Can Vitamin D Reduce the Burden of COVID-19?

I. Background:

COVID-19, caused by the SARS-CoV-2 coronavirus, has resulted in over 1,290,000 deaths worldwide, with nearly 11 million cases and over 240,000 deaths in the US.[1] The burden of COVID-19 in the US has fallen disproportionately on racial minorities and socioeconomically disadvantaged groups. For example, 30% of Chicago's population is Black, but 60% of COVID-19 deaths are among Black people.[2] The Latinx community has also experienced a disproportionate burden of COVID deaths, both in Chicago and elsewhere.[3] Social distancing remains a critical strategy, but may be particularly difficult for minority and disadvantaged populations, which have higher proportions of multigenerational households and essential workers who cannot avoid exposure to COVID-19. Despite the global need for effective biomedical strategies to prevent COVID-19, an effective and widespread vaccine could take a year or more.

It has recently been proposed that vitamin D deficiency may increase vulnerability to COVID-19 and vitamin D supplementation may be valuable in prevention and treatment.[4] COVID-19 presents complex challenges with respect to immune function, requiring activation of innate immunity for prevention and modulation of adaptive immunity in treating inflammatory stages of the disease.[5] [6] In addition to its well-known roles in bone health, vitamin D activates innate immunity and suppresses excess inflammation in adaptive immunity, decreases acute respiratory infections and improves inflammatory conditions.[7] [8] A meta-analysis found viral respiratory infections decreased 70% in persons with vitamin D deficiency randomized to receive vitamin D and 10% in persons with "sufficient" levels.[9] These findings motivate the hypothesis that vitamin D may be important in COVID-19.

Growing epidemiological evidence points to potential effects of vitamin D in COVID-19. Nearly half the US population is vitamin D deficient, with higher rates in older adults and African-Americans, Latinx individuals, and other racial or ethnic groups with darker skin, and persons with less sun exposure, including persons who live in higher latitudes in winter, nursing home residents and health care workers, which also have higher risk of COVID-19.[10] [11] [12] [13] COVID-19 is less common in populations with more vitamin D intake – pregnant women, children[14] and persons who eat more fish[15]. Analyzing internal data at the University of Chicago Medicine, we found that the risk of testing positive for COVID-19 is 77% higher for vitamin D deficient persons who are not treated.[16] Several studies find COVID-19 outcomes are better with higher vitamin D levels.[17] [18] [19]

Experimental studies of vitamin D in COVID-19 are needed, especially since efforts to treat some infectious or inflammatory diseases with vitamin D have failed.[20] Negative findings have been attributed to vitamin D doses that are too low, infrequent or do not account for baseline vitamin D levels, dietary sources or sun exposure. Dose is important; the recommended daily allowance (RDA) of 600 IU/day and minimum sufficient level (30 ng/ml) are based on bone heath, not immune function, for which needs are unknown. The Institute of Medicine (IOM) has recommended 4,000 IU/day as safe for broad consumption, noting the lack of clear evidence of benefit and some evidence of toxicity at higher doses, including hypercalcemia and resulting falls in older persons.[21] They also noted that vitamin D supplements are often variable in dosing. For example, even the US Pharmacopia standards for vitamin D, which likely exceed usual practice, specify variation from 90% to 165% of the stated dose.[22] Notably, however, side effects are rare even with 10,000 IU/day,[23] and humans evolved with sun exposure expected to produce 25,000 IU/day. Vitamin D levels in lifeguards are 50-100 ng/ml.[24] Light skin and different vitamin D binding globulin in persons of northern European origin compared to populations more recently from equatorial regions may reflect evolutionary pressures related to sunlight and vitamin D in northern latitudes and may explain increased rates of vitamin D deficiency in Black and Latinx populations,[25] and perhaps increase COVID-19 risk, which is 1.5-4 times greater for Black and Latinx Chicagoans than for White Chicagoans.[26] Experimental studies of vitamin D in diverse populations are urgently justified.

In this context, *the proposed study is innovative most importantly because it proposes to perform a randomized clinical trial to prevent COVID-19.*

II. Purpose:

Our overall aim of this study is to perform a randomized clinical trial (RCT) in a diverse, largely minority, cross-section of at least 2,000 US residents to test the hypothesis that vitamin D supplementation with 4,000 IU/day compared to minimal supplementation with 400 IU/day will decrease COVID-19 incidence (primary outcome) and adverse health outcomes (secondary outcome).

Primary Outcome: patient report of lab-confirmed COVID-19 (and date). Secondary Outcomes: patient report of hospitalization (and date), ICU use, mechanical ventilation, death, patient-reported adherence to vitamin D supplementation, and results of COVID-19 antibody blood spot tests. Key milestones for success will include partner and participant recruitment, including intake surveys, baseline Vitamin D levels and antibody testing for 20% of the sample, vitamin D distribution to the participants, completion of quarterly surveys, end-of-study antibody testing for full sample, and analysis and publication of the study results.

