 (24.3%)	15,719 (20.3%)
- weekly	86,927 (49.0%)	10,200 (40.5%)	36,306 (48.5%)	40,421 (52.3%)
- daily	34,678 (19.6%)	4,447 (17.7%)	13,976 (18.7%)	16,255 (21.0%)
Drinking status				
- Never	7,910 (4.5%)	2,137 (8.5%)	3,421 (4.6%)	2,352 (3.0%)
- Previous	6,832 (3.9%)	1,419 (5.6%)	2,926 (3.9%)	2,487 (3.2%)
- Current	162,451 (91.7%)	21,594 (85.9%)	68,462 (91.5%)	72,395 (93.7%)
Smoking status				
- non-smoker	95,117 (53.8%)	13,088 (52.2%)	40,404 (54.1%)	41,625 (54.1%)
- ex-smoker	62,720 (35.5%)	7,729 (30.8%)	26,170 (35.1%)	28,821 (37.4%)
- current-smoker	18,922 (10.7%)	4,278 (17.0%)	8,081 (10.8%)	6,563 (8.5%)
Index of Multiple Deprivation				
- Least deprived	31,711 (18.3%)	3,440 (13.9%)	12,800 (17.5%)	15,471 (20.6%)
- 2nd deprived	33,707 (19.5%)	3,890 (15.8%)	14,082 (19.3%)	15,735 (20.9%)
- 3rd deprived	36,033 (20.8%)	4,589 (18.6%)	15,110 (20.7%)	16,334 (21.7%)
- 4th deprived	36,265 (21.0%)	5,582 (22.6%)	15,454 (21.1%)	15,229 (20.3%)
- Most deprived	35,286 (20.4%)	7,162 (29.0%)	15,691 (21.5%)	12,433 (16.5%)
Vitamin D and associated mineral supplement				
- No vitamin D supplement	1,804 (2.2%)	326 (5.2%)	834 (2.7%)	644 (1.5%)

- With vitamin D and mineral supplement	79,073 (97.8%)	5,974 (94.8%)	29,539 (97.3%)	43,560 (98.5%)
Received vitamin D prescription from GP				
- No vitamin D prescription	174,148 (98.1%)	25,049 (99.1%)	74,130 (98.9%)	74,969 (96.9%)
- Had vitamin D prescription	3,424 (1.9%)	225 (0.9%)	833 (1.1%)	2,366 (3.1%)
Vitamin D testing seasons				
- Summer	43,629 (24.6%)	2,090 (8.3%)	14,478 (19.3%)	27,061 (35.0%)
- Spring	52,211 (29.4%)	10,665 (42.2%)	24,995 (33.3%)	16,551 (21.4%)
- Autumn	42,962 (24.2%)	3,447 (13.6%)	16,786 (22.4%)	22,729 (29.4%)
- Winter	38,770 (21.8%)	9,072 (35.9%)	18,704 (25.0%)	10,994 (14.2%)
Regions of UK Biobank assessment centres				
- East Midlands	20,867 (11.8%)	2,142 (8.5%)	8,433 (11.2%)	10,292 (13.3%)
- London	15,519 (8.7%)	2,554 (10.1%)	6,475 (8.6%)	6,490 (8.4%)
- Northeast	25,650 (14.4%)	2,979 (11.8%)	10,071 (13.4%)	12,600 (16.3%)
- Northwest	13,078 (7.4%)	1,940 (7.7%)	5,723 (7.6%)	5,415 (7.0%)
- Southeast	3,365 (1.9%)	295 (1.2%)	1,287 (1.7%)	1,783 (2.3%)
- Southwest	5,516 (3.1%)	256 (1.0%)	1,834 (2.4%)	3,426 (4.4%)
- West Midlands	11,117 (6.3%)	2,030 (8.0%)	4,985 (6.6%)	4,102 (5.3%)
- Yorkshire and The Humber	46,645 (26.3%)	5,925 (23.4%)	19,674 (26.2%)	21,046 (27.2%)
- Wales	13,764 (7.8%)	1,997 (7.9%)	6,251 (8.3%)	5,516 (7.1%)
- Scotland	22,051 (12.4%)	5,156 (20.4%)	10,230 (13.6%)	6,665 (8.6%)
Asthma				

- No Asthma	153,815 (86.6%)	21,458 (84.9%)	64,693 (86.3%)	67,664 (87.5%)
- Asthma	23,757 (13.4%)	3,816 (15.1%)	10,270 (13.7%)	9,671 (12.5%)
CKD				
- No CKD	176,164 (99.2%)	25,017 (99.0%)	74,369 (99.2%)	76,778 (99.3%)
- CKD	1,408 (0.8%)	257 (1.0%)	594 (0.8%)	557 (0.7%)
COPD				
- No COPD	172,444 (97.1%)	24,261 (96.0%)	72,838 (97.2%)	75,345 (97.4%)
- COPD	5,128 (2.9%)	1,013 (4.0%)	2,125 (2.8%)	1,990 (2.6%)
Depression				
- No depression	157,163 (88.5%)	21,658 (85.7%)	66,171 (88.3%)	69,334 (89.7%)
- depression	20,409 (11.5%)	3,616 (14.3%)	8,792 (11.7%)	8,001 (10.3%)
DM				
- No DM	166,395 (93.7%)	22,843 (90.4%)	69,887 (93.2%)	73,665 (95.3%)
- Have DM	11,177 (6.3%)	2,431 (9.6%)	5,076 (6.8%)	3,670 (4.7%)
Inflammatory bowel diseases				
- No inflammatory bowel disease	172,794 (97.3%)	24,551 (97.1%)	72,937 (97.3%)	75,306 (97.4%)
- Inflammatory bowel disease	4,778 (2.7%)	723 (2.9%)	2,026 (2.7%)	2,029 (2.6%)
Rheumatoid arthritis				
- No RA	175,034 (98.6%)	24,841 (98.3%)	73,905 (98.6%)	76,288 (98.6%)
- RA	2,538 (1.4%)	433 (1.7%)	1,058 (1.4%)	1,047 (1.4%)
Systematic lupus erythematosus				
- No SLE	177,254 (99.8%)	25,218 (99.8%)	74,840 (99.8%)	77,196 (99.8%)

- SLE	318 (0.2%)	56 (0.2%)	123 (0.2%)	139 (0.2%)
Immunosuppression				
- Not immunosuppressive	171,903 (96.8%)	24,354 (96.4%)	72,611 (96.9%)	74,938 (96.9%)
- Immunosuppression	5,669 (3.2%)	920 (3.6%)	2,352 (3.1%)	2,397 (3.1%)
Mean follow-up year (SD)	10.1 (1.9)	10.1 (1.9)	10.1 (1.9)	10.0 (1.8)

Appendix 6.9. Figure S1.

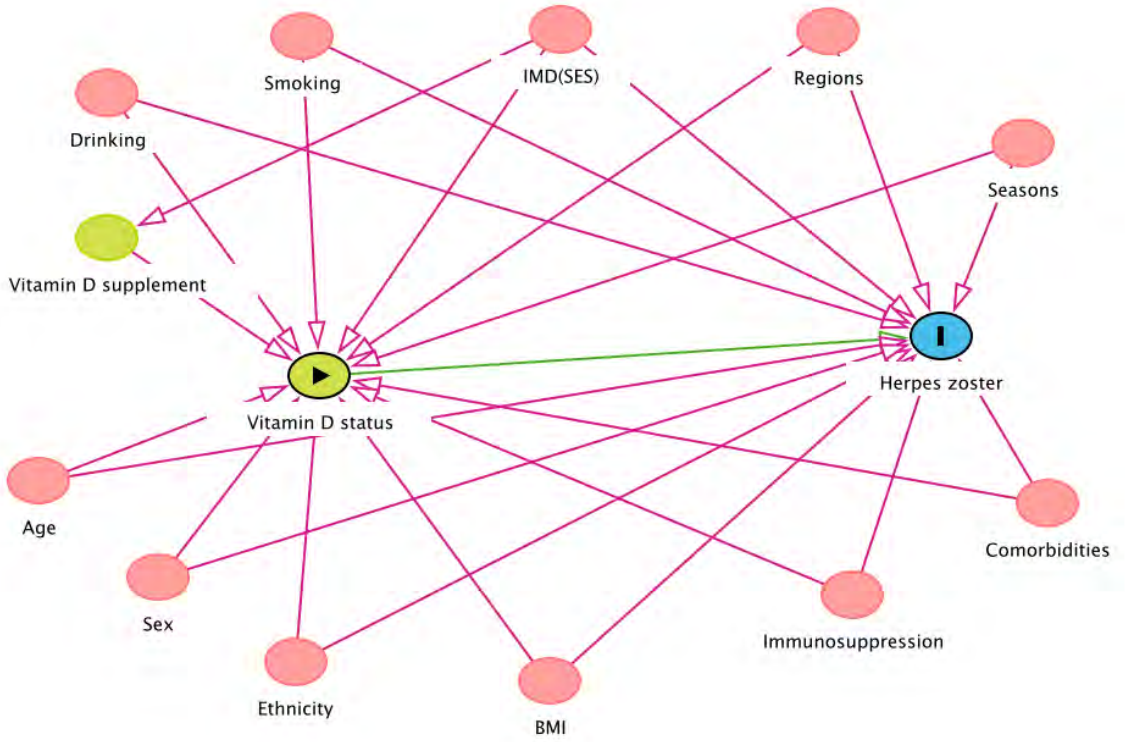


Figure S1. The directed acyclic graph for selecting potential confounders. The green circles (vitamin D status and supplement) are exposure, and the blue circle (herpes zoster) is the outcome of the study. Circles in red colour are adjustment covariate sets for estimating the total effect of the exposure on the outcome.

Appendix 6.10. Figure S2.

