Protocol Title:Low Versus Moderate to High Dose Vitamin D for Prevention of COVID-19
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I. Background

There is an urgent need for effective interventions to prevent and treat COVID-19.Vitamin D deficiency has recently been identified as potentially contributing to the spread of COVID-19 and vitamin D supplementation has been identified as having potential in prevention and treatment.[1] There is evidence that vitamin D can prevent or improve outcomes in many infectious and inflammatory conditions, including acute and chronic respiratory infections, and an increasing understanding of its immunomodulatory and anti-inflammatory functions. A recent meta-analysis found a 70% reduction in viral respiratory tract infections among persons with vitamin D deficiency randomized to vitamin D treatment.[2] The potential importance of vitamin D in the spread of COVID is supported by the fact that nearly half the US population is vitamin D deficient, with higher rates among persons with darker skin and/or lower sun exposure, including those living in higher latitudes in the winter, nursing home residents and health care workers, who also have greater risk of COVID-19.[3,4,5,6] In contrast, populations with greater vitamin D intake, including pregnant women, children[7] and persons who eat more fish,[8] have lower rates of COVID-19. Also, we have recently analyzed internal data at the University of Chicago Medicine and found that the risk of testing positive for COVID-19 is 77% higher for vitamin D deficient persons who are not treated.[9]

Though these findings are suggestive, experimental studies are needed to establish if vitamin D treatment can decrease the risk or improve outcomes of COVID-19. Such data are especially important because efforts to treat some infectious or inflammatory conditions with vitamin D have not been successful.[10] Negative findings have been attributed to vitamin D dosages that are too low, infrequent, or fail to account for baseline vitamin D levels, dietary sources or sun exposure. Dose is very important; the recommended daily allowance (RDA) of 600 IU/day and definition of sufficient level (≥30 ng/ml) are based on effects on bone heath, not immune function, for which needs are not known. The Institute of Medicine (IOM) has recommended 4,000IU/day as safe for regular consumption but did not recommend higher doses based on the lack of clear evidence of benefit for higher doses and some evidence of possible toxicity, especially hypercalcemia and resulting falls in older persons.[11] However, humans evolved under conditions of sun exposure that would produce 10,000-25,000 IU/day – far more than recommended by the IOM, and lifeguards have vitamin D levels of 50-100 ng/ml.[12] Moreover, large case series have found low rates of side effects (e.g., hypercalcemia) with doses as high as 10,000 IU/day.[13] Experimental studies to define ideal vitamin D dosing are justified in populations with different needs for, risks of, and preferences related to vitamin D treatment. Randomizing subjects to a minimal dose of vitamin D (e.g., 400 IU/day) or a high dose (e.g., 10,000 IU/day) might seem to maximize statistical power to assess the effectiveness of vitamin D in COVID-19. However, some potential research subjects may consider the high dose to carry unnecessary risks, so we propose offering subjects the option to be randomized to moderate dose, 4,000IU/day, if they are not comfortable to maximize participation.

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

We propose to seek to recruit participants to be randomized to low versus high (10,000 IU/day) or low versus moderate (4,000 IU/day) dose vitamin D and pool these populations for our primary analyses. The study will focus on health care workers and other individuals from Chicagoland communities with elevated risk of exposure to COVID-19. In addition to their high exposure rates to COVID-19, focusing on health care workers is also useful because they have high rates of vitamin D deficiency due to indoor work and long hours, are likely to tolerate high doses of vitamin D given their generally good health, and have ready access to monitoring for dose toxicity. In addition to HCWs, we have decided to expand our focus to include other adults in Chicagoland communities. Due to vaccine distribution in the coming months prioritizing HCWs, we want to offer the potential benefits of vitamin D in COVID-19 prevention to those who will not have prioritized access to vaccines, or choose to be vaccinated at present. **Thus, our overall aim is to compare the risks of COVID-19 in health care workers and other Chicagoland adults at increased risk of COVID-19 randomized to low (400 IU/day) vs. moderate (4,000 IU/day) or high (10,000 IU/day) dose vitamin D. Our specific aims are:**

Aim 1: To compare the risk of developing COVID-19 in health care workers and other Chicagoland community members at increased risk of COVID-19 randomized to low vs. moderate or high dose vitamin D. We hypothesize that moderate and high dose therapy will reduce rates of COVID-19 compared to low dose therapy because vitamin D will reduce symptomatic infection that prompts testing for COVID-19. Our primary analysis will pool subjects randomized to the moderate or high dose and compare them to low dose subjects, with secondary analyses comparing low to moderate and low to high. The design thus seeks to maximize potential to find statistically significant results between different dosages given uncertainty in subject willingness to be randomized to different doses.

Aim 2: To compare COVID-19 seroconversion in health care workers and other Chicagoland community members at increased risk of COVID-19 randomized to low vs. moderate or high dose vitamin D. As we suspect the main effect of vitamin D may be to decrease symptomatic disease we hypothesize that there may be little or no difference in seroconversion.

Aim 3: To compare COVID-19 outcomes (hospitalization, ICU stay, ventilator use, death) in health care workers and other Chicagoland community members at increased risk of COVID-19 randomized to low vs. moderate or high dose vitamin D. Consistent with several recent observational analyses, we expect higher doses to improve COVID-19 outcomes.

Comparing the effectiveness of low to moderate and high dose vitamin D dosing strategies for COVID-19 is important and innovative given the global impact of COVID-19 and need for studies that examine the effects of higher doses of vitamin D and the focus on higher risk HCWs and other individuals in high-density, racially diverse metropolitan areas, both groups which already have increased risk relative to the general population.

We are additionally interested in the relationship between vitamin A and vitamin D, and how they impact immune response in the context of COVID-19. Therefore, we are adding additional Aims 4-6.