III. Description of protocol methodology:

Summary

Our RCT will test the hypothesis that vitamin D supplementation with 4,000 IU/day compared to minimal supplementation with 400 IU/day will decrease the primary outcome of COVID-19 incidence and secondary outcomes of COVID-19 adverse health outcomes (hospitalization, ICU use, mechanical ventilation and death) in a diverse, largely minority, sample of 2,000 US residents. It has been developed and will be implemented in partnership with key community stakeholders in Chicago and other US cities.

We propose the 4,000 IU/day higher dosage because it is the highest dose the IOM recommended as safe to consume without clinical supervision and we wish to maximize the likelihood of enhancing immune function while minimizing risk of harm in the absence of clinical supervision so the intervention can be broadly scaled at low cost. Based on needs for bone health, the NIH and IOM recommend 600–800 IU/day as adequate for 97.5% of people,[27] so we have chosen the low dosage to be the amount in most multivitamins (400 IU, 2/3 of the RDA), instead of placebo. This ensures almost all participants have total vitamin D intake (including diet) that meets the RDA. As participants might alter their adherence to vitamin D or personal protective guidelines if they knew the dose to which they had been randomized, we propose a blinded-research design.

We will target recruitment of a population that has a high fraction of racial minorities and socioeconomically disadvantaged individuals since these populations have been disproportionately impacted by COVID-19. Latinx and Black residents each constitute about 1/3 of Chicago's population and have COVID-19 incidence (5,626/100,000 and 2,845/100,000, respectively), 1.5-4 times that of White Chicagoans (2,115/100,000).[28] COVID-19 mortality differentials by race are even higher. Moreover, 75% of Black and Latinx persons in the US are vitamin D deficient.[29] [30] Targeting these populations is important most critically because of their urgent needs for interventions to address COVID-19 but also because the high incidence of both COVID-19 and vitamin D deficiency in Chicago’s racially and ethnically diverse population and known northern location make it a scientifically advantageous place to assess the potential benefits of vitamin D supplementation in COVID-19.

Study recruitment will be conducted online to minimize COVID-19 exposure, with vitamin D distribution primarily delivered by mail to participants’ home addresses or by pick-up from community partner locations. Supported by student volunteers and cost-shared staff effort as needed, we will partner with Chicago community organizations to promote and disseminate information about the study to their clients/members during a 2-month promotional campaign. We have established community partners in the Black and Latinx community through our prior work that we will engage as delineated below under Partner Engagement. Promotion and information-sharing strategies will vary by partner, but we expect that, at minimum, partners will send communications to encourage participation and share information about eligibility criteria and how to enroll in the study. The study website will also list pick-up sites for interested participants. Interested participants will be guided to a secure REDCap platform that will provide information about the study and allow

them to virtually enroll and consent to the study, using their email address for enrollment and later survey participation. Enrollment will require completing a 15-minute baseline survey in REDCap that asks about demographics, health and health behaviors (including vitamin D intake and sun exposure), COVID-19 risks. Persons reporting heath conditions indicating risk from vitamin D treatment (hypercalcemia, hyperparathyroidism, kidney stones, pregnancy, lactation) or an allergy to vitamin D will be excluded.

Due to lower than anticipated enrollment in both this study and our other NIH Vitamin D study (IRB20-1302), we will merge study populations. Participants graduating from the 6-month NIH study will be contacted via email and offered the opportunity to participate in this study. These “transitional” participants will then be directed to a new consent form to be enrolled in this study. For participants that have graduated from the NIH study prior to the approval of this amendment (amendment #24 for #IRB20-0847), the study team will reach out via email to invite them to participate. These previous graduates will be considered new participants and will complete the same eligibility screening, consent, and baseline survey process as other new participants.

A minimum of 2,000 eligible participants will be randomized to the 400 IU/day or 4,000 IU/day treatment arms. Each lot of vitamin D supplements will be tested to ensure they are within the dosing tolerance that meets USP standards. Transitional participants will maintain their dosing arm from the previous NIH study; those in the high or moderate dosing arms (10,000 IU/day or 4,000 IU/day) will receive 4,000 IU/day and those who were previously in the low arm (400 IU/day) will receive 400 IU/day. The UCM pharmacy will prepare and package blind-label safety-top medication bottles to be distributed to participants via mail to either their home address or for pick-up from community partners. During the 12-month implementation period, we will ask participants to complete follow-up surveys every 3 months. A sample of the participants (20%) who complete the surveys also have the opportunity to receive a free home COVID-19 antibody blood spot test and Vitamin D level tests at the beginning of the study. Both tests are done at home by the participant themselves. Testing this subset will allow the research team to capture baseline levels of Vitamin D and immunity to COVID-19. We suspect 10% will have positive antibodies and 80-90% will be Vitamin D deficient; these baseline sample tests will confirm the general hypothesis. Originally, we planned for the entire sample to be be offered the opportunity to receive a free, in home COVID-19 antibody blood spot tests at the end of the study. Due to low return rate, we paused end of study testing in early 2024 and now wish to permanently discontinue it. Over the course of this three year study, it is likely that most participants have COVID-19 antibodies, therefore testing is no longer scientifically valuable. Results of the antibody test will help inform research outcomes and may increase completion of quarterly self-reported surveys.