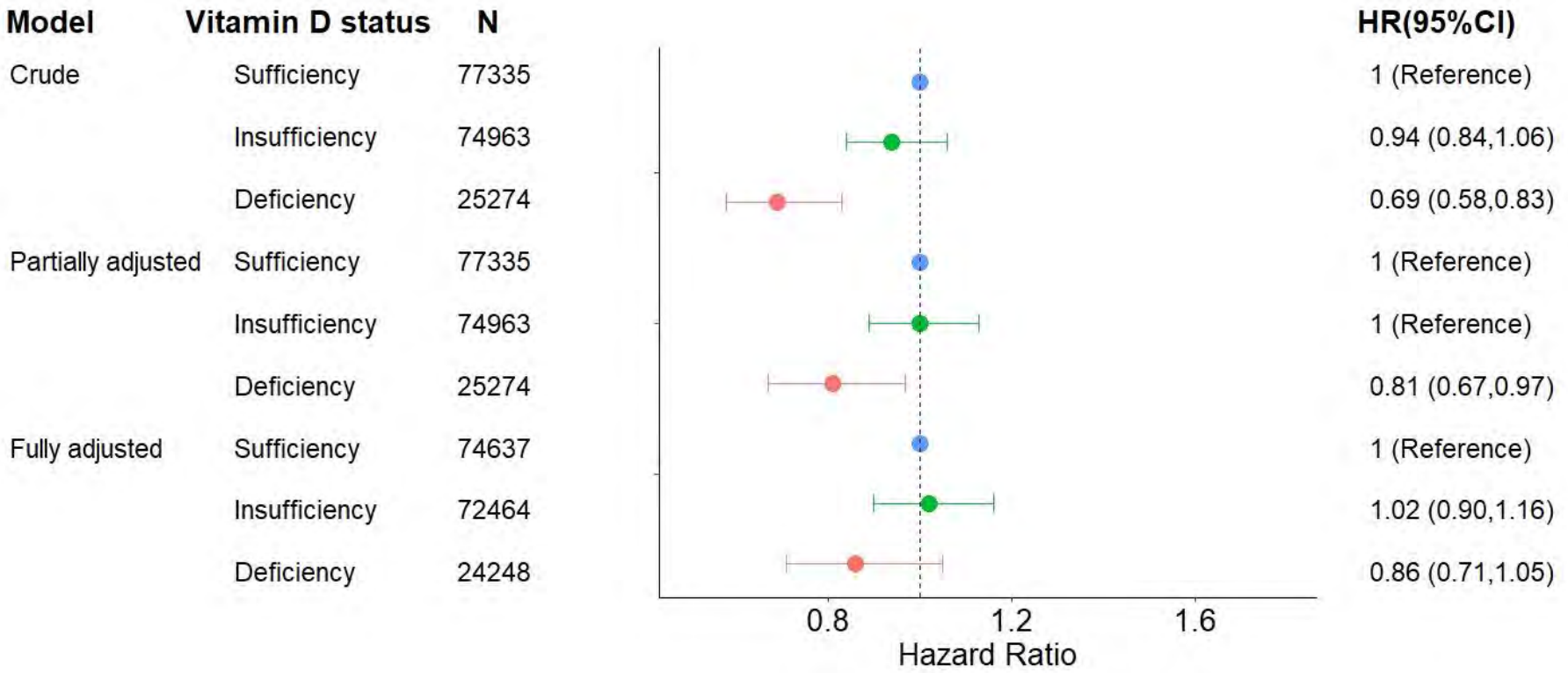


Figure S2. The association between vitamin D status and the risk of herpes zoster excluding records after September 2013. Crude: Poisson regression model without adjustment; Partially adjustment: Poisson regression model adjusted for sex and age; Fully adjusted: multivariable Poisson regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 6.11. Figure S3.

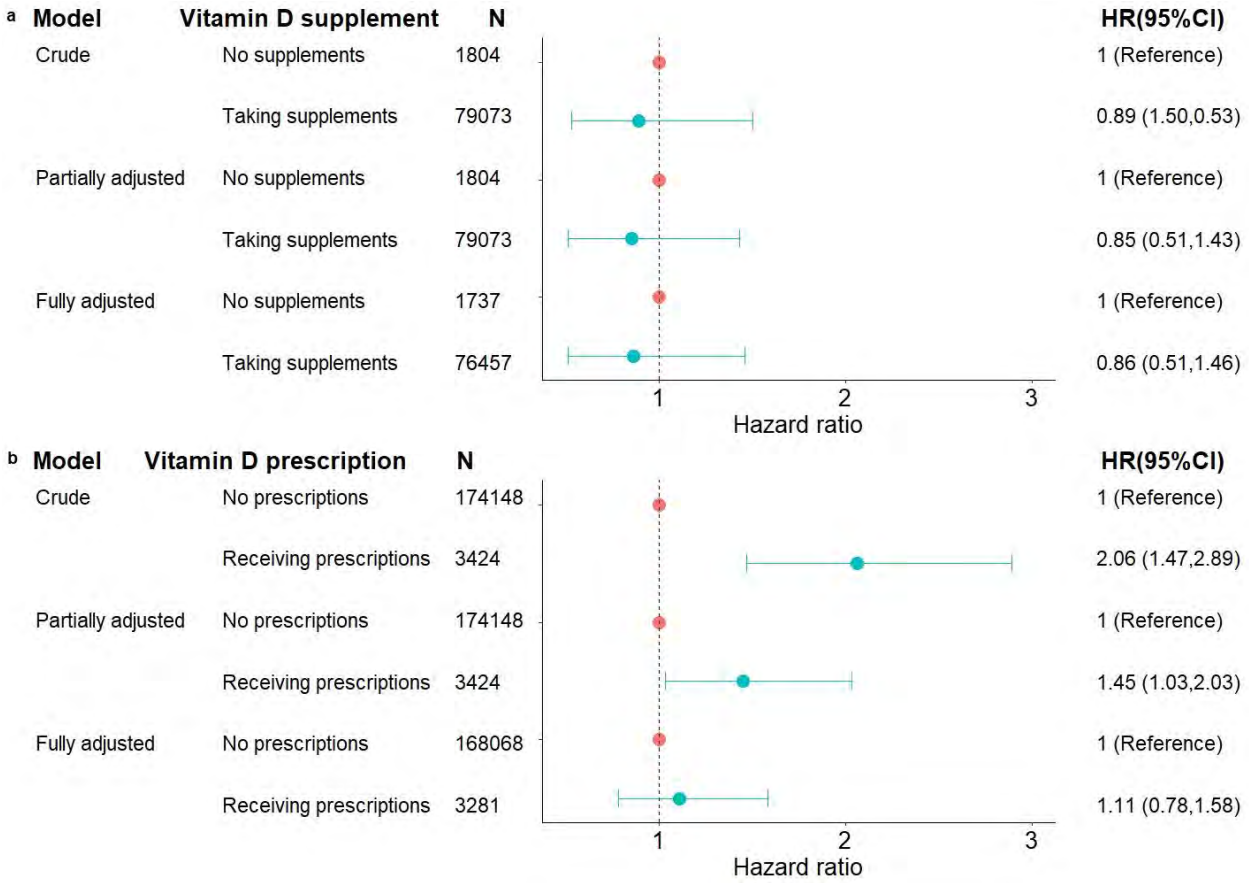


Figure S3. Vitamin D intake and the risk of herpes zoster excluding records after September 2013. Crude: Poisson regression model without adjustment; Partially adjustment: Poisson regression model adjusted for sex and age; Fully adjusted: multivariable Poisson regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 6.12. Figure S4.

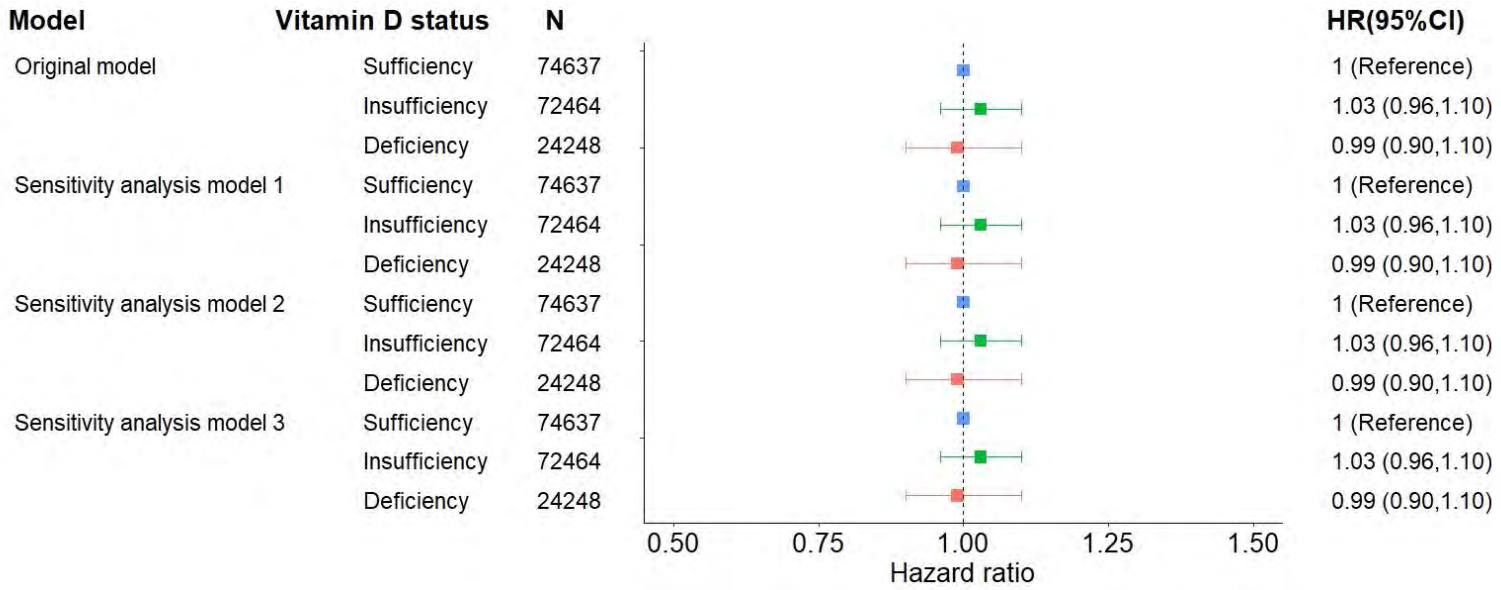


Figure S4. Sensitivity analysis of using different definitions of clinical covariates. All models were adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions. Original model: the comorbidities were defined by diagnostic records and self-reported non-cancer health conditions; Model 1: the comorbidities were only defined by diagnostic records; Model 2: the comorbidities were identified by both diagnostic codes and self-reported non-cancer health conditions. The oral steroid users among immunosuppressive conditions, we only included participants using high-dose steroids with daily dose more than 20 mg. Model 3: the comorbidities were identified without self-reported health conditions, and only people taking high dose oral steroid (> 20mg/day) were defined as steroid users among immunosuppressive conditions.