Aim 4: Create a biobank of PBMCs from a panel of 500 individuals with well characterized levels of vitamin A and D. We propose to cryopreserve PBMCs derived from ~20 ml of blood to test the impact of vitamin A and D levels in the regulation of immune responses. PBMCs will be used as described in Aims 2 and 3 below. In addition, and upon the availability of additional funds, we will collect genetic data from all individuals to test for the impact of genetics to inter-individual differences in immune response and its interaction with vitamin A and D levels.

Aim 5: Evaluate the impact of vitamin A and D levels on immune function. We will perform immunophenotyping of PBMC using a panel of 30 antibodies that allow characterizing the different immune cell populations found in circulation, as well as their functional status. Using these data we will ask if

there is an association between the prevalence of certain immune cell population or functional potential and vitamin A and D levels.

Aim 6: Evaluate the impact of vitamin A and D levels on PBMC gene expression levels. We will perform transcriptional profiling of PBMC from a panel of 100 individuals enriched for individuals on the two extremes of the distributions of vitamin A and D levels. These data will be used to identify changes in gene expression levels that differ between individuals showing low- vs high-vitamin A/D levels.

Other protocol linkages

Linkage to protocol # 20-2183:
Protocol 20-2183 will use existing samples drawn under this protocol (further described in Section III) to analyze the following aims:

Aim 7: To test the hypothesis that oral vitamin D3 intervention will inhibit the renin- angiotensin system and reduce the incidence and/or severity of COVID-19 infections. A dose response will be tested in this study by comparing 400 IU to 4,000 IU to 10,000 IU vitmin D3.

III. Description of protocol methodology

Summary

To meet our aims, we propose a 2,000-person two-arm, double-blinded randomized controlled trial in HCWs and other Chicagoland community adults at University of Chicago Medicine (UCM) and Rush University (RU) selected to ensure safe administration of low compared to moderate or high dose vitamin D that will randomize half the subjects to low dose vitamin D therapy (400 IU/day) and half to moderate (4,000 IU/day) or high (10,000 IU/day).

During the consent process, study participants will be able to choose between being randomized between the low versus high dose strategy or the low dose versus moderate dose strategy. Moderate and high dose treatment participants will be combined for analysis as described below. The moderate and high treatment doses were chosen with help from our clinical advisory committee because they are generally considered safe and potentially effective among persons who meet our inclusion criteria based on medical history and baseline laboratory values (normal calcium and PTH, and negative COVID-19 antibodies). 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).[14,15] All patients will be informed of whether their baseline calcium and PTH are normal. Patients who are still eligible for study participation after initial testing will be asked prior to randomization whether they are still willing to participate to maximize retention and minimize dropout. After randomization, subjects will be instructed to pick up their 9-month supply of vitamin D at either UCMC or Rush University corresponding to their randomized, blinded dose. For those unable to pick up their bottles in-person, the UCM IDS pharmacy will mail the subject their bottle. We propose a 3 to 9-month study period, depending on when the subject was recruited, as described in the study timeline of Section IV. Subjects recruited after September 30th will participate in the intervention 6 months to align with study timeline and funding. Subjects enrolled between January 1, 2023 and March 31, 2023 will take the study drug until June 2023, so at least 3 and no more than 6 months. Chart review for subjects will occur for 12 months after the active intervention period has ended. This study timeline is further detailed in Tables 1-4 of Section IV in the currently approved protocol document. Adverse events, defined as hypercalcemia or unexplained increased falls in either study arm will prompt discontinuation of the study medication and further evaluation as reported in the monitoring section below.

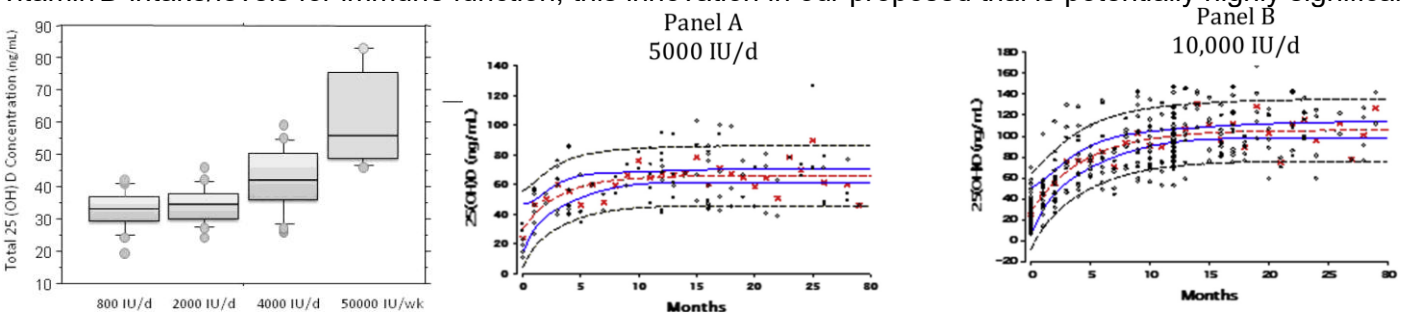
On the advice of our Clinical Advisory Council faculty members, patients who test for PTH >85pg/dL but have calcium levels <10mg/dL and low vitamin D (<30ng/mL) will be eligible to continue in the study because those

who have elevated PTH, but low vitamin D and normal calcium likely have secondary hyperparathyroidism due to low vitamin D levels. However, since there is some risk that the PTH elevation in these patients is not due to low vitamin D, but instead primary hyperparathyroidism, we will check their calcium levels 30 days after they begin vitamin D supplementation and will follow the referral algorithm in the protocol should their calcium level become abnormal. Given this, we plan to reach out to subjects who have been previously disqualified but now meet the inclusion criteria to continue in our study to see if they still would like to participate. If they would like to, we will dispense them their randomized dose of vitamin D and follow up with calcium testing in approximately one month. Our exclusion criteria has therefore been modified to exclude those with PTH>85pg/dL but with calcium ≥10mg/dL and normal levels of vitamin D (≥30ng/mL).