In addition to these questions, we have added some questions on the baseline and follow-up surveys that evaluate sleep habits as well as additional possible risk factors for COVID-19 outcomes. These risk factors—hypertension, diabetes, and mood—are shown to have possible linkages to vitamin D levels. These factors are receiving increased attention in the context of COVID-19 risk and its relationship to low vitamin D levels.[33] While available evidence suggests there is a link between hypertension/cardiovascular disease[34,35,36], diabetes and metabolism,[37,38] depression[39,40,41]/anxiety[42], and vitamin D levels, to-date no causal relationship has been established. This is supported by both evidence as recently as this year,[34,35,41] as well as earlier studies[36,39,40]. Questions about hypertension and diabetes are of our own construction, and questions about depression and anxiety come from validated instruments, the GAD-2 and the PHQ-2. We hope to shed light on the relationship between our outcomes of interest, vitamin D, and these risk factors by asking subjects, both already enrolled and new, about these factors.

Our data management plan will specify procedures to collect, organize, handle, clean, analyze, describe, preserve, destroy, report and share data. We will review self-reported survey data regularly to prevent and monitor missing data. We will record and report all known reasons for study dropout and missing data. For our primary outcome of COVID-19 infection as measured by patient report of clinically confirmed COVID-19 (or viral PCR if available) and COVID-19 seroconversion tests that data collection, hazard models will be used to assess the effect of vitamin D dosing on the outcome. We will first develop hazard ratios for between-group analyses on the primary outcome using log-rank tests, and then Cox proportional hazard models with covariates including vitamin D dose, age, gender, race, ethnicity, sun exposure, exposure of cohabitants,

employment status, and geographic location. Secondary study outcomes of hospitalization, ICU use, mechanical ventilation use and death will be analyzed with longitudinal mixed models with these covariates.

We estimate statistical power using a simulation model based on conservative estimates of the fraction of Chicagoans already immune but unaware they have had COVID-19 (≤10%), monthly incidence of COVID-19 (3%/month for Black and Latinx persons and 1%/month for White persons), size of the true effect of vitamin D on incidence we seek to assess, confidence we wish in that assessment, and length of time participants are followed.[31] [32] Based on this we propose to recruit at least 2,000 participants, yielding 80% power at $p<0.05$ to demonstrate a reduction of 20% or more in COVID-19 incidence due to vitamin D within 12 months of randomization.

IV. Probable duration:

The study period, including recruitment, enrollment, and analysis, will last 36 months. We expect the recruitment period to last 18 months to enroll our target of 2,000 subjects. Once enrolled, subjects' participation will last 12 months total. This includes 12 months of taking a daily dose of vitamin D, answering surveys, analysis to measure outcomes.

Recruitment: December 1, 2020 – February 29, 2023
Research Objective(s) to be achieved: Obtain IRB approval, launch project website, recruit 5-10 community partner organizations to be able to help recruit a total of 2000 participants, recruit 2000 participants with target of completing all recruitment by mid 2022. Complete enrollment, consent and baseline survey and distribute Vitamin D to all participants' homes or other locations at the participant's choosing. Consent at least 20% of sample (200 in treatment arm; 200 in control arm) to complete baseline Vitamin D level tests and baseline antibody tests; mail out at-home testing to consented participants within the first two quarters of the study.

Follow Up, Safety Monitoring and Analysis: March 1, 2021 – February 292025
Research Objective(s) to be achieved: Complete four quarterly follow-up survey. Begin analysis of data.

Project Close: December 31, 2025
Endpoint/Success Measure 1: Successful recruitment of 2000 subjects

Endpoint/Success Measure 2: Successful follow up of at least 80% of subjects to end of study period or study outcome of patient report of clinically confirmed COVID-19, with no difference in follow up rates between arms. Successful testing of COVID-19 antibodies of at least 80% of subjects.

Endpoint/Success Measure 3: Valid assessment of statistical significance of difference in COVID-19 risk (hazard) between treatment and control groups.

Endpoint/Success Measure 4: Collect, analyze and report Vitamin D levels for at least 80% of the 20% of the sample that will receive at-home test kits. We will work closely with the VitD testing distributor (Everlywell) to ensure compliance and test completion among participants and will use text and call reminders if needed.