Appendix 6.13. Figure S5.

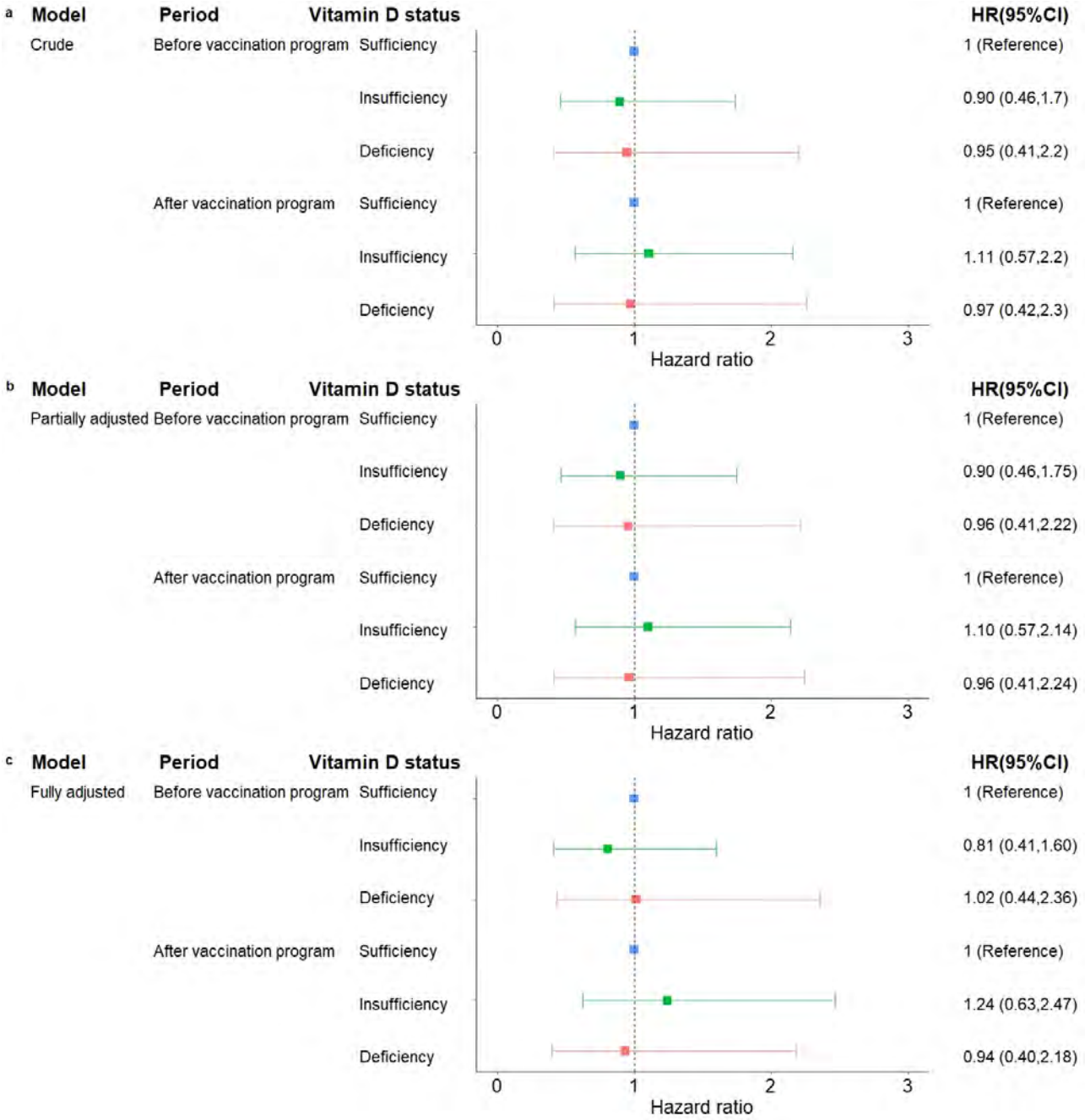


Figure S5. Sensitivity analysis of using stratified Cox regression to assess the association between vitamin D status and the hazards of incident herpes zoster before and after the vaccination program initiated. Partially adjustment: Poisson regression model adjusted for sex and age; Fully adjusted: multivariable Poisson regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 6.14. Figure S6.

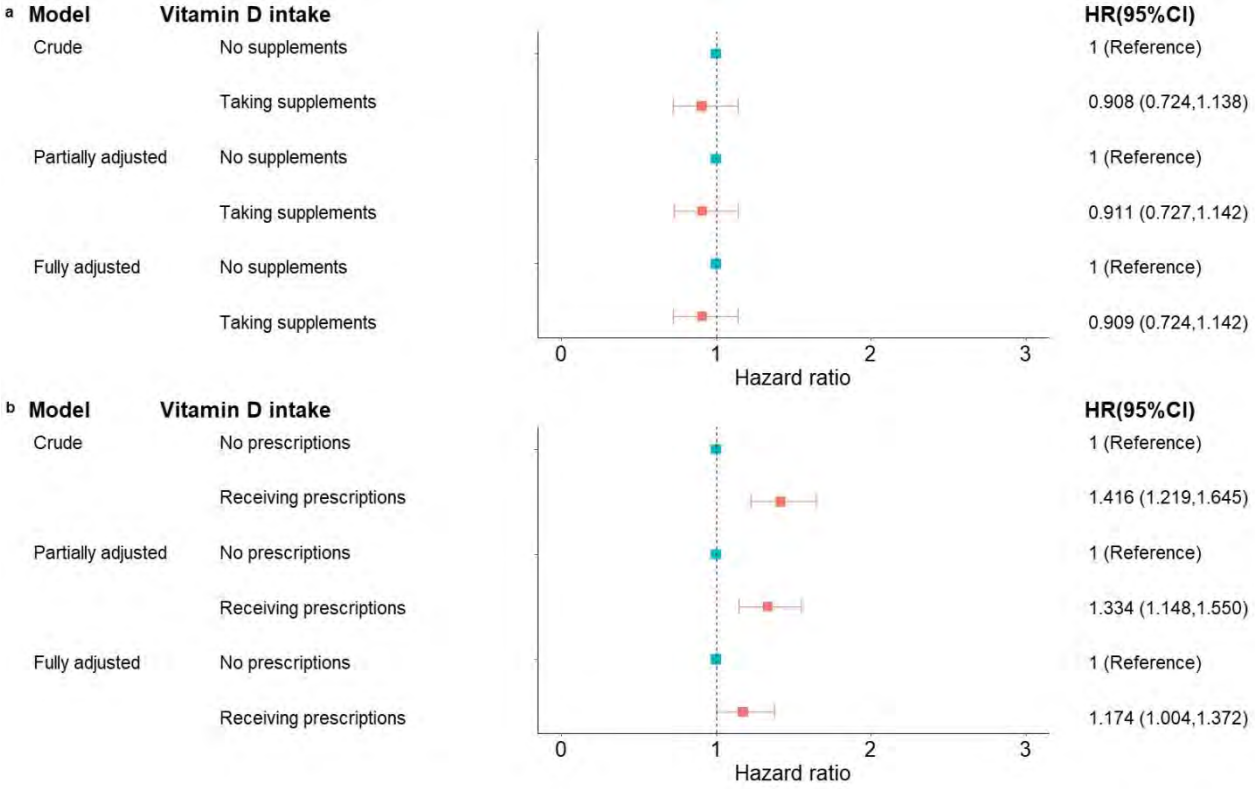


Figure S6. Sensitivity analysis of using Cox proportional-hazards model to examine the association between vitamin D intake and the risk of herpes zoster. a. The association between self-reported vitamin D supplementation and the risk of herpes zoster; b. the association between receiving vitamin D prescriptions and the risk of herpes zoster. Model explanation: Crude: Cox regression model adjusted for age; Partially adjustment: Cox regression model adjusted for sex and age; Fully adjusted: multivariable Cox regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 6.15. Figure S7.

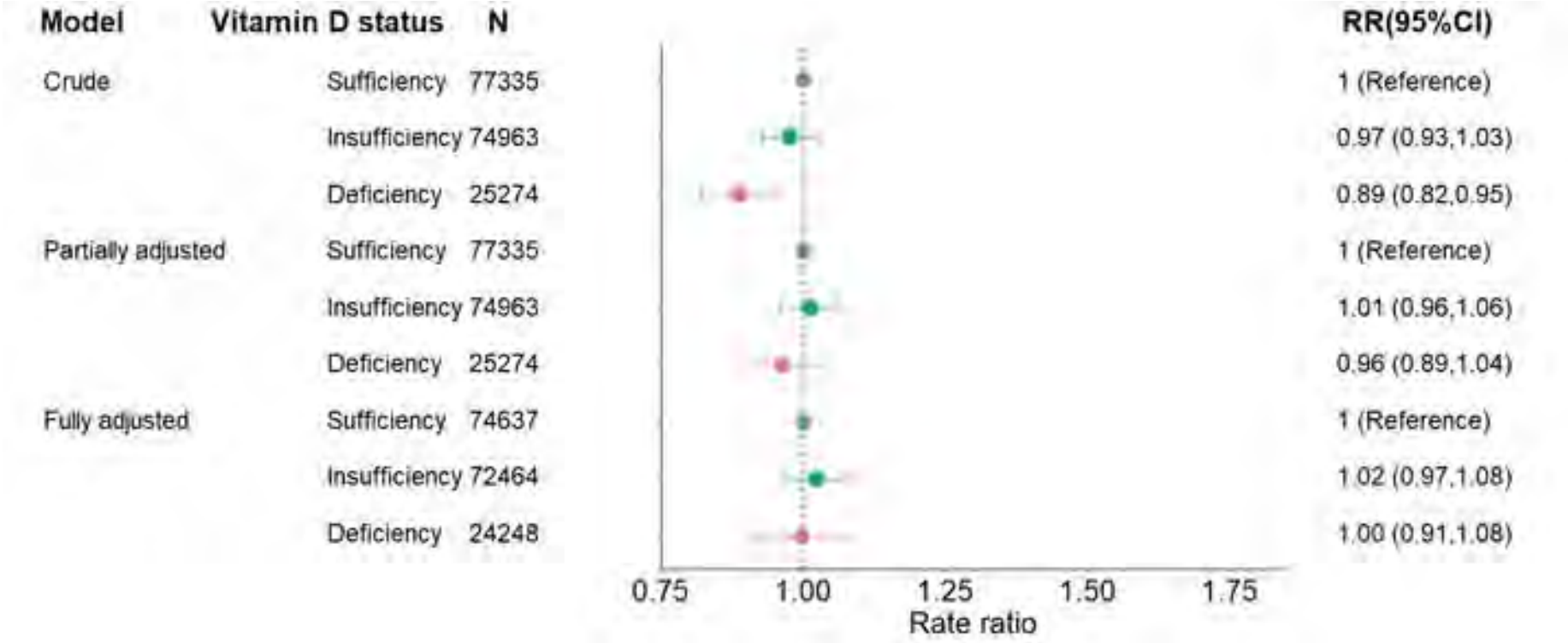


Figure S7. The association between vitamin D status and the risk of herpes zoster. Crude: simple Poisson regression model without adjustment; Partially adjustment: Poisson regression model adjusted for sex and age; Fully adjusted: multivariable Poisson regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 6.16. Figure S8.