Dose, Frequency, and Administration of the Intervention

Subjects will receive one bottle of a 9-month supply of a vitamin D dose depending on the arm they are randomized to and contingent on their preference for moderate vs. high vitamin D dose. At the end of the study, subjects will be asked to discard any remaining pills in their bottle given that some subjects participate for 3-6 months of follow-up if recruited after October 1, 2022. A low-dose treatment arm (400 IU) will be compared with those in the moderate (4,000 IU/day) to high (10,000 IU/day) treatment arm. Subjects will retrieve blinded bottles containing a 9-month supply of vitamin D at the DCAM pharmacy if they live or work closer to UCM, and at the Rush University Professional Office building, 1725 W. Harrison St. Suite 739 if they live or work closer to Rush. In the event subjects are unable to pick up their vitamin D bottles in these physical locations, bottles will be mailed to an address they provide. The source of the intervention will be the University of Chicago Investigational Drug Service where the bottles will be distributed to DCAM and James Moy's lab at Rush.

1) Dosing. The proposed trial will compare high dose (10,000 IU/d) or moderate dose (4,000 IU/d) to low dose (400 IU/d) vitamin D as opposed to lower doses, e.g., 2,000 IU/d maximum dose in the Harvard trial. Schwartz et al. (left panel below) show that 800 or 2000 IU/d produce average levels of only about 35 ng/ml compared to 40-45 ng/ml for 4000 IU/d and 55 ng/ml for 50,000 IU/week. Data from Mc Cullough et al. (Panel A, B below) shows that long-term administration of 10,000 IU/d produces much higher steady state vitamin D levels (about 95 ng/ml) compared to 5000 IU/d, producing about 60 ng/ml. Given the data discussed above on uncertainty about the optimal vitamin D intake/levels for immune function, this innovation in our proposed trial is potentially highly significant.



2) Safety for higher dosing. As stated above, there is strong evidence that safety risks up to 10,000 IU/d are minimal. Nevertheless, our study provides both frequent lab monitoring for side effects and education to enable subjects to recognize and respond to any symptoms of side effects if they occur. We also screen out participants with co-morbidities that predispose them to adverse outcomes from higher dose vitamin D supplementation. We have another vitamin D/COVID-19 study in progress with medically complex patients that will provide data on effectiveness and safety under intensive monitoring in that population.

Procedures and Materials

Blood labs will be taken from subjects at intake, 3 months, 6 months and, if in the 9-month cohort, 9 months. If subjects are in the 2023 cohort (defined as recruited on or after January 1, 2023) blood labs will be taken from

subjects at intake, 3 months, and, if they are recruited in January or Feburary 2023, they will have one final blood draw in June 2023. Labs for UCM subjects will be drawn at DCAM phlebotomy, River East phlebotomy, or Ingalls Hospital, depending on the location most convenient for the subject. Labs for Rush subjects will be drawn by James Moy at his Rush lab.

Intake labs will measure baseline calcium and PTH levels, presence of COVID-19 antibodies, baseline vitamin D levels, and vitamin A levels. Where possible given constraints related to sample storage, analysis and available funding for free vitamin D measurement, vitamin D measurements will include free vitamin D levels measured by ELISA assay, as well as total vitamin D levels.

For Aim 7, Intake labs for UCM subjects will additionally measure vitamin D-binding protein (DBP), RAS components (Renin, Ang II, soluble ACE and ACE2), bradykinin, inflammation markers (IL-6, TNF-a, IL-1b and CRP), and SARS-CoV-2 antibodies agains S-protein and N-protein.

Follow-up labs (excluding the final lab) will measure PTH levels, calcium levels, vitamin A levels for those who have high baseline vitamin A, and vitamin D levels for monitoring of the intervention's safety and outcomes, and COVID-19 antibodies for monitoring of the outcome. Records of these levels will be kept by the UCM and RU PIs and laboratories.

Final labs will measure calcium levels, vitamin D levels for monitoring of the intervention's safety and outcomes, and COVID-19 antibodies for monitoring of the outcome. Records of these levels will be kept by the UCM and RU PIs and laboratories. Final labs will not measure PTH levels. During the intervention period, our study monitors PTH levels for active participants, because primary hyperparathyroidism is a risk factor for vitamin D toxicity. Given that participants will no longer be taking study-provided vitamin D after their final lab draw, we do not believe it is medically necessary or an appropriate use of limited study resources to continue checking PTH levels with this final draw.

Those who enroll in March 2023 will have baseline labs in March 2023 and final labs in June 2023 (3 months later). This is due to the end of study funding in June 2023. This is further specified in Section IV, Table 4 below.

Testing for calcium, PTH, and vitamin D will be done at UCM (UCMC, River East, or Ingalls) or Rush (James Moy's lab), with the site for each subject selected based on which site is most convenient for the subject. Vitamin A, which will be drawn only from UCM subjects, will be sent to an outside lab for analysis. Throughout the study period we will tell subjects about calcium, PTH and vitamin D lab results only if they suggest a safety concern for which we would like them to seek care from a medical professional. Except in the instance of a safety concern, subjects will be blinded to their vitamin A& D levels, calcium and PTH throughout the study to ensure protocol compliance. At the end of their time in the study, we will provide test results to subjects as indicated in "Study Completion Email V4". At the end of the intervention period for all participants in this protocol, we will unblind participants to their vitamin D dose assignment as indicated in "Dose Unblinding Email V1". We will do this at the end of the intervention for all participants because we, as the study team, are also currently blinded to dose assignments until the end of the intervention period for all participants. For participants who transition to participate in protocol 20-0847, unblinding will occur at the end of the intervention period for all participants in that study. Blood draws for COVID-19 antibodies will also occur at same location as their other lab draws for convenience, consolidated with other labs, but antibody testing will occur at UCM using the Roche assay for consistency given the differing ranges of different COVID-19 assays. Subjects will be instructed during the consent process to seek COVID-19 PCR testing on their own at a site convenient for them if they become symptomatic or were exposed to COVID-19, which is aligned with recommendations from CDC and IDPH. COVID-19 PCR tests will not be part of the research labs, and thus will not be paid for by the study. Subjects will self-report positive COVID-19 PCR results in the quarterly survey. Laboratory records will be monitored regularly by site PIs, Co-Investigators, and members of the DSMB and Clinical Advisory Committee for safety. Patient-reported outcome measures will be measured by a RedCap-based survey