V. Location of research

The research will take place at the University of Chicago Health Lab. Participants will be recruited through emails and flyers from partner organizations asking for their participation in the study. The recruitment materials will vary by partner, but we will submit an amendment with the final materials used. Actual study recruitment and follow-up processes will be conducted completely online to minimize exposure to COVID-19. Community partners will focus on communicating information about the study and distributing vitamin D supplements if needed. Expected organizational partners currently include Ingalls Hospital, St. Anthony Hospital, Heartland Health, the Greater Chicago Food Depository, and the YWCA. Subjects will be recruited from all of these specified locations via promotional materials.

The UCM pharmacy will prepare and package blind-label safety-top medication bottles to be distributed to participants via mail to a home address or to be picked-up from community partners. For transitional participants, the pharmacy will maintain the previous dosing arm from the NIH study. Those in the high or moderate dosing arms (10,000 IU/day or 4,000 IU/day) will receive 4,000 IU/day and those who were previously in the low arm (400 IU/day) will receive 400 IU/day. Each bottle will contain a 12-month supply of vitamin D tablets.

For 20% of the subjects, blood samples to test for Vitamin D levels and COVID-19 antibodies will be obtained at the beginning of the study by two different vendors: Everlywell and Rush Hospital.Originally, the entire study sample was offered antibody testing at the end of the study**.** We paused end of study antibody testing in early 2024 due to low return rate and now wish to permanently discontinue it. Over the course of this three year study, it is likely that most participants have COVID-19 antibodies, therefore testing is no longer scientifically valuable. The vitamin D test is a standard commercially available vitamin D home test and provides a number level measured in ng/ml. We currently plan to source this from Everlywell (https://www.everlywell.com). We will register vitamin D test kits with Everlywell on each subject's behalf, using subject's name and the test kit ID. Our current plan for the antibody test is to use the 'Rush Covid-19 Multi-analyte Serological Assay', which evaluates an immune response to four SARS-CoV-2 antigens, including the spike S1, nuclocapsid, matrix, and envelope glycoproteins in individuals convalescing from Covid-19. This assay has been developed by Dr. Jeff Borgia at Rush and is built on the Luminex immunobead platform and was specifically engineered to provide greater confidence in diagnostic results through the use of an algorithm integrating results from the four independent assay findings and requires no less than two analytical findings to deliver a diagnostic result to significantly reduce the rate of 'False Negative' misclassifications. The analytical performance characteristics of the Rush Covid-19 Multianalyte Serological Assay have been calculated to be 100% sensitivity and 99.6% specificity with a 99.62% accuracy when evaluated against 44 cases with clinically-diagnosed Covid-19 within a window of 8 and 30 days from testing and 998 cases of pre-Covid-19 era (i.e. prior to 6/2019) specimens. This assay will be performed in Dr. Borgia's Biomarker Development Facility, which falls under Rush Pathology's CLIA license. Both the Vitamin D and antibody blood tests will be self-administered, at-home, with no in-person contact, and will be mailed back to the vendors.

Survey data will be collected quarterly by the study team via a HIPAA-secure Redcap survey. Data will be stored on encrypted-password protected computers, locked filing cabinets, and secured encrypted drives in these offices.

VI. Special precautions, including dose modifications

We will not make modifications of the vitamin D dose based on the study design. As described in Section XI, "Monitoring Subject Safety", subjects will be advised to cease vitamin D supplementation if they experience adverse effects commensurate with hypercalcemia. Subjects will be asked to discontinue taking any non-doctor recommended supplements containing more than 1,000 IU of vitamin D when they begin taking study-provided supplements.

VII. Description of experimental controls and use of placebos:

In this 2,000-person two-arm, double-blinded randomized controlled trial, we will randomize half the subjects to low dose vitamin D therapy (400 IU/day), which will serve as the control group, and half to a moderate (4,000 IU/day) dose. Participants will have the option of being randomized to the moderate versus low strategy. Transitional participants will maintain their dosing arm from the previous NIH study; those in the high or moderate dosing arms (10,000 IU/day or 4,000 IU/day) will receive 4,000 IU/day and those who were previously in the low arm (400 IU/day) will receive 400 IU/day. The low dose was selected to ensure that all subjects had a high likelihood of a total vitamin D intake that met the recommended daily allowance (RDA).

VIII. Type and number of experimental subjects:

As noted, we plan to recruit 2,000 participants. Recruitment information will vary by partner, but recruitment materials and emails will have links to the research website for additional information and enrollment. On the website, interested participants will complete a 2-minute eligibility questionnaire to determine their eligibility for the study. Persons reporting heath conditions indicating risk from vitamin D treatment (hypercalcemia, hyperparathyroidism, some medications, kidney stones, pregnancy, lactation) or an allergy to vitamin D will be ineligible.