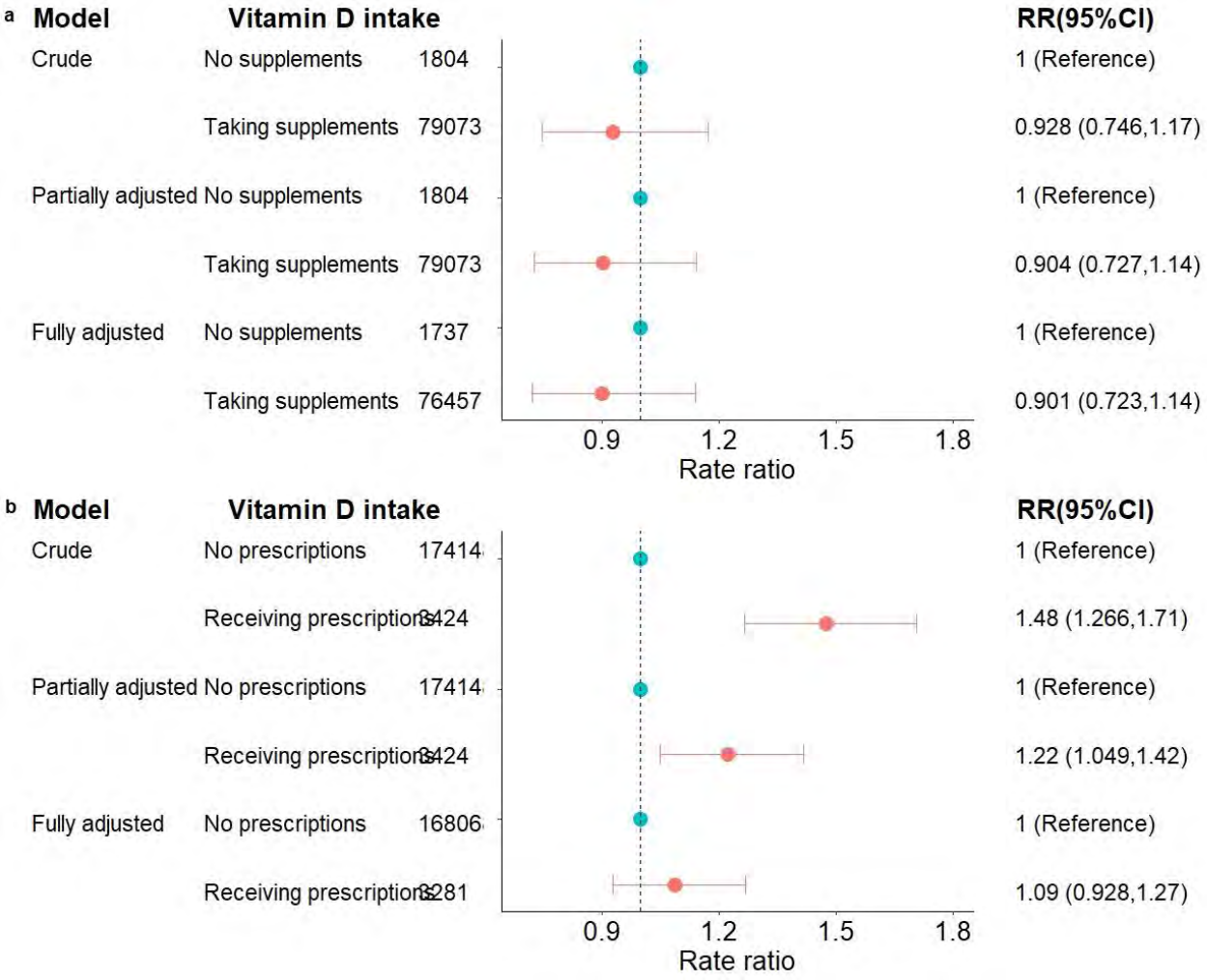


Figure S8 a. The association between self-reported vitamin D supplementation and the risk of herpes zoster; b. the association between receiving vitamin D prescriptions and the risk of herpes zoster. Model explanation: Crude: simple Poisson regression model without adjustment; Partially adjustment: Poisson regression model adjusted for sex and age; Fully adjusted: multivariable Poisson regression model adjusted for all covariates including sex, age, ethnicity, BMI, smoking status, drinking frequency, IMD, regions, seasons, comorbidities, and immunosuppressive conditions.

Appendix 7. Chapter 7: Supplementary materials

Appendix 7.1. Supplementary table 1

Supplementary table 1. The comparison of inclusion and exclusion participants

	Included (N=307,512)	Excluded (N=194,977)
Sex		
- Female	169,018 (55.0%)	104,357 (53.5%)
- Male	138,494 (45.0%)	90,619 (46.5%)
Age[1]		
- Under 70 years old	150,428 (48.9%)	96,903 (49.7%)
- Greater and equal to 70 years old	157,084 (51.1%)	98,073 (50.3%)
Ethnicity		
- White	289,165 (94.0%)	183,514 (94.1%)
- Black	5,310 (1.7%)	2,751 (1.4%)
- Asian and others	13,037 (4.2%)	8,712 (4.5%)
BMI[2]		
- Healthy weight	1,480 (0.5%)	1,146 (0.6%)
- Underweight	97,499 (31.8%)	62,790 (32.5%)
- Overweight	130,370 (42.6%)	81,870 (42.4%)
- Obese	76,989 (25.1%)	47,254 (24.5%)
Drinking frequency		
- Never	24,394 (8.0%)	16,245 (8.4%)
- Sometimes	70,806 (23.1%)	43,056 (22.2%)

- Weekly	149,866 (48.8%)	94,857 (48.9%)
- Daily	61,770 (20.1%)	39,997 (20.6%)
Drinking status		
- Never	13,434 (4.4%)	8,951 (4.6%)
- Previous	10,867 (3.5%)	7,235 (3.7%)
- Current	282,442 (92.1%)	177,910 (91.7%)
Smoking status		
- Non-smoker	167,513 (54.8%)	106,004 (54.8%)
- Ex-smoker	108,326 (35.4%)	64,724 (33.4%)
- Current-smoker	30,105 (9.8%)	22,872 (11.8%)
IMD[3]		
- Least deprived	59,870 (20.0%)	38,261 (20.1%)
- 2 deprived	59,219 (19.8%)	38,773 (20.3%)
- 3 deprived	60,261 (20.1%)	37,570 (19.7%)
- 4 deprived	60,255 (20.1%)	37,634 (19.7%)
- Most deprived	59,490 (19.9%)	38,419 (20.2%)
Vitamin D and mineral supplementation[4]		
- Not taking supplement	22417 (21.2%)	14395 (22.8%)
- Taking vitamin D supplement	83131 (78.8%)	48692 (77.2%)
Vitamin D prescription		
- Not receiving prescriptions	234411 (76.2%)	176220 (90.4%)
- Had vitamin D prescriptions	73101 (23.8%)	18757 (9.6%)
Regions		
- East Midlands	24,467 (8.0%)	5,869 (4.1%)

- London	44,374 (14.4%)	16,917 (11.8%)
- North East	43,707 (14.2%)	8,570 (6.0%)
- North West	50,808 (16.5%)	19,454 (13.6%)
- South East	28,859 (9.4%)	10,955 (7.7%)
- South West	29,445 (9.6%)	9,424 (6.6%)
- West Midlands	31,522 (10.3%)	8,506 (5.9%)
- Yorkshire and The Humber	54,330 (17.7%)	11,768 (8.2%)
- Wales	0 (0.0%)	19,154 (13.4%)
- Scotland	0 (0.0%)	32,419 (22.7%)
Clinically vulnerable to COVID-19[5,6]		
- Not extremely vulnerable	249,944 (81.3%)	171,488 (88.0%)
- Clinically extremely vulnerable	57,568 (18.7%)	23,489 (12.0%)
Underlying comorbidities[5,7]		
- No chronic diseases	94,237 (30.6%)	107,046 (54.9%)
- Chronic diseases	213,275 (69.4%)	87,931 (45.1%)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. Vitamin D supplement includes vitamin D, multivitamin, fish oil and calcium supplementation. 5. Health conditions were identified from linked electronic health records. 6. The clinically extremely vulnerable groups were defined by using Public Health England’s definition. 7. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma.