administered to subjects post-randomization over the phone or via web-based survey at baseline, depending on subject preference, and every 3 months thereafter via a web-based instrument. Subjects will be given the option to call the study team and complete the survey over the phone in reminder emails. Survey and laboratory records will be maintained and monitored by study staff on encrypted, password-protected devices in compliance with UCM and RU IRB Information Security Office standards. Electronic Medical Records will be reviewed for predictors and indicators of COVID-19 and for severity of infection if subject has had a positive COVID-19 test result.

The intake survey will collect baseline data on subjects' current medications and supplements with doses (e.g., multivitamin, zinc, calcium, vitamin D), average time outside per day (specifying direct vs. indirect sun exposure), Fitzpatrick skin type, exercise habits, diet, sleep habits, possible COVID-19 symptoms such as fever, cough, GI problems, and other influenza-like symptoms. Subjects will also be asked to report suspected exposure to the virus (e.g. if a family member falls ill), specific occupation, and demographic information. Subjects will also be asked to evaluate risks of exposure. Participants will also be asked in the intake surveys for the name and contact of information of 1-2 people who could reach them and answer follow-up questions if they cannot be reached or answer questions themselves. Follow-up surveys at months 3, 6, and 9 will ask the participant (or proxy if needed) whether the participant has had a clinically 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, ICU-use and duration, and need for mechanical ventilation and duration, death and COVID-19 symptom severity using the BEAT-19 adapted to reflect the worst symptomology during their period of active COVID-19. In addition to these questions, we have added some questions on the baseline and follow-up surveys that evaluate 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.[16] While available evidence suggests there is a link between hypertension/cardiovascular disease[17,18,19], diabetes and metabolism,[20,21] depression[22,23,24]/anxiety[25], and vitamin D levels, to-date no causal relationship has been established. This is supported by both evidence as recently as this year,[17,18,24] as well as earlier studies[19,22,23]. 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.

For Aims 4-6, we will draw baseline vitamin A for 1,000 UCM subjects and draw vitamin A 3 months later for those UCM subjects who are high at baseline. Of these initial vitamin A samples, we aim to cryopreserve samples from 500 individuals, and perform RNA sequencing on a subset of 100 individuals. Once we reach this number, we will submit an amendment removing these analyses from the consent form and the blood draw/analysis protocol. Because cryopreservation and RNA sequencing involves obtaining 20mLs of additional blood from subjects and involves genetic sequencing, we will ask subjects in the consent form whether they would like to opt-in to provide more blood during their baseline visit for these measurements.

IV. Probable duration

The study period, including recruitment, enrollment, and analysis, will last 36 months. If we are granted a no-cost extension from our funder, NCATS, we expect the recruitment period to last until March 31, 2023, so one year longer, to enroll our target of 2,000 subjects. Once enrolled, subjects' participation will last up to 21 months total. This includes up to 9 months of taking a daily dose of vitamin D, attending laboratory appointments for testing, and answering surveys, and 12 months of chart review thereafter to measure outcomes.

NCATS has granted us an initial no-cost extension to continue the study through June 30, 2022 and our study timeline according to that funding period is specified in Table 1 and Table 2. Given delays in recruitment due in large part to the changing landscape of COVID risk, more infectious variants, the current flu season, and vaccine rollout, we wish to further exend our recruitment period through the end of March, 2023 but this is contingent on an additional no-cost extension from NCATS. We have requested an additional no-cost extension through June 30th, 2023 and anticipate hearing back sometime Spring 2022 with an answer. Should we receive this second extension, we will continue the study through June 2023. In this case, we will adapt to the study timeline specified in Table 3 and Table 4, rather than Tables 1 & 2, specified below. In either scenario, some subjects will participate in our study for 9 months, and others for 3-6 months, depending on when they are recruited to the study (see tables for these dates)_.Based on our understanding of vitamin D levels in the blood over time including seasonal variation, we believe subjects who participate on treatment for 3 to 6 months will achieve appropriate serum vitamin D levels for analysis. We plan to adjust our analyses for this cohort accordingly.

Table 1: Subject timeline in study: For subjects recruited before January 1, 2022

Date	Activity	Description
Baseline (Month 0)	Recruitment and enrollment	• Subjects will be screened for eligibility, undergo initial blood draws, and undergo randomization. • Subjects will be given 9-month supply of vitamin D with instructions for daily intake. Subjects will complete the intake survey via telephone or web.
Month 3	Wave 1	• Subjects will visit the lab for their follow-up Wave 1 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will also be sent the first follow-up web-based survey via email or text message. CRCs will call and email subjects as needed to remind them to complete the survey.
Month 6	Wave 2	• Subjects will visit the lab for their follow-up Wave 2 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will be sent the second follow-up web-based survey. CRCs will call and email subjects as needed to remind them to complete the survey. For the 6-month subject cohort, this set of labs will be their final set of labs and surveys.
Month 9	Wave 3	• Subjects in the 9-month cohort will visit the lab for their final blood draws in this wave. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will also be sent the final follow-up web-based survey. CRCs will call or text subjects to remind them to complete the survey.
Months 10-23	Final analysis & chart review	• Final analyses and reporting of the intervention effects on outcome measures will take place during this period to prepare for dissemination. • Chart review evaluating outcomes (e.g., hospitalization, positive COVID test)