Individuals are eligible to enroll in the study if they:

1. Are 18 years or older.

Individuals are excluded from the study if they:

1. Report being pregnant, planning to become pregnant, and/or report breastfeeding during the study period.
2. Report a history of chronic kidney disease, including a history of abnormal GFR and/or creatinine.
3. Report a history of hyperparathyroidism.
4. Report a history of increased falls.
5. Report a history of hypercalcemia.
6. Report a history of gastrointestinal absorptive disorders, including having undergone bariatric surgery.
7. Report a history of kidney stones (1 in past year or 2 in lifetime).
8. Report already taking more than 400 IU of vitamin D daily as recommended by a medical professional, and/or taking D2.
9. Report a history of sarcoidosis.
10. Report an allergy to vitamin D.
11. Are unwilling to take daily vitamin D.

Eligible participants will be guided to a secure REDCap website where they will complete a electronic consent form and a 15-minute baseline survey that asks about demographics, health and health behaviors (including vitamin D intake and sun exposure), and COVID-19 risks. For transitional participants from the NIH study, those who have recently completed the NIH study will not take a new baseline survey, as they will have recently completed a follow-up survey from the previous study that includes the same questions. These participants will be directed to a new consent form that includes eligibility criteria to be enrolled in this study. Participants that have graduated from the NIH study prior to the approval of this amendment (amendment #24 for #IRB20-0847), will be considered new participants and will complete the same eligibility screening, consent, and baseline survey process as other new participants.

As noted, we plan to recruit 20% (200 participants) of the eligible participants at the beginning of the study to receive and complete a self-guided, at-home vitamin D level and a COVID-19 anti-body test at their places of residence. At the end of the study, all study participants were to be offered an at-home COVID-19 antibody test kits. Please note that these tests were halted in early 2024. Participants will have the option to opt in or out of these tests on the study consent form. Participants who opt out of the at-home antibody test will still be able to participate in the study and receive Vitamin D supplements.

Participants will have the option to receive their vitamin D results and their antibody test results at the end of the study. If positive, communication of the antibody results will clearly indicate that there is evidence of past exposure to SARS Cov2 virus but that it is not known whether such indication is related to the presence or absence of immunity to future infections. To ensure only secure release of information, request for release of information will only be honored when communicated by email or text from the email address or cell phone number used to register the participant, and will be provided by mail only to the address registered by that individual upon enrollment.

During the 12-month implementation period, we will ask participants to complete online follow-up surveys every 3 months. The follow-up surveys at months 3, 6, and 9 will ask the participant whether the they have had a

confirmed diagnosis of COVID-19, and the date if so, ask about rates of study medication adherence, and assess for changes in the intake questions about use of other supplements, sun exposure, diet, exercise, and COVID-19 exposures. If the patient is reported to have had COVID-19, we ask about severity, including hospitalization and duration, type of COVID-19 testing used, and need for mechanical ventilation and duration, and COVID-19 symptom severity using the BEAT-19 adapted to reflect the worst symptomology during their period of active COVID-19.

IX. Description of statistical analysis

The higher doses we propose, 4,000 IU/d is expected to maximize the vitamin D treatment effect if one exists compared to a lower high dose as in a Harvard study currently underway which explores 2,000IU/day vitamin D. While the Harvard study gives vitamin D in the context of acute infection or household exposure so vitamin D levels may not respond quickly enough to affect outcomes (perhaps both for treatment and prevention), our proposed design seeks to raise vitamin D levels in advance of sustained risk in community members who are disproportionately at increased risk of COVID-19, increasing the underlying risk of COVID-19 that increases the expected treatment effect, and therefore increases overall statistical power.

For our primary outcome of COVID-19 infection as measured by patient report of clinically confirmed COVID-19 (or viral PCR when available) and secondary outcomes of COVID-19 antibody seroconversion and disease severity (hospitalization, ICU stay, ventilator use, death), hazard models will be employed to assess the effect of each vitamin D dosing strategy on the outcome. We will first develop hazard ratios for between-group analyses on the primary outcome using log-rank tests, and then develop Cox proportional hazard models to model the hazard function on a set of covariates including but not limited to vitamin D dose, baseline vitamin D levels, age, gender, race, ethnicity, sun exposure, sleep habits, exposure of cohabitants, job type, and study site. We will also control for randomization date to adjust the underlying hazard function for COVID-19 prevalence over time. As noted above, we estimate statistical power using a simulation model based on conservative estimates of the fraction of Chicagoans already immune but unaware they have had COVID-19 (≤10%), monthly incidence of COVID-19 (3%/month for Black/Latinx persons and 1%/month for White persons), size of the true effect of vitamin D on incidence we seek to assess, confidence we wish in that assessment, and length of time participants are followed. Based on this we propose to recruit at least 2,000 participants, yielding 80% power at $p<0.05$ to demonstrate a reduction of 20% or more in COVID-19 incidence due to vitamin D within 12 months of randomization.

X. Potential risks and benefits to subjects:

There are four minimal risks associated with the baseline survey and overall study participation.