Appendix 7.2. Supplementary table 2

Supplementary table 2. the association between receiving vitamin D tests during British summer time and serum vitamin D status

	Vitamin D deficiency (25OHD<25nmol/L)		Vitamin D insufficiency (25OHD<50nmol/L)	
British summer time[1]	OR (crude)	OR (adjusted for all covariates[2])	OR (crude)	OR (adjusted for all covariates[2])
Non-British summer time	-	-	-	-
British summer time	0.34 (0.33-0.35)	0.51 (0.58-0.75)	0.4 (0.39-0.4)	0.58 (0.57-0.59)

1. British summer time: from April to October; non-British summer time: from November to March; 2. Including sex, age, ethnicity, smoking, drinking frequency, index of multiple deprivation

Appendix 7.3. Supplementary table 3

Supplementary table 3. The association between vitamin D prescription and Covid-19 diagnosis

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Without vitamin D prescription	-	-	-
	Had vitamin D prescription	1.28 (1.19-1.37)	1.43 (1.33-1.54)	1.22 (1.13-1.32)
Non-British summer time (26 October to 18 January 2021)	Without vitamin D prescription	-	-	-
	Had vitamin D prescription	0.90 (0.82-0.99)	0.90 (0.82-0.98)	0.90 (0.82-0.98)
Sex	Female	-	-	-
	Male	1.10 (1.06-1.15)	-	1.11 (1.06-1.15)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	0.59 (0.56-0.61)	-	0.57 (0.54-0.59)
Ethnicity	White	-	-	-
	Black	1.79 (1.60-2.01)	1.58 (1.41-1.78)	1.32 (1.16-1.49)
	Asian and others	1.72 (1.60-1.86)	1.57 (1.45-1.70)	1.37 (1.26-1.50)
BMI[2]	Healthy weight	-	-	-
	Underweight	1.10 (0.81-1.49)	1.08 (0.80-1.47)	1.04 (0.77-1.41)
	Overweight	1.23 (1.17-1.29)	1.26 (1.19-1.32)	1.20 (1.14-1.26)

	Obese	1.60 (1.52-1.69)	1.62 (1.54-1.71)	1.42 (1.35-1.50)
Drinking frequency	Never	-	-	-
	Sometimes	0.87 (0.81-0.94)	0.86 (0.80-0.92)	0.95 (0.88-1.03)
	Weekly	0.80 (0.74-0.85)	0.77 (0.72-0.82)	0.95 (0.88-1.02)
	Daily	0.65 (0.60-0.70)	0.64 (0.60-0.70)	0.83 (0.76-0.90)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.09 (1.04-1.14)	1.16 (1.11-1.21)	1.15 (1.10-1.20)
	Current smoker	1.23 (1.15-1.31)	1.15 (1.08-1.23)	1.05 (0.98-1.12)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.98 (0.94-1.02)	0.99 (0.95-1.03)	1.01 (0.97-1.06)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.18 (1.10-1.27)	1.18 (1.10-1.27)	1.09 (1.01-1.18)
	3 deprived	1.38 (1.28-1.48)	1.36 (1.27-1.46)	1.21 (1.12-1.30)
	4 deprived	1.63 (1.53-1.75)	1.59 (1.49-1.70)	1.34 (1.25-1.44)
	Most deprived	2.16 (2.03-2.30)	2.05 (1.92-2.19)	1.57 (1.46-1.68)
Regions	North East	-	-	-
	East Midlands	0.87 (0.80-0.95)	0.88 (0.80-0.96)	0.92 (0.84-1.01)

	London	1.04 (0.97-1.12)	1.01 (0.94-1.09)	0.91 (0.84-0.98)
	North West	1.30 (1.22-1.39)	1.31 (1.22-1.39)	1.23 (1.15-1.31)
	South East	0.57 (0.52-0.63)	0.57 (0.52-0.63)	0.66 (0.60-0.73)
	South West	0.66 (0.60-0.73)	0.65 (0.59-0.71)	0.71 (0.64-0.78)
	West Midlands	1.03 (0.96-1.12)	1.02 (0.94-1.10)	0.95 (0.87-1.02)
	Yorkshire and The Humber	0.97 (0.91-1.04)	0.96 (0.90-1.03)	0.95 (0.89-1.02)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-		-
	Extremely vulnerable	1.28 (1.23-1.35)	1.42 (1.36-1.49)	1.26 (1.20-1.33)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	1.07 (1.02-1.12)	1.21 (1.15-1.26)	1.01 (0.96-1.06)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.4. Supplementary table 4

Supplementary table 4. The association between vitamin D prescription and hospitalization due to Covid-19

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Without vitamin D prescription	-	-	-
	Had vitamin D prescription	1.99 (1.76-2.26)	2.36 (2.08-2.68)	1.59 (1.39-1.82)
Non-British summer time (26 October to 18 January 2021)	Without vitamin D prescription	-	-	
	Had vitamin D prescription	0.93 (0.71-1.21)	0.93 (0.71-1.22)	0.95 (0.72-1.25)
Sex	Female	-	-	-
	Male	1.96 (1.76-2.18)	-	1.90 (1.68-2.14)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	1.80 (1.61-2.01)	-	1.45 (1.28-1.64)
Ethnicity	White	-	-	-
	Black	2.22 (1.67-2.95)	2.75 (2.07-3.66)	2.07 (1.52-2.81)
	Asian and others	1.59 (1.28-1.97)	1.77 (1.43-2.20)	1.28 (0.99-1.65)
BMI[2]	Healthy weight	-	-	-
	Underweight	2.00 (0.94-4.23)	2.31 (1.09-4.89)	1.87 (0.88-3.98)
	Overweight	1.80 (1.55-2.10)	1.56 (1.33-1.82)	1.44 (1.23-1.70)

	Obese	3.05 (2.61-3.55)	2.76 (2.37-3.22)	2.07 (1.76-2.44)
Drinking frequency	Never	-	-	-
	Sometimes	0.67 (0.56-0.80)	0.70 (0.58-0.83)	0.81 (0.67-0.98)
	Weekly	0.53 (0.45-0.63)	0.48 (0.41-0.57)	0.72 (0.60-0.86)
	Daily	0.53 (0.43-0.64)	0.43 (0.35-0.52)	0.66 (0.53-0.81)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.61 (1.44-1.81)	1.41 (1.26-1.59)	1.29 (1.14-1.46)
	Current smoker	1.94 (1.65-2.28)	1.88 (1.60-2.22)	1.42 (1.20-1.69)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.99 (0.89-1.11)	1.01 (0.90-1.13)	1.03 (0.92-1.16)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.19 (0.96-1.46)	1.19 (0.96-1.47)	1.06 (0.85-1.31)
	3 deprived	1.37 (1.12-1.68)	1.39 (1.13-1.70)	1.11 (0.90-1.37)
	4 deprived	1.82 (1.50-2.20)	1.88 (1.55-2.28)	1.39 (1.14-1.70)
	Most deprived	2.87 (2.39-3.43)	3.04 (2.54-3.64)	1.74 (1.43-2.12)
Regions	North East	-	-	-
	East Midlands	1.18 (0.94-1.48)	1.17 (0.93-1.47)	1.29 (1.02-1.63)

	London	0.88 (0.71-1.09)	0.92 (0.74-1.13)	0.76 (0.60-0.95)
	North West	1.54 (1.28-1.84)	1.52 (1.27-1.83)	1.35 (1.12-1.63)
	South East	0.49 (0.37-0.66)	0.49 (0.37-0.66)	0.66 (0.49-0.90)
	South West	0.58 (0.44-0.76)	0.60 (0.45-0.78)	0.68 (0.51-0.91)
	West Midlands	1.34 (1.09-1.65)	1.33 (1.08-1.64)	1.19 (0.96-1.47)
	Yorkshire and The Humber	1.20 (1.00-1.45)	1.21 (1.00-1.46)	1.24 (1.03-1.51)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	3.50 (3.14-3.89)	3.20 (2.87-3.57)	2.37 (2.10-2.66)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	2.84 (2.43-3.31)	2.41 (2.06-2.82)	1.56 (1.32-1.85)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.5. Supplementary table 5

Supplementary table 5. The association between vitamin D prescription and mortality due to Covid-19

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Without vitamin D prescription	-	-	-
	Had vitamin D prescription	2.55 (1.89-3.43)	3.07 (2.26-4.16)	2.31 (1.68-3.18)
Non-British summer time (26 October to 18 January 2021)	Without vitamin D prescription	-		-
	Had vitamin D prescription	0.91 (0.57-1.48)	0.92 (0.57-1.48)	0.91 (0.56-1.48)
Sex	Female	-	-	-
	Male	2.86 (2.22-3.68)	-	2.89 (2.19-3.81)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	6.50 (4.60-9.18)	-	5.30 (3.65-7.68)
Ethnicity	White	-	-	-
	Black	2.25 (1.23-4.11)	3.93 (2.14-7.21)	3.15 (1.65-5.99)
	Asian and others	0.83 (0.44-1.57)	1.12 (0.60-2.11)	0.75 (0.36-1.56)
BMI[2]	Healthy weight	-	-	-
	Underweight	4.72 (1.46-15.24)	6.38 (1.97-20.63)	4.70 (1.45-15.23)
	Overweight	2.05 (1.44-2.92)	1.59 (1.11-2.27)	1.39 (0.97-2.00)

	Obese	3.75 (2.64-5.32)	3.17 (2.23-4.50)	2.23 (1.55-3.20)
Drinking frequency	Never	-	-	-
	Sometimes	0.51 (0.34-0.76)	0.55 (0.37-0.81)	0.58 (0.39-0.88)
	Weekly	0.49 (0.34-0.69)	0.43 (0.30-0.61)	0.62 (0.43-0.90)
	Daily	0.58 (0.39-0.86)	0.41 (0.28-0.62)	0.65 (0.42-0.99)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	2.05 (1.59-2.64)	1.54 (1.19-1.99)	1.35 (1.03-1.76)
	Current smoker	2.13 (1.48-3.06)	2.16 (1.50-3.12)	1.55 (1.06-2.29)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.97 (0.76-1.23)	0.99 (0.78-1.26)	1.04 (0.81-1.34)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.16 (0.73-1.86)	1.17 (0.74-1.87)	1.00 (0.62-1.61)
	3 deprived	1.36 (0.86-2.12)	1.40 (0.89-2.19)	1.11 (0.70-1.76)
	4 deprived	1.78 (1.16-2.72)	1.94 (1.27-2.97)	1.33 (0.86-2.07)
	Most deprived	3.21 (2.17-4.74)	3.75 (2.53-5.55	2.05 (1.35-3.11)
Regions	North East	-	-	-
	East Midlands	1.02 (0.64-1.60)	0.98 (0.62-1.55)	1.13 (0.71-1.79)

	London	0.46 (0.28-0.75)	0.51 (0.31-0.82)	0.43 (0.25-0.71)
	North West	0.81 (0.55-1.20)	0.80 (0.54-1.18)	0.68 (0.45-1.01)
	South East	0.18 (0.08-0.41)	0.18 (0.08-0.41)	0.21 (0.08-0.54)
	South West	0.32 (0.17-0.61)	0.34 (0.18-0.65)	0.43 (0.22-0.83)
	West Midlands	1.11 (0.74-1.68)	1.12 (0.74-1.69)	0.95 (0.62-1.45)
	Yorkshire and The Humber	1.21 (0.85-1.73)	1.24 (0.87-1.77)	1.24 (0.86-1.77)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	4.29 (3.40-5.40)	3.32 (2.63-4.20)	2.24 (1.74-2.87)
Underlying comorbidities[5]	No chronic diseases	-		-
	Chronic diseases	4.64 (3.08-7.00)	2.97 (1.96-4.49)	1.78 (1.15-2.74)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.6. Supplementary table 6