		• For both the 6-month and 9-month cohorts, chart review will occur for 12 months after their participation

Table 2: Subject Timeline in Study: For subjects recruited in the study on or after January 1, 2022 but before March 31, 2022

Date	Activity	Description
Baseline (Month 0)	Recruitment and enrollment	• Subjects will be screened for eligibility, undergo initial blood draws, and undergo randomization. • Subjects will be given a bottle of vitamin D with instructions for daily intake. Subjects will complete the intake survey via telephone or web.
Month 3	Wave 1	• Subjects will visit the lab for their follow-up Wave 1 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will also be sent the first follow-up web-based survey via email or text message. CRCs will call and email subjects as needed to remind them to complete the survey. • For subjects recruited March 1-31, 2022, this will be their final blood draw and survey
Final month (June 1-30, 2022)	Wave 2	• This second round of follow-up labs will apply to those recruited in January and February 2022 • Subjects will visit the lab for final blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will be sent the second follow-up web-based survey. CRCs will call and email subjects as needed to remind them to complete the survey. For the 6-month subject cohort, this set of labs will be their final set of labs and surveys.
Months 4-18	Final analysis & chart review	• Final analyses and reporting of the intervention effects on outcome measures will take place during this period to prepare for dissemination. • Chart review evaluating outcomes (e.g., hospitalization, positive COVID test) • Chart review will occur for 12 months after this cohort's participation

Table 3: Subject timeline in study: For subjects recruited after March 31, 2022 and before January 1, 2023

Date	Activity	Description
Baseline (Month 0)	Recruitment and enrollment	• Subjects will be screened for eligibility, undergo initial blood draws, and undergo randomization.

		• Subjects will be given 9-month supply of vitamin D with instructions for daily intake. Subjects will complete the intake survey via telephone or web.
Month 3	Wave 1	• Subjects will visit the lab for their follow-up Wave 1 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will also be sent the first follow-up web-based survey via email or text message. CRCs will call and email subjects as needed to remind them to complete the survey.
Month 6	Wave 2	• Subjects will visit the lab for their follow-up Wave 2 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will be sent the second follow-up web-based survey. CRCs will call and email subjects as needed to remind them to complete the survey. For the 6-month subject cohort, this set of labs will be their final set of labs and surveys.
Month 9	Wave 3	• Subjects in the 9-month cohort (those recruited before October 1, 2022) will visit the lab for their final blood draws in this wave. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will also be sent the final follow-up web-based survey. CRCs will call or text subjects to remind them to complete the survey.
Months 10-23	Final analysis & chart review	• Final analyses and reporting of the intervention effects on outcome measures will take place during this period to prepare for dissemination. • Chart review evaluating outcomes (e.g., hospitalization, positive COVID test) • For both the 6-month and 9-month cohorts, chart review will occur for 12 months after their participation

Table 4: Subject Timeline in Study: For subjects recruited in the study on or after January 1, 2023 but before March 31, 2023 (end of recruitment period)

Date	Activity	Description
Baseline (Month 0)	Recruitment and enrollment	• Subjects will be screened for eligibility, undergo initial blood draws, and undergo randomization. • Subjects will be given a bottle of vitamin D with instructions for daily intake. Subjects will complete the intake survey via telephone or web.
Month 3	Wave 1	• Subjects will visit the lab for their follow-up Wave 1 blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed.

		• Subjects will also be sent the first follow-up web-based survey via email or text message. CRCs will call and email subjects as needed to remind them to complete the survey. • For subjects recruited March 1-31, 2023, this will be their final blood draw and survey
Final month (June 1-30, 2023)	Wave 2	• This second round of follow-up labs will apply to those recruited in January and February 2023 • Subjects will visit the lab for final blood draws. CRCs will call or email subjects to remind them of the lab visit as needed until the lab visit is confirmed completed. • Subjects will be sent the second follow-up web-based survey. CRCs will call and email subjects as needed to remind them to complete the survey. For the 6-month subject cohort, this set of labs will be their final set of labs and surveys.
Months 4-18	Final analysis & chart review	• Final analyses and reporting of the intervention effects on outcome measures will take place during this period to prepare for dissemination. • Chart review evaluating outcomes (e.g., hospitalization, positive COVID test) • Chart review will occur for 12 months after this cohort's participation

Note: While all participants will receive their final survey invitations in advance of the end of the active intervention date asking them to complete their survey by the study's end date, June 30, 2023, it is possible that some participants may not complete their surveys in time ("overdue participants"). We plan to continue to follow up with overdue participants beyond the study's end date to ensure that participants have the opportunity to complete their final survey. We will do so via multiple modalities (text, email and phone) and at different times of the day to increase response.

Table 5: Monitoring timeline

Any dates specified after June 2022 are contingent on granting of second no-cost extension in Spring 2022

Date	Activity	Description
September 2020	Clinial Advisory Committee meeting	9 faculty members of the CAC meet to create guidelines for safety testing and cutoffs, and to create the exclusion criteria
February 2021	Clinical Advisory Committee meeting	Faculty members of the CAC meet to review preliminary testing data and any clinical concerns. Will also discuss strategies for recruitment of healthy volunteers
June 2021	DSMB first meeting	DSMB meet with the PI and analysts to discuss study aims, outcomes, survey

		instruments, testing measurements, and data requests
August 2021	DSMB second meeting	DSMB meet to ensure randomization has worked: review of baseline testing and demographic characteristics by arm and review of subject complaints
December 2021	DSMB interim efficacy analysis (closed meeting)	DSMB will review longitudinal testing data by arm and conduct an interim efficacy analysis
May 2022	DSMB meeting	DSMB will meet to review trst results and PRO measurements aligned with the aims
January 2023	DSMB meeting	DSMB will meet to review test results and PRO measurements aligned with the aims.
May 2023	Final DSMB meeting	DSMB will meet to review test results and PRO measurements aligned with the aims. Will discuss dissemination strategies

V. Location of research

The research will take place at University of Chicago Medicine at the Hyde Park campus and River East, and at Rush University (RU) on the near west side campus, Oak Park, and South Loop locations. Subjects will be recruited from all of these specified locations.