Confidentiality risk: There is a small chance that someone could find out that a subject is participating in this study or access the study data. However, we will take every precaution to protect subject privacy. In addition to using the Health Labs limiting data access to specified research staff, each person on the research team will be trained in human subject's protections and HIPAA policies and best practices. Additionally, we will minimize the risk of exposure or accidental disclosure to the fullest extent possible by maintaining all research data only on secure, password and firewall protected network computer accounts, limiting access to those data files to members of the research team, and only communicating data findings in the aggregate form. All names and other protected health information will be deleted from study records whenever possible. For example, in analytic datasets, participants will typically be identified by their unique study numbers.

Vitamin D toxicity risk: There is a very small chance that a participant could develop symptoms of vitamin D toxicity, including hypercalcemia, falls, hyperparathyroidism, or kidney stones. The health issues above are included in our exclusion criteria to further minimize this risk. Risk of toxicity could also be increased if patients take other vitamin D supplements, which they will be advised not to do. All patients will be informed about symptoms of vitamin D toxicity and advised to contact their physician if symptoms occur. The highest dose

administered as part of the study is considered by the National Academy of Medicine as low risk for the vast majority of the population without direct medical supervision,

Vitamin D insufficiency risk: There is a risk that some people in the 400 IU group may consume less than the daily RDA (600 IU) once diet (~200-300 IU/day) and the supplement are accounted for, but this risk is very low and reduced compared to not taking any vitamin D supplement. Persons whose doctors have advised higher doses will be excluded from the study.

Blood spot testing risk: The risks of a finger-prick required for blood spot testing are minor and include slight discomfort, the possibility of a bruise on the participant's fingertip and, rarely, infection or fainting. A small amount of blood may ooze from the fingertip, which can be easily stopped with gauze.

Protections Against Potential Risks
We have strict data security and confidentiality procedures in place as described below. All research staff are required to sign a confidentiality pledge and to complete human subject's research training. Moreover, the data will be stored on a firewall protected, restricted access server that is only accessible to select Health Lab researchers. Additionally, the survey data will be collected through University of Chicago's REDCap system which meets all HIPAA requirements for data security. For the potential risks relating to vitamin D toxicity and insufficiency, we think these are very low but will inform participants of them prior to their participation in the study.

Furthermore, participants will be given a contact number and email address for study staff and encouraged to monitor for and report any possible signs of vitamin D toxicity, and to report any COVID-19 symptoms or positive tests. Participants will also be given instructions for any home testing-related risks such as bruising or bleeding risk. Participants will be advised to seek medical care immediately for any problem they view as urgent and the PI will be informed within 24 hours. In the event of an unanticipated problem, study staff will inform the PI within 24 hours, and document study protocol number, participant's initials, date of event, and description of event. Study staff will then formally submit an "Unanticipated Problem Event Form" to the UChicago Biological Sciences Division IRB within 5 days.

Potential Benefits
We believe this research will have large benefits to society. One of the key challenges in addressing COVID-19 is the lack of rigorous RCT's on potential pharmacologic solutions to pandemic. Thus, this research has the potential to contribute to the ongoing solutions being tested to alleviate the health impacts of COVID19 in our society. Furthermore, as the world awaits a potential vaccine, many policymakers and communities will be interested in understanding how low-cost preventative measures can help people at risk of contracting COVID-19.

XI. Monitoring of safety:

The current study represents a single-site clinical trial in which the risks inherent to the intervention are of minimal risk to participants given the long-standing use of vitamin D supplementation with few adverse events. The protocol's exclusion criteria and monitoring plan limit the likelihood that subjects will develop a severe medical condition during the course of this study. Commensurate with these risks, the PI Dr. Meltzer, with support from the Clinical Advisory Committee and co-investigator Dr. Schram, will incorporate this study into an already established formal Data and Safety Monitoring Board (DSMB). Given the low reported COVID rates and low reported rates of potential side effects from vitamin D treatment, we will utilize the DSMB we have already convened for our NIH Vitamin D study (IRB20-1302), which currently meet quarterly. This independent DSMB has been set up and is comprised of three experts, a Chair and two members, who have been providing regular oversight for that study. These individuals are senior faculty members and hold expertise in clinical trial oversight and design, biostatistics, endocrinology, pulmonology and critical care medicine. Further, these individuals are the same experts we identified in the DSMB Charter below.

To date, the NIH DSMB has met twice, once in June 2021 and again in August 2021, and has identified no patient safety concerned (note the NIH study includes the same doses as Vitamin D as this study, as well as one arm with a higher dose of 10,000 IU per day). We plan to bring more data analysis from this study to next quarterly joint-DSMB In December 2021. We believe this is justified given the absence of any evidence of adverse outcomes in this study to date. In addition, due to lower than anticipated enrollment and the studies being so complementary, we plan to eventually combine the two studies samples at a later date and will submit additional amendments to reflect this change. In the meantime, introducing this study to the existing DSMB is one of the first steps of merging these studies.