Supplementary table 6. The association between vitamin D supplementation and Covid-19 diagnosis

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	0.84 (0.74-0.97)	0.83 (0.73-0.95)	0.88 (0.77-1.01)
Non-British summer time (26 October to 18 January 2021)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	1.25 (1.05-1.48)	1.25 (1.05-1.48)	1.23 (1.03-1.47)
Sex	Female	-	-	-
	Male	1.10 (1.06-1.15)	-	1.12 (1.04-1.21)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	0.59 (0.56-0.61)	-	0.58 (0.54-0.63)
Ethnicity	White	-	-	-
	Black	1.79 (1.60-2.01)	1.58 (1.41-1.78)	1.36 (1.12-1.64)
	Asian and others	1.72 (1.60-1.86)	1.57 (1.45-1.70)	1.43 (1.24-1.65)
BMI[2]	Healthy weight	-	-	-
	Underweight	1.10 (0.81-1.49)	1.08 (0.80-1.47)	0.98 (0.60-1.60)
	Overweight	1.23 (1.17-1.29)	1.26 (1.19-1.32)	1.15 (1.06-1.26)
	Obese	1.60 (1.52-1.69)	1.62 (1.54-1.71)	1.39 (1.26-1.53)

Drinking frequency	Never	-	-	-
	Sometimes	0.87 (0.81-0.94)	0.86 (0.80-0.92)	0.94 (0.83-1.08)
	Weekly	0.80 (0.74-0.85)	0.77 (0.72-0.82)	0.93 (0.82-1.06)
	Daily	0.65 (0.60-0.70)	0.64 (0.60-0.70)	0.82 (0.71-0.95)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.09 (1.04-1.14)	1.16 (1.11-1.21)	1.12 (1.04-1.22)
	Current smoker	1.23 (1.15-1.31)	1.15 (1.08-1.23)	0.95 (0.84-1.08)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.98 (0.94-1.02)	0.99 (0.95-1.03)	0.93 (0.87-1.00)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.18 (1.10-1.27)	1.18 (1.10-1.27)	1.15 (1.02-1.31)
	3 deprived	1.38 (1.28-1.48)	1.36 (1.27-1.46)	1.23 (1.09-1.40)
	4 deprived	1.63 (1.53-1.75)	1.59 (1.49-1.70)	1.28 (1.13-1.45)
	Most deprived	2.16 (2.03-2.30)	2.05 (1.92-2.19)	1.52 (1.34-1.72)
Regions	North East	-	-	-
	East Midlands	0.87 (0.80-0.95)	0.88 (0.80-0.96)	0.98 (0.83-1.16)
	London	1.04 (0.97-1.12)	1.01 (0.94-1.09)	1.04 (0.91-1.18)
	North West	1.30 (1.22-1.39)	1.31 (1.22-1.39)	1.32 (1.17-1.50)
	South East	0.57 (0.52-0.63)	0.57 (0.52-0.63)	0.72 (0.60-0.86)

	South West	0.66 (0.60-0.73)	0.65 (0.59-0.71)	0.69 (0.57-0.82)
	West Midlands	1.03 (0.96-1.12)	1.02 (0.94-1.10)	0.97 (0.83-1.12)
	Yorkshire and The Humber	0.97 (0.91-1.04)	0.96 (0.90-1.03)	1.04 (0.92-1.18)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-		-
	Extremely vulnerable	1.28 (1.23-1.35)	1.42 (1.36-1.49)	1.33 (1.22-1.44)
Underlying comorbidities[5]	No chronic diseases	-		-
	Chronic diseases	1.07 (1.02-1.12)	1.21 (1.15-1.26)	1.01 (0.93-1.09)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.7. Supplementary table 7

Supplementary table 7. The association between vitamin D supplementation and hospitalization due to Covid-19

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	0.67 (0.53-0.84)	0.71 (0.56-0.89)	0.83 (0.65-1.06)
Non-British summer time (26 October to 18 January 2021)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	0.86 (0.52-1.44)	0.86 (0.52-1.44)	0.83 (0.49-1.42)
Sex	Female		-	-
	Male	1.96 (1.76-2.18)	-	1.72 (1.41-2.11)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	1.80 (1.61-2.01)	-	1.29 (1.04-1.60)
Ethnicity	White	-	-	-
	Black	2.22 (1.67-2.95)	2.75 (2.07-3.66)	1.97 (1.23-3.15)
	Asian and others	1.59 (1.28-1.97)	1.77 (1.43-2.20)	1.34 (0.89-2.03)
BMI[2]	Healthy weight	-	-	-
	Underweight	2.00 (0.94-4.23)	2.31 (1.09-4.89)	1.41 (0.35-5.75)
	Overweight	1.80 (1.55-2.10)	1.56 (1.33-1.82)	1.39 (1.06-1.83)
	Obese	3.05 (2.61-3.55)	2.76 (2.37-3.22)	1.93 (1.46-2.56)

Drinking frequency	Never	-	-	-
	Sometimes	0.67 (0.56-0.80)	0.70 (0.58-0.83)	0.85 (0.62-1.18)
	Weekly	0.53 (0.45-0.63)	0.48 (0.41-0.57)	0.68 (0.50-0.94)
	Daily	0.53 (0.43-0.64)	0.43 (0.35-0.52)	0.63 (0.44-0.92)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.61 (1.44-1.81)	1.41 (1.26-1.59)	1.34 (1.08-1.67)
	Current smoker	1.94 (1.65-2.28)	1.88 (1.60-2.22)	1.40 (1.02-1.93)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.99 (0.89-1.11)	1.01 (0.90-1.13)	0.98 (0.80-1.20)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.19 (0.96-1.46)	1.19 (0.96-1.47)	1.48 (1.01-2.17)
	3 deprived	1.37 (1.12-1.68)	1.39 (1.13-1.70)	1.48 (1.01-2.16)
	4 deprived	1.82 (1.50-2.20)	1.88 (1.55-2.28)	1.63 (1.12-2.36)
	Most deprived	2.87 (2.39-3.43)	3.04 (2.54-3.64)	1.97 (1.37-2.85)
Regions	North East	-	-	-
	East Midlands	1.18 (0.94-1.48)	1.18 (0.94-1.48)	1.60 (1.02-2.52)
	London	0.88 (0.71-1.09)	0.88 (0.71-1.09)	1.17 (0.79-1.74)
	North West	1.54 (1.28-1.84)	1.54 (1.28-1.84)	1.63 (1.14-2.33)
	South East	0.49 (0.37-0.66)	0.49 (0.37-0.66)	0.86 (0.50-1.49)

	South West	0.58 (0.44-0.76)	0.58 (0.44-0.76)	0.63 (0.35-1.13)
	West Midlands	1.34 (1.09-1.65)	1.34 (1.09-1.65)	1.53 (1.02-2.30)
	Yorkshire and The Humber	1.20 (1.00-1.45)	1.20 (1.00-1.45)	1.53 (1.05-2.22)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	3.50 (3.14-3.89)	3.20 (2.87-3.57)	2.75 (2.25-3.36)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	2.84 (2.43-3.31)	2.41 (2.06-2.82)	2.05 (1.50-2.80)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.8. Supplementary table 8

Supplementary table 8. The association between vitamin D supplementation and mortality due to Covid-19

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	0.49 (0.27-0.89)	0.55 (0.30-1.00)	0.64 (0.35-1.16)
Non-British summer time (26 October to 18 January 2021)	No vitamin D supplementation	-	-	-
	Taking vitamin D supplementation	1.64 (0.58-4.65)	1.64 (0.58-4.64)	1.88 (0.63-5.59)
Sex	Female	-	-	-
	Male	2.86 (2.22-3.68)	-	2.53 (1.53-4.18)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	6.50 (4.60-9.18)	-	3.48 (1.84-6.59)
Ethnicity	White	-	-	-
	Black	2.25 (1.23-4.11)	3.93 (2.14-7.21)	1.30 (0.30-5.56)
	Asian and others	0.83 (0.44-1.57)	1.12 (0.60-2.11)	0.52 (0.12-2.23)
BMI[2]	Healthy weight	-	-	-
	Underweight	4.72 (1.46-15.24)	6.38 (1.97-20.63)	9.80 (2.18-44.11)
	Overweight	2.05 (1.44-2.92)	1.59 (1.11-2.27)	1.48 (0.77-2.83)
	Obese	3.75 (2.64-5.32)	3.17 (2.23-4.50)	1.61 (0.81-3.22)
Drinking frequency	Never	-	-	-

	Sometimes	0.51 (0.34-0.76)	0.55 (0.37-0.81)	0.43 (0.21-0.90)
	Weekly	0.49 (0.34-0.69)	0.43 (0.30-0.61)	0.40 (0.21-0.78)
	Daily	0.58 (0.39-0.86)	0.41 (0.28-0.62)	0.39 (0.18-0.84)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	2.05 (1.59-2.64)	1.54 (1.19-1.99)	1.23 (0.73-2.06)
	Current smoker	2.13 (1.48-3.06)	2.16 (1.50-3.12)	1.76 (0.86-3.60)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.97 (0.76-1.23)	0.99 (0.78-1.26)	0.88 (0.55-1.40)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.16 (0.73-1.86)	1.17 (0.74-1.87)	2.10 (0.80-5.49)
	3 deprived	1.36 (0.86-2.12)	1.40 (0.89-2.19)	1.92 (0.73-5.05)
	4 deprived	1.78 (1.16-2.72)	1.94 (1.27-2.97)	2.07 (0.80-5.37)
	Most deprived	3.21 (2.17-4.74)	3.75 (2.53-5.55	2.66 (1.04-6.77)
Regions	North East	-	-	-
	East Midlands	1.02 (0.64-1.60)	0.98 (0.62-1.55)	1.64 (0.65-4.18)
	London	0.46 (0.28-0.75)	0.51 (0.31-0.82)	0.92 (0.37-2.26)
	North West	0.81 (0.55-1.20)	0.80 (0.54-1.18)	0.77 (0.33-1.82)
	South East	0.18 (0.08-0.41)	0.18 (0.08-0.41)	0.41 (0.09-1.92)
	South West	0.32 (0.17-0.61)	0.34 (0.18-0.65)	0.19 (0.02-1.49)