Subjects closer to UCM will pick up their vitamin D bottles at DCAM pharmacy, and subjects closer to RU will pick up their vitamin D bottles from the Rush University Professional Office building, 1725 W. Harrison St. Suite 739, Chicago, IL. Each bottle will contain a 9-month supply of vitamin D tablets. In the event subjects are unable to pick up their vitamin D bottles in these physical locations, bottles will be mailed to an address they provide by the UCM IDS pharmacy.

For all subjects, blood samples will be obtained by venipuncture and collected in the appropriate blood tubes. For UCM subjects, we anticipate that venipuncture and laboratory tests will be collected at the DCAM laboratory and River East laboratory and all tests will be analyzed at UCMC. We may also choose to establish temporary phlebotomy locations in additional locations for subject convenience in CCD, Mitchell, DCAM, and at Ingalls outpatient labs. If we do expand to the Ingalls phlebotomy site, we will submit an amendment to provide the required lab contracts. Laboratory tests for RU subjects will be collected by Dr. James Moy, site PI. For these RU subjects, samples for PTH, vitamin D, and calcium will be collected and analyzed by Dr. Moy's laboratory on site. Venipuncture will be performed by research nurses or clinical research coordinators in the Allergy & Immunology Clinical Research Site of Rush University Medical Center, in Suite 739 of the Rush Professional Building. One of the tubes of blood will be transported by a research assistant to the Rush University Medical Center's Clinical Chemistry Laboratory for determination of PTH, calcium and vitamin D levels. Another tube of blood will be kept at the Allergy & Immunology Clinical Research Site for pick up/drop

off three times per week by CRCs for testing of SARS-COV-2 IgG antibodies using the either the nucleocapsid or spike protein Roche Elecsys® Total Antibody test at UCM. This is to maintain internal consistency of the test across UCM and Rush, which use different assays.

Samples for Aims 4, 5, and 6, after collection will be stored in liquid nitrogen tanks in Luis Barreiro's laboratory, located on the 9th floor of KCBD on the UChicago Hyde Park campus.

Samples for Aim 7 will be stored in a lab utilized by Dr. Bissonnette and his colleagues in the Biological Sciences Division.

Survey data will be collected by the study team at UCM's Hyde Park campus and RU's near west side campus in the W300 corridor in the Section of Hospital Medicine at UCM and in Suite 739 of the RU Professional Office Building. 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 specific lab values

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 moderate (4,000 IU/day) or high (10,000 IU/day). Participants will have the option of being randomized to the high versus low strategy or the moderate versus low strategy. 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

We plan to recruit 2,000 health care workers and other Chicagoland community members. HCWs will be primarily employed at the University of Chicago Medicine (UCM)- Hyde Park, River East, or Ingalls, and Rush University (RU) at the Rush University Medical Center, Rush Oak Park Hospital, and Rush South Loop clinics. At UCM, ~250 HCWs have been diagnosed with COVID-19 as of late June, out of a reduced pool working non-remotely of about 3,000 persons over 4 months, for a monthly incidence of about 2% of working HCWs per month. During the summer months, as COVID-19 rates declined and stabilized in Chicago, the monthly incidence at UCM has fallen to about 1% per working HCW per month, but this reflects continued closure of most ambulatory services, which are now among the highest risk services given COVID-19 testing of all inpatients but not outpatients. Indeed, our inpatient COVID-19 unit has reported zero HCW infections. Thus the relative odds of infection appear to be several times higher in high risk HCWs than low risk HCWs. In the presence of appropriate personal protective equipment, high risk HCW groups based on the UCM experience and the literature are persons working in high volume ambulatory settings (e.g., primary care, ophthalmology), the emergency department, radiology, respiratory services, nursing, patient transport, custodial and food services. We will prioritize recruitment of these groups, and since ambulatory care is rapidly reopening now, we think that 3% is a reasonable estimate for the baseline monthly incidence in our study subjects. We think this is a conservative estimate as it is similar to the rate in the general local population. As COVID-19 infection rates have spiked in Chicago and neighboring communities over the past few months, the vast majority HCWs who test positive report community exposure, predominantly from transmission in their household or from those in their close

social network. Further, since two COVID-19 vaccines are now available we will expand our population to also include Chicagoland community members, who continue to be at increased risk for COVID-19 infection, with increased rates from 15 to 70 per 100,000 in recent months, trust gaps in vaccines, and lower prority access to vaccines for many outside the health care/front line professions—all resulting in potential lower rates of vaccination among the general population and a continued risk of infection.

The sample size was chosen to have at least 80% power to detect a 25% or larger decrease in the hazard of developing COVID-19 between study arms at $p<0.05$ assuming a 30% baseline COVID-19 incidence rate over the study period, in line with the 3% per month incidence cited above.

Individuals are eligible to enroll in the study if they:

1. Are 18 years or older.
2. Live or work in the Chicagloland area (Illinois counties: Cook, Lake, McHenry, DuPage, Kane, Kendall, Grundy, Will, and Kankakee; Indiana counties: Lake and Porter).
3. Are interested in vitamin D as a potential preventive measure against COVID-19 in which they self-administer a daily dose of vitamin D during the 9-month study period.
4. Are willing to attend the laboratory for drop-in appointments at UChicago Medicine, Ingalls Hospital, or Rush University Medical Center every 3 months at up to 4 time points over up to a 9-month period for blood draws measuring COVID-19 antibodies, calcium, vitamin D and PTH levels.
5. Are willing to complete self-report measures at up to 4 time points over the course of up to 9 months by completing a 15-minute survey at intake by telephone or via web and 10-minute web-based follow-up surveys.