The protocol's safety and monitoring guidelines as described below have been created in collaboration with the already-identified members of the Clinical Advisory Committee, a committee of interdisciplinary clinicians who will provide the PI clinical support and guidance. The protocol will be overseen by the DSMB which will provide continued guidance on administration of the study protocol and management of any adverse events. The DSMB will work closely with the PI, co-investigators, and Clinical Advisory Committee, monitoring the intervention on an ongoing basis and meeting quarterly to review intervention data and provide recommendations as needed for modifying the intervention.

Ensuring subject safety
The proposed study will be supervised by PI and the Clinical Advisory Committee, with oversight by the University of Chicago Biological Sciences Division Institutional Review Board (UCM BSD IRB). All subjects will sign an electronic consent. Additional safety precautions include: 1) exclusion of individuals for whom higher dose vitamin D supplementation is more likely to be unsafe, 2) written materials on possible side effects of vitamin D supplementation provided at consent (I.e., risk of falls, symptoms of hypercalcemia), 3) participants will be advised to report adverse events (email and study phone number provided), 4) independent testing of vitamin D supplements to ensure accurate potency, 5) participation in the study is entirely online and remote, mitigating any additional spread of COVID-19 and 6) well-trained research staff.

Monitoring of the intervention
The study will be monitored by the study investigator(s). Based on conversations with our Clinical Advisory Board and prior studies, we expect very few people to become hypercalcemic due to study medication. The PI(s) will also conduct an interim analysis of COVID-19 incidence among the two study arms approximately 6 months into the study to determine study continuation in addition to routine safety review.

<u>Data and Safety Monitoring Board (DSMB) Charter</u>

Trial Monitoring
The trial will be monitored by an independent Data and Safety Monitoring Board for this study made up of three members of the UC BSD faculty with experience in clinical trial management and expertise in critical care, pulmonology, and biostatistics. The DSMB members are: Edward Naureckas, MD, DSMB Chair, Theodore Karrison, PhD, and George Bakris, MD.

Monitoring Frequency
The DSMB will meet to review initial laboratory values and quarterly thereafter to review the overall protocol administration, review adverse events, and trends in laboratory values for determination of safe progression of the study. DSMB members will schedule additional meetings as needed to review adverse events.

Adverse Event Management
Adverse Events, Serious Adverse Events and Unanticipated Problems will be reported to members of DSMB by the PI immediately for independent review. These events will be managed by the PI and Co-Investigator at the direction of the DSMB and UChicago Medicine Biological Sciences Division Institutional Review Board.

<u>XII. Payment to subjects:</u>

Subjects will not receive payment or gifts of any kind for participation in the study.

XIII. Procedures to obtain and record informed consent:

After completing the eligibility questionnaire on the REDCap page, participants will be redirected to the electronic consent form which they'll have to complete prior to beginning the baseline survey. Participants will also be informed that the electronic consent form will cover all subsequent study participation. The electronic consent form will detail the voluntary nature of participating in the research, procedures, potential risks as well as our prepared measures and protocols to maintain their confidentiality. In the consent form, subjects will be informed that they will be receiving a daily dose of vitamin D, either low or moderate, and must self-administer this dose each day and that they are blinded to the dose they receive. The consent form will also ask participants if they are interested in the at-home blood testing for Vitamin D levels and COVID-19 antibodies at the beginning or end of the study. There is no consequence for opting out of the blood tests.

When participants have read through the consent form, they will indicate their consent by signing their name and entering the current date and time. If they do not consent, they may simply exit the page. When a participant chooses to consent to the study, a signed PDF copy of their eConsent form will be automatically emailed to them upon completion with the following text: "Thank you for consenting to participate in the study. Attached is a copy of the consent form you signed, which you may keep for your personal records. If you would like a copy that includes the investigator's signature, please email us at vitd@uchicago.edu." After consenting, subjects will be redirected to begin the baseline survey. Once a subject finishes the baseline survey, the research team will reach out to them to confirm next steps, as well as whether the subject is ready or not to receive test kit(s) should they have consented to optional vitamin D level and/or COVID-19 antibody testing. This communication step both ensures any optional testing is readily performed before vitamin D is sent to subjects and to avoid further delays before vitamin D is sent.

XIV. Procedures to maintaining confidentiality:

Health Lab has strict data security and confidentiality procedures in place as described below. All research staff are required to sign a confidentiality pledge and to complete human subject's research training. Moreover, the data will be stored on a firewall protected, restricted access server that is only accessible to select Health Lab researchers. Additionally, the survey data will be collected through University of Chicago's REDCap system which meets all HIPAA requirements for data security. For the potential risks relating to vitamin D toxicity and insufficiency, we think these are very low but will inform participants of them prior to their participation in the study.

Furthermore, participants will be given a contact number and email address for study staff and encouraged to monitor for and report any possible signs of vitamin D toxicity, and to report any COVID-19 symptoms or positive tests. Participants will also be given instructions for any home testing-related risks such as bruising or bleeding risk. Participants will be advised to seek medical care immediately for any problem they view as urgent and the PI will be informed within 24 hours. In the event of an unanticipated problem, study staff will inform the PI within 24 hours, and document study protocol number, participant's initials, date of event, and description of event. Study staff will then formally submit an "Unanticipated Problem Event Form" to the UChicago Biological Sciences Division IRB within 5 days.