	West Midlands	1.11 (0.74-1.68)	1.12 (0.74-1.69)	1.42 (0.60-3.35)
	Yorkshire and The Humber	1.21 (0.85-1.73)	1.24 (0.87-1.77)	1.78 (0.83-3.81)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	4.29 (3.40-5.40)	3.32 (2.63-4.20)	3.02 (1.87-4.85)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	4.64 (3.08-7.00)	2.97 (1.96-4.49)	3.05 (1.20-7.72)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.9. Supplementary table 9

Supplementary table 9. The association between vitamin D status and laboratory-confirmed Covid-19 diagnosis

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	1.09 (1.01-1.18)	1.05 (0.97-1.13)	0.95 (0.88-1.02)
	Vitamin D deficiency	1.16 (1.04-1.29)	1.06 (0.95-1.18)	0.84 (0.75-0.95)
Non-British summer time (26 October to 18 January 2021)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	0.95 (0.86-1.04)	0.95 (0.87-1.04	0.95 (0.86-1.04)
	Vitamin D deficiency	1.17 (1.02-1.33)	1.17 (1.03-1.33)	1.15 (1.01-1.32)
Sex	Female	-	-	-
	Male	1.09 (1.05-1.14)	-	1.07 (1.03-1.12,)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	0.56 (0.54-0.58)	-	0.55 (0.52-0.57)
Ethnicity	White	-	-	-
	Black	1.79 (1.59-2.02)	1.57 (1.39-1.76)	1.35 (1.19-1.53)
	Asian and others	1.73 (1.60-1.87)	1.57 (1.45-1.70)	1.44 (1.32-1.58)
BMI[2]	Healthy weight	-	-	-
	Underweight	1.05 (0.77-1.44)	1.04 (0.76-1.41)	1.02 (0.74-1.40)
	Overweight	1.22 (1.16-1.28)	1.25 (1.19-1.31)	1.20 (1.14-1.26)

	Obese	1.58 (1.50-1.67)	1.61 (1.52-1.69	1.43 (1.35-1.51)
Drinking frequency	Never	-	-	-
	Sometimes	0.89 (0.82-0.96)	0.87 (0.81-0.94)	0.96 (0.88-1.04)
	Weekly	0.82 (0.76-0.88)	0.79 (0.73-0.84)	0.95 (0.88-1.03)
	Daily	0.65 (0.60-0.71)	0.65 (0.60-0.71)	0.82 (0.76-0.90)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.08 (1.03-1.13, p<0.001)	1.15 (1.10-1.20)	1.14 (1.09-1.20)
	Current smoker	1.21 (1.13-1.29, p<0.001)	1.13 (1.06-1.21)	1.04 (0.97-1.12)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.98 (0.94-1.02)	0.99 (0.95-1.03)	1.00 (0.96-1.04)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.18 (1.10-1.28)	1.18 (1.10-1.27)	1.09 (1.02-1.18)
	3 deprived	1.38 (1.28-1.48)	1.36 (1.27-1.46)	1.21 (1.13-1.30)
	4 deprived	1.65 (1.54-1.76)	1.60 (1.49-1.72)	1.36 (1.27-1.46)
	Most deprived	2.16 (2.03-2.31)	2.04 (1.91-2.18)	1.59 (1.48-1.71)
Regions	North East	-	-	-
	East Midlands	0.83 (0.76-0.91)	0.84 (0.76-0.92)	0.87 (0.80-0.96)

	London	1.02 (0.95-1.09)	0.99 (0.92-1.06)	0.90 (0.83-0.97)
	North West	1.29 (1.21-1.38)	1.29 (1.21-1.38)	1.23 (1.15-1.32)
	South East	0.56 (0.51-0.62)	0.57 (0.51-0.62	0.65 (0.59-0.72)
	South West	0.65 (0.59-0.71)	0.64 (0.58-0.70)	0.69 (0.63-0.76)
	West Midlands	1.02 (0.94-1.10)	1.00 (0.93-1.08)	0.94 (0.87-1.02)
	Yorkshire and The Humber	0.93 (0.87-1.00)	0.92 (0.86-0.99)	0.92 (0.86-0.99)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	1.23 (1.18-1.29)	1.37 (1.31-1.44)	1.25 (1.19-1.31)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	1.05 (1.00-1.09)	1.19 (1.14-1.25)	1.01 (0.96-1.06)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. Health conditions were identified from linked electronic health records. 5. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 6. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma.

Appendix 7.10. Supplementary table 10

Supplementary table 10. The association between vitamin D status and hospital admission among patients with Covid-19 diagnosis

		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	1.07 (0.94-1.22)	1.13 (0.99-1.29)	1.01 (0.88-1.16)
	Vitamin D deficiency	1.18 (0.99-1.40)	1.31 (1.10-1.56)	1.07 (0.88-1.31)
Non-British summer time (26 October to 18 January 2021)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	1.05 (0.80-1.40)	1.06 (0.80-1.40)	1.12 (0.84-1.49)
	Vitamin D deficiency	0.92 (0.63-1.36)	0.93 (0.63-1.37)	0.92 (0.61-1.37)
Sex	Female	-	-	-
	Male	1.86 (1.67-2.08)	-	1.57 (1.39-1.76)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	3.36 (3.01-3.76)	-	2.55 (2.25-2.89)
Ethnicity	White	-	-	-
	Black	1.32 (0.99-1.75)	1.84 (1.39-2.45)	1.66 (1.22-2.26)
	Asian and others	0.92 (0.74-1.15)	1.09 (0.88-1.36)	0.98 (0.75-1.27)
BMI[2]	Healthy weight	-		-
	Underweight	1.88 (0.89-3.99)	1.90 (0.89-4.03)	1.74 (0.82-3.71)
	Overweight	1.51 (1.29-1.75)	1.25 (1.07-1.46)	1.19 (1.02-1.40)

	Obese	2.00 (1.72-2.33)	1.71 (1.46-1.99)	1.47 (1.25-1.74)
Drinking frequency	Never	-	-	-
	Sometimes	0.75 (0.63-0.90)	0.86 (0.72-1.03)	0.90 (0.75-1.09)
	Weekly	0.64 (0.54-0.75)	0.63 (0.53-0.74)	0.75 (0.63-0.91)
	Daily	0.79 (0.65-0.96)	0.65 (0.54-0.79)	0.77 (0.63-0.96)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.52 (1.36-1.71)	1.21 (1.08-1.36)	1.15 (1.01-1.30)
	Current smoker	1.63 (1.38-1.91)	1.56 (1.33-1.84)	1.37 (1.15-1.63)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	1.01 (0.91-1.13)	1.03 (0.93-1.16)	1.06 (0.94-1.20)
IMD[3]	Least deprived	-	-	-
	2 deprived	1.00 (0.81-1.23)	1.00 (0.81-1.24)	0.98 (0.79-1.22)
	3 deprived	0.99 (0.81-1.22)	0.99 (0.81-1.21)	0.89 (0.72-1.10)
	4 deprived	1.13 (0.93-1.36)	1.14 (0.94-1.38)	0.98 (0.80-1.20)
	Most deprived	1.36 (1.14-1.63)	1.44 (1.21-1.73)	1.09 (0.89-1.32)
Regions	North East	-	-	-
	East Midlands	1.38 (1.10-1.74)	1.25 (0.99-1.57)	1.31 (1.03-1.66)
	London	0.85 (0.69-1.05)	0.87 (0.71-1.08)	0.81 (0.65-1.02)
	North West	1.19 (0.99-1.42)	1.13 (0.94-1.36)	1.11 (0.92-1.34)

	South East	0.85 (0.63-1.14)	0.83 (0.62-1.11)	0.92 (0.68-1.25)
	South West	0.85 (0.65-1.12)	0.89 (0.68-1.17)	0.93 (0.70-1.24)
	West Midlands	1.32 (1.07-1.62)	1.30 (1.06-1.60)	1.26 (1.01-1.56)
	Yorkshire and The Humber	1.26 (1.04-1.52)	1.24 (1.03-1.49)	1.30 (1.07-1.58)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	3.03 (2.73-3.38)	2.28 (2.04-2.55)	1.98 (1.76-2.23)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	2.81 (2.41-3.29)	1.93 (1.64-2.26)	1.54 (1.30-1.83)

1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.11. Supplementary table 11

Supplementary table 11. The association between vitamin D status and mortality among patients with Covid-19 diagnosis