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 their health care provider, excluding multivitamins and excluding supplements that include vitamin D and calcium together, unless permission is received from the prescribing health care provider to participate. In this case, no more than 1,000 IU vitamin D may be taken daily in addition to the study medication and we would limit the subjects to participation in the low vs. moderate arm of the study, producing a maximum dose of 5,000 IU per day. We note that the NAM recommended a maximum dose of 4,000 IU per day without medical supervision based on evidence that 5,000 was safe and known uncertainty about the precision of dosing in 5,000 IU supplements available over the counter. However, since we are testing the dosage of our 4,000 IU supplements to high precision and these patients would be taking their original supplements with a labelled dose of 1,000 IU or less, we think that the combination of our 4,000 IU supplements and the patient's original supplement of up to 1,000 IU should not provide a maximum dose meaningfully above the 5,000 IU actual dose the NAM has considered safe for taking without medical supervision. We note further that we are measuring vitamin D levels and PTH in these patients regardless in this study. We do, however, have another study of vitamin D an COVID (IRB# 20-0847), in which we are not measuring these levels, for which it is more important to not substantially exceed NAM maximum level of 5,000 IU per day. The approach described here allows us to align the approach in these two studies.
9. Report taking D2.
10. Report a history of sarcoidosis.

11. Screen positive for hypercalcemia (at UCM, defined as >10.4 mg/dL and at Rush, defined as >11.2 mg/dL)) during the initial blood test.
12. Screen positive for hyperparathyroidism (PTH>85 pg/dL) during the initial blood test with calcium ≥10mg/dL and normal levels of vitamin D (≥30ng/mL)
13. Have vitamin D levels of >100ng/mL at study start, or >250ng/mL during follow-up labs.
14. Are unwilling to provide blood samples during quarterly blood tests.
15. Are unwilling to take daily vitamin D.

IX. Description of statistical analysis

The higher doses we propose, with 4,000 or 10,000 IU/d are 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 a set of HCWs and other community members at increased risk of COVID-19, increasing both the likelihood of having therapeutic dosing and the underlying risk of COVID-19 that increase 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, moderate or high 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. While our primary analysis will pool subjects randomized to either the moderate or high dose and compare them to low dose subjects, we will also perform secondary analyses comparing low to moderate and low to high and additional analyses that use post randomization vitamin D levels as time varying covariates. The design thus seeks to maximize potential to find statistically significant results between different dosages given uncertainty in subject willingness to be randomized to different doses. As noted above, the sample size was chosen to have at least 80% power to detect a 25% or larger decrease in the hazard of developing COVID-19 between study arms at $p<0.05$ assuming a 30% baseline 10-month COVID incidence over the study period, in line with the 3% monthly incidence cited above. As finding that a higher dose has no effect or a paradoxical adverse effect on COVID incidence would both lead to use of a lower dose given even the small risks of higher dose vitamin D, Ruxson and Neuhauser would argue for use of a 1-sided test, resulting in 80% power to detect a 20% or larger decrease.

The proportional hazards assumption will be assessed using the *estat phtest* command in Stata with rejection of proportion hazards defined by $p<0.05$ for any variable or the global test, or by visual assessment of Schoenfeld residuals. Continuous variables which fail proportional hazards will be categorized. For any categorical variable or categorized continuous variable which fail proportional hazards, we will perform Cox regression which stratifies based on that variable. If moderate or high dose vitamin D variables fail the proportional hazards assumption, we will estimate non-proportional hazards models. Assuming missingness at random, we will impute missing values of covariates with multiple imputation by chained equations with 20 imputations. Covariates in the imputation will include the covariates in the primary analysis excluding moderate or high dose vitamin D variables. Data analysis will be executed in Stata 18.0.

X. Potential risks and benefits to subjects

Potential Risks

A potential risk of this intervention is vitamin D-mediated hypercalcemia. Given the exclusion criteria which screens for those at increased risk for developing hypercalcemia, this risk is anticipated to be low and will be monitored for throughout the intervention period via quarterly lab draws regularly reviewed by investigators, the DSMB, and the Clinical Advisory Committee. In addition, high doses of vitamin D have been associated with an increased risk of falls. Our exclusion criteria also attempts to minimize the likelihood of this risk by screening for those before enrollment at increased risk for developing this side effect. This risk will also be monitored in subject self-reports during survey administration. We believe this risk to be low in this study population. As with any study that involves blood draws, participants will also be exposed to the minimal risks of phlebotomy including pain, infection, and venous damage. These risks will be mitigated by using professionally trained phlebotomists. Since protected health information (PHI) is collected on web-based software, there is also a risk to loss of confidentiality, which we believe to be minimal. Precautions to minimize these risks are detailed below.

Protections Against Potential Risks

The risks as described in “Potential Risks” we believe to be unlikely and will be mitigated whenever possible. The nature of the intervention and all risks will be fully described to subjects, moreover, subjects will be informed that they can choose to discontinue and withdraw from the study at any time. Subjects can also choose to not answer any question on the survey. For the risk of loss to confidentiality, all members of the study team will maintain strict standards for confidentiality, further described in Section XIV and thus not repeated here. The risk of complications due to venipuncture will be mitigated by use of trained professional phlebotomists at UCM and RU. The risks of complications due to higher doses of vitamin D will be mitigated with the exclusion criteria as outlined above during preliminary screening and through subject education of symptoms for fall risk. Vitamin D-mediated hypercalcemia will be mitigated by regular monitoring by investigators for critical calcium values during blood draws and subject education of symptoms related to hypercalcemia. Subjects at UCM will be referred to their primary care provider if they experience these symptoms or have calcium levels of 10.5-12 mg/dL and subjects at Rush will be referred to their primary care provider if they experience these symptoms or have calcium levels of 11.3-12mg/dL. For all subjects, if calcium laboratory values are 12.1mg/dL-14mg/dL, subjects will be advised to go to urgent care and if values are greater than 14 mg/dL subjects will be advised to seek care at an emergency room. Subjects will be provided with the pager number of an on-call physician at UCM and Rush primary care group that they can use in the event of emergent symptoms.

Potential Benefits

Health care workers (HCWs) and those who reside in diverse metropolitan communities with high community spread of COVID-19 are of special public health relevance because of their 1) increased exposure to COVID-19, 2) frequent contacts with individuals at increased risk of infection and adverse outcomes if infected, and 3) frequent contact with others who could spread COVID-19. We intentionally do not seek a representative sample because our primary goal is to learn if vitamin D treatment is efficacious in reducing COVID-19 incidence and improving outcomes and the higher risk populations we will focus on facilitates that. We think that if we find evidence of effectiveness among our study population, the low risks and costs of vitamin D treatment would encourage widespread use of vitamin D more broadly, producing health benefits throughout the population. Nevertheless, even if one thought the results could only be generalized to HCWs and those in metropolitan communities, the public health impact of this work would be significant for the reasons above.

XI. Monitoring of safety

The current study represents a multi-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-investigators Drs. Moy, Schram, Bandi, and Prochaska, will conduct regularly monitoring of safety which will be overseen by a formal Data and Safety Monitoring Board (DSMB). The independent DSMB will be comprised of three experts, a Chair and two members, who will provide regular oversight for this multi-site study. The PI has already identified all three DSMB members, one of whom will be the Chair. These individuals are senior faculty members and hold expertise in clinical trial oversight and design, biostatistics, endocrinology, pulmonology and critical care medicine.

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. If funded, 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. Study analysts will work with the PI and Co-Investigators to send laboratory data to the DSMB regularly for review.

Ensuring subject safety

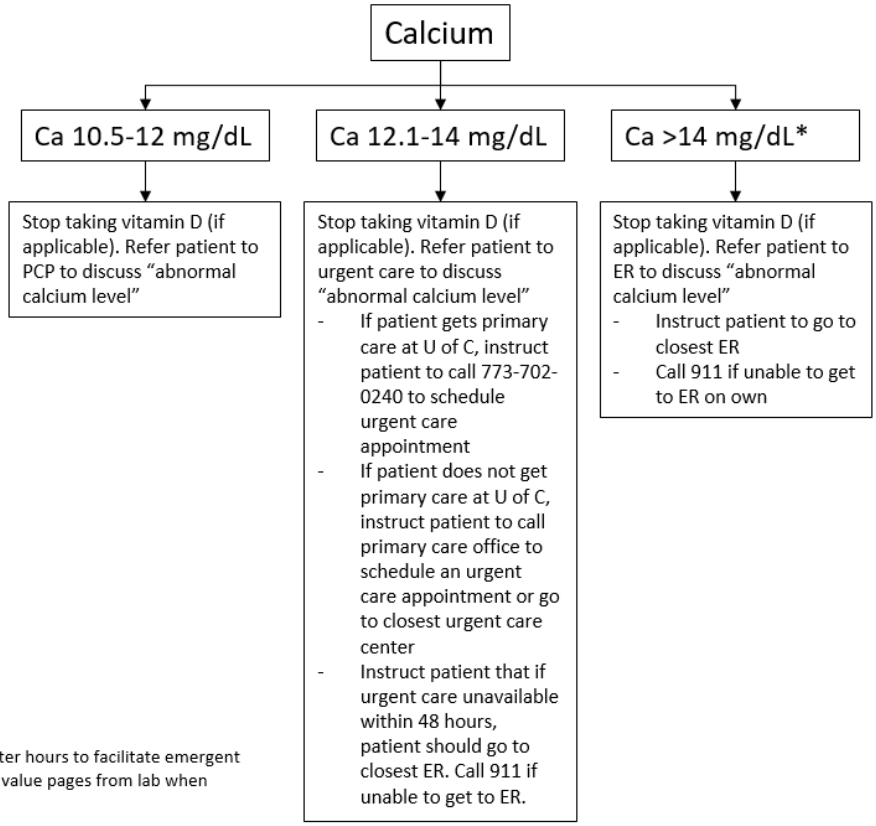
The proposed study will be supervised by PI and the Clinical Advisory Committee, with oversight by the DSMB and the University of Chicago Biological Sciences Division Institutional Review Board (UCM BSD IRB). All subjects will undergo written informed consent. Additional safety precautions include: 1) 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) monitoring of calcium levels to detect toxicity, 4) participants will be advised to report adverse events (email and study phone number provided), 5) independent testing of vitamin D supplements to ensure accurate potency, 6) rigorous infection control practices to mitigate the spread of COVID-19 at UChicago Medicine where testing is performed and 7) well-trained research staff.

Monitoring of the intervention

To monitor for toxicity, calcium levels will be checked at study initiation to assess for baseline hypercalcemia and at each subsequent blood draw (3, 6, and 9 months) to monitor for incidental or study-induced hypercalcemia. If study participants are found to be hypercalcemic, they will be told to cease vitamin D supplementation. For UCM patients with a calcium level 10.5-12 mg/dL and for Rush patients with a calcium level of 11.3-12mg/dL, patients will be instructed to consult with their primary care physician. For patients with a calcium level of 12.1-14 mg/dL, patients will be referred to urgent care. For patients with a calcium level greater than 14 mg/dL, patients will be referred to the emergency room. These participants will continue to undergo monitoring blood draws and surveys and may resume vitamin D supplementation per study protocol with a physician note. Based on conversations with our Clinical Advisory Board and prior studies, we expect very few people to become hypercalcemic due to study medication. The DSMB 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.

UCM Laboratory Abnormality Referral Algorithm