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XVI. Description of recruiting methods:

Script from end of NIH study for transitional subjects
Recruitment from NIH pool

Given the current risk of COVID-19, we will use virtual recruiting as much as possible. Recruitment information will vary by partner, but recruitment materials and emails will have links to the research website for additional information and enrollment. On the website, interested participants will complete a 2-minute eligibility questionnaire to determine their eligibility for the study. Persons reporting heath conditions indicating risk from vitamin D treatment (hypercalcemia, hyperparathyroidism, some medications, kidney stones, pregnancy, lactation) or an allergy to Vitamin D will be ineligible. Eligible participants will be guided to a secure REDCap website where they will complete a consent form and a 15-minute baseline survey that asks about demographics, health and health behaviors (including vitamin D intake and sun exposure), and COVID-19 risks. We plan to recruit 20% (200 participants) of the eligible participants at the beginning of the study to receive and complete a vitamin D level and a COVID-19 anti-body test at their places of residence. At the end of the study, all study participants will be offered an at-home COVID-19 antibody test kits. Participants will have the option to opt in or out of these tests on the study consent form.

We will also operate a telephone line staffed by our research staff on which potential participants can have questions answered and assistance with assessing eligibility and complete the online intake survey. Study recruitment will take place within the first 2 months of the study period. Though our primary recruitment approaches will be virtual, the community partners will also hang posters and flyers throughout their agencies.

Health Lab researchers and analysts will monitor survey completion at each wave and send out email and telephone reminders to subjects who have not completed the survey.

XVII. Notification of primary physician:

Subjects' primary care physicians will not be notified of subjects' involvement in the proposed research. One exception to this is if a subject is counseled to seek care from their primary care physician due to an abnormal laboratory result or symptom as described above.

XVIII. Anticipated coordination between inter-departmental faculty:

We have created a Clinical Advisory Committee to support our multiple research lines investigating the relationship between vitamin D and COVID-19 and plan to regularly coordinate with the faculty on this Committee.

Our multidisciplinary clinical advisory committee will provide continued guidance on administration of the study protocol and will support the PI and DSMB in monitoring safety. Committee members and expertise include: Raphael Lee MD, ScD (surgery, dermatology, basic science), Tamara Vokes MD (endocrinology), Marc Bissonnette MD (gastroenterology), Tipu Puri MD, PhD (nephrology), Julian Solway MD (pulmonary, translational research), Mark Ratain MD (oncology, clinical pharmacology), and Kevin Colgan MA (pharmacy). Members of the Committee will meet quarterly with the PI to review study progression and results to ensure broad application and dissemination of findings.

XIX. Pregnancy test

Subjects will not be asked to take a pregnancy test before or during the study. We will ask potential subjects during recruitment screening to self-report pregnancy. If a potential subject self-reports pregnancy, they will be excluded as noted above. Upon enrollment, subjects will be asked to inform the study team if they become pregnant during the study.

XX. Rationale for excluding women/minorities and/or children:

We will not recruit those who are considered special populations. We are excluding pregnant and breastfeeding women because they have unique guidelines for supplementation and to protect the fetus and infants. We are excluding children, prisoners, and neonates due to the study design and study population.

XXI. Unblinding protocol

At the end of the intervention period for all participants in this protocol, we will unblind participants to their vitamin D dose assignment as detailed in "Dose Unblinding Email". We will do this at the end of the intervention for all participants because we, as the study team, are blinded to dose assignments until the end of the intervention period for all participants.

XXII. Notification of Publication

Upon publication of results related to this study, we will notify all participants in this protocol of the availability of these results. In that correspondence, we will attach the most-final version of the publication as allowed by the publisher.

APPENDIX A: Virtual consent method

1. Once interest has been expressed and the individual has been screened for eligibility in the REDCap intake form, the subject will be redirected to an electronic consent form. For transitional NIH study partipants, eligibility will be affirmed on the consent form. Participants that have graduated from the NIH study prior to the approval of this amendment (amendment #24 for #IRB20-0847), will be considered new participants and will complete the same eligibility screening, consent, and baseline survey process as other new participants

2. After a subject has opened the electronic consent form, the subject will read through the form, which will detail the voluntary nature of participating in the research, procedures, potential risks as well as our prepared measures and protocols to maintain their confidentiality.

3. When participants have read through the consent form, they will indicate their consent by signing their name and entering the current date and time. If they do not consent, they may simply exit the page.

4. When a participant chooses to consent to the study, a signed PDF copy of their eConsent form will be automatically emailed to them upon completion.

5. Within three working days, a Health Lab RC/RA will review to make sure consent form is valid.