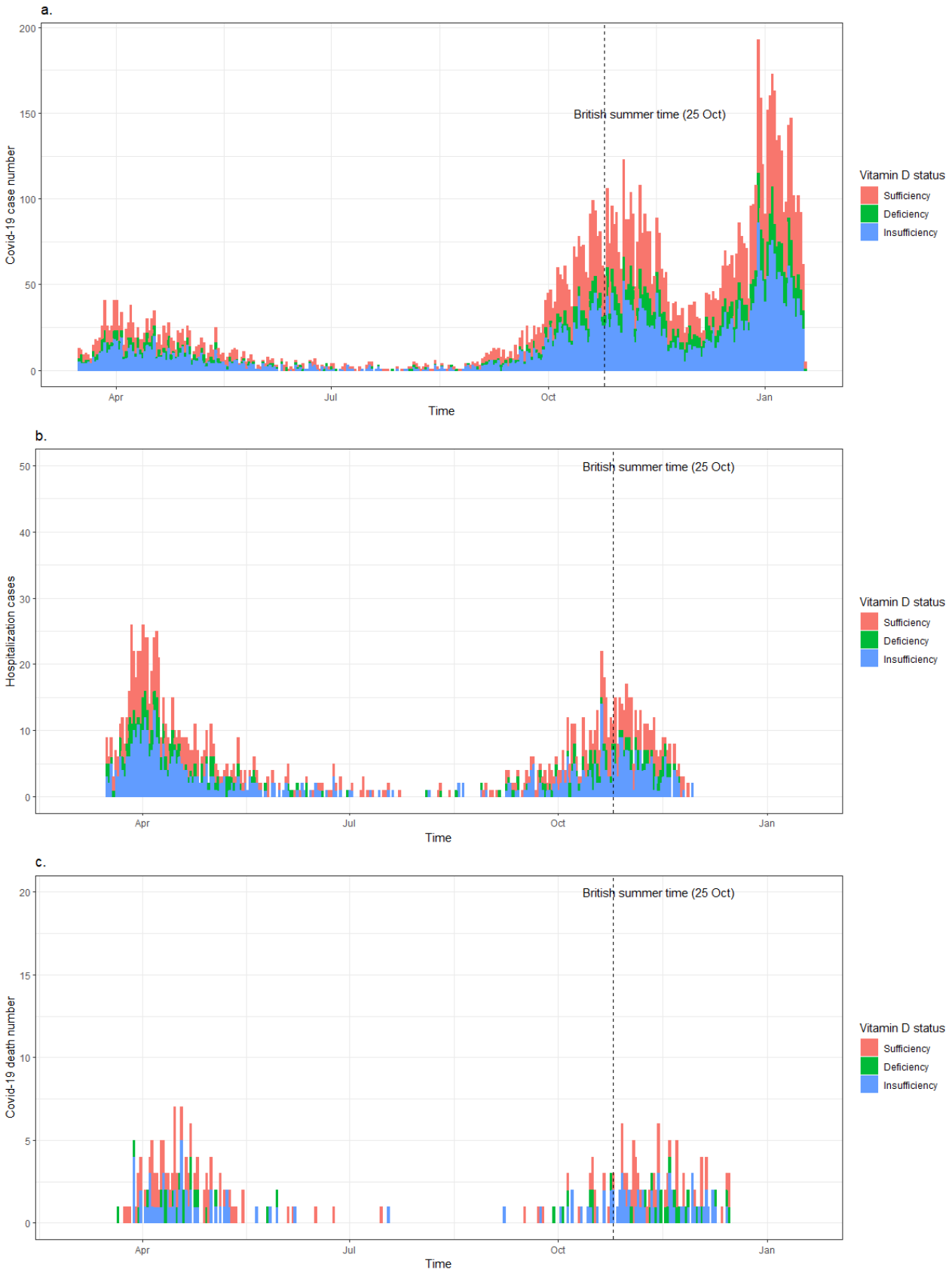
		HR (crude)	HR (adjusted for sex and age)	HR (adjusted for all covariates)
British summer time (15 March to 25 October 2020)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	0.89 (0.65-1.23)	0.99 (0.72-1.37	0.89 (0.63-1.24)
	Vitamin D deficiency	0.98 (0.63-1.51)	1.19 (0.77-1.84)	1.00 (0.63-1.60)
Non-British summer time (26 October to 18 January 2021)	Vitamin D sufficiency	-	-	
	Vitamin D insufficiency	1.22 (0.72-2.06)	1.23 (0.73-2.07)	1.37 (0.80-2.33)
	Vitamin D deficiency	1.34 (0.68-2.65)	1.34 (0.68-2.66)	1.48 (0.74-2.95)
Sex	Female	-	-	-
	Male	2.64 (2.05-3.40)	-	2.08 (1.59-2.72)
Age[1]	Under 70 years old	-	-	-
	Greater and equal to 70 years old	11.50 (8.14)	-	8.92 (6.12-12.99)
Ethnicity	White	-	-	-
	Black	1.27 (0.70-2.33)	2.37 (1.29-4.34)	2.35 (1.24-4.44)
	Asian and others	0.48 (0.26-0.91)	0.66 (0.35-1.24)	0.62 (0.29-1.30)
BMI[2]	Healthy weight	-	-	-
	Underweight	4.41 (1.37-14.23)	4.84 (1.49-15.67)	3.92 (1.20-12.88)
	Overweight	1.68 (1.18-2.39)	1.27 (0.89-1.81)	1.16 (0.80-1.67)

	Obese	2.38 (1.68-3.38)	1.85 (1.30-2.64)	1.53 (1.06-2.21)
Drinking frequency	Never	-	-	-
	Sometimes	0.58 (0.39-0.86)	0.73 (0.49-1.09)	0.69 (0.45-1.04)
	Weekly	0.60 (0.42-0.86)	0.60 (0.42-0.86)	0.68 (0.47-1.00)
	Daily	0.88 (0.59-1.32)	0.66 (0.44-0.99)	0.75 (0.49-1.16)
Smoking status	Non-smoker	-	-	-
	Ex-smoker	1.91 (1.48-2.46)	1.27 (0.98-1.64)	1.19 (0.91-1.56)
	Current smoker	1.75 (1.22-2.53)	1.59 (1.11-2.30)	1.37 (0.93-2.02)
Vitamin D status testing time	During non-British summer time	-	-	-
	During British summer time	0.99 (0.78-1.26)	1.02 (0.80-1.30)	1.10 (0.84-1.42)
IMD[3]	Least deprived	-	-	-
	2 deprived	0.98 (0.62-1.57)	0.99 (0.62-1.57)	0.89 (0.55-1.44)
	3 deprived	0.98 (0.63-1.54)	1.00 (0.64-1.56)	0.87 (0.55-1.38)
	4 deprived	1.10 (0.72-1.68)	1.14 (0.74-1.74)	0.90 (0.58-1.41)
	Most deprived	1.51 (1.02-2.23)	1.67 (1.13-2.46)	1.22 (0.80-1.85)
Regions	North East	-	-	-
	East Midlands	1.18 (0.75-1.86)	1.00 (0.63-1.57)	1.09 (0.68-1.72)
	London	0.44 (0.27-0.71)	0.47 (0.29-0.76)	0.46 (0.27-0.76)
	North West	0.62 (0.42-0.91)	0.58 (0.39-0.86)	0.55 (0.37-0.82)

	South East	0.30 (0.13-0.71)	0.29 (0.13-0.69)	0.29 (0.11-0.73)
	South West	0.47 (0.25-0.90)	0.51 (0.26-0.97)	0.56 (0.29-1.08)
	West Midlands	1.07 (0.71-1.62)	1.09 (0.72-1.65)	0.98 (0.64-1.50)
	Yorkshire and The Humber	1.26 (0.89-1.80)	1.24 (0.87-1.76)	1.24 (0.86-1.79)
Clinically vulnerable to COVID-19[4]	Not vulnerable	-	-	-
	Extremely vulnerable	3.50 (2.77-4.41)	2.08 (1.65-2.63)	1.77 (1.38-2.27)
Underlying comorbidities[5]	No chronic diseases	-	-	-
	Chronic diseases	4.45 (2.95-6.70)	2.11 (1.39-3.21)	1.62 (1.05-2.52)

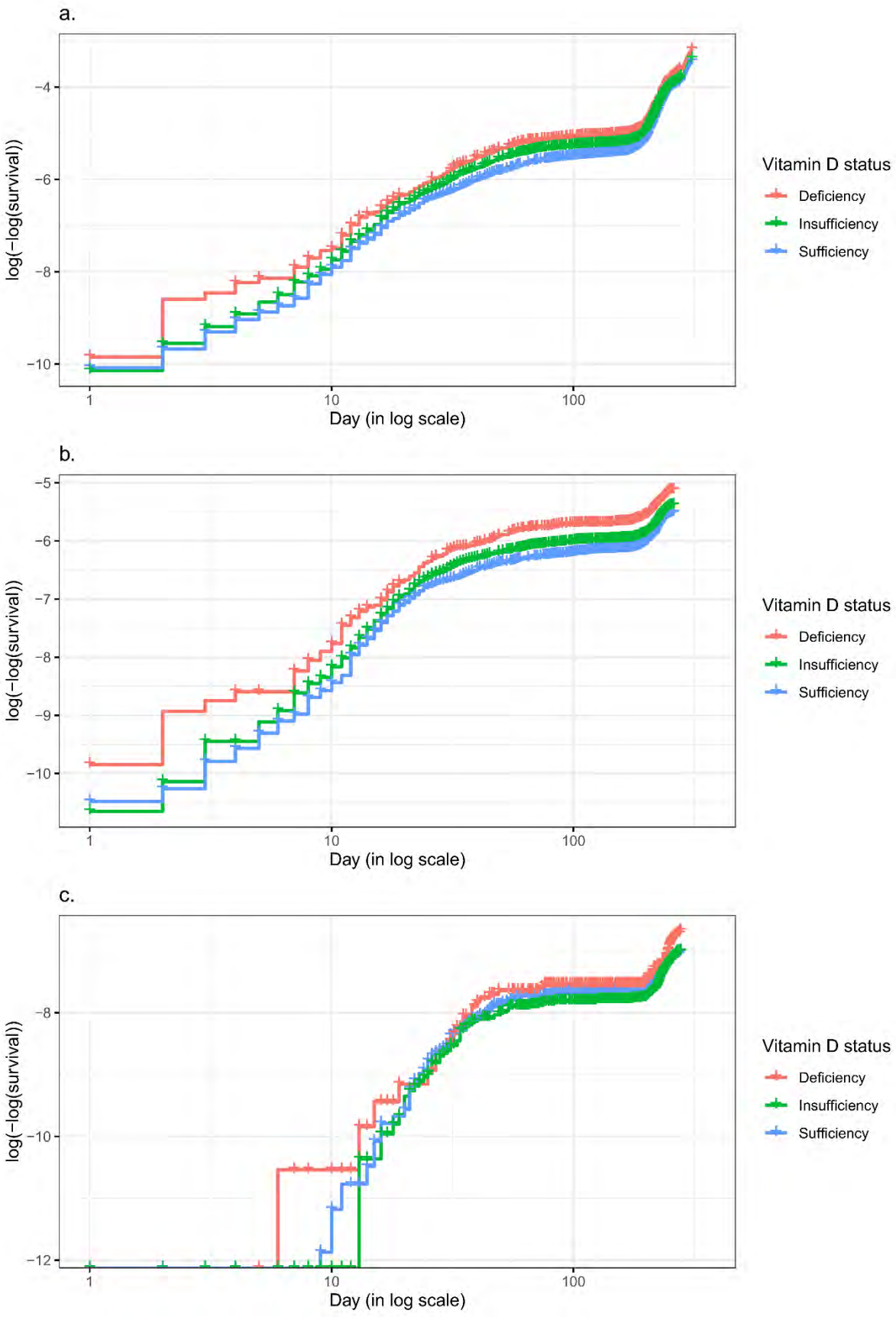
1. Calculated from participants' year of birth. 2. The classification is suggested by NICE guidelines. 3. IMD scores were classified by quintile. 4. The clinically extremely vulnerable groups were defined by using Public Health England's definition. 5. Including hypertension, cardiovascular diseases, diabetes mellitus, and asthma

Appendix 7.12. Supplementary Figure 1.



Supplementary Figure 1. The distribution of COVID-19 diagnosis, hospitalisation, and mortality by vitamin D status.

Appendix 7.13. Supplementary Figure 2



Supplementary Figure 2. The Log(-log[survival]) plots for examining proportional hazard assumptions between vitamin D status and COVID-19 diagnosis, hospitalisation, and mortality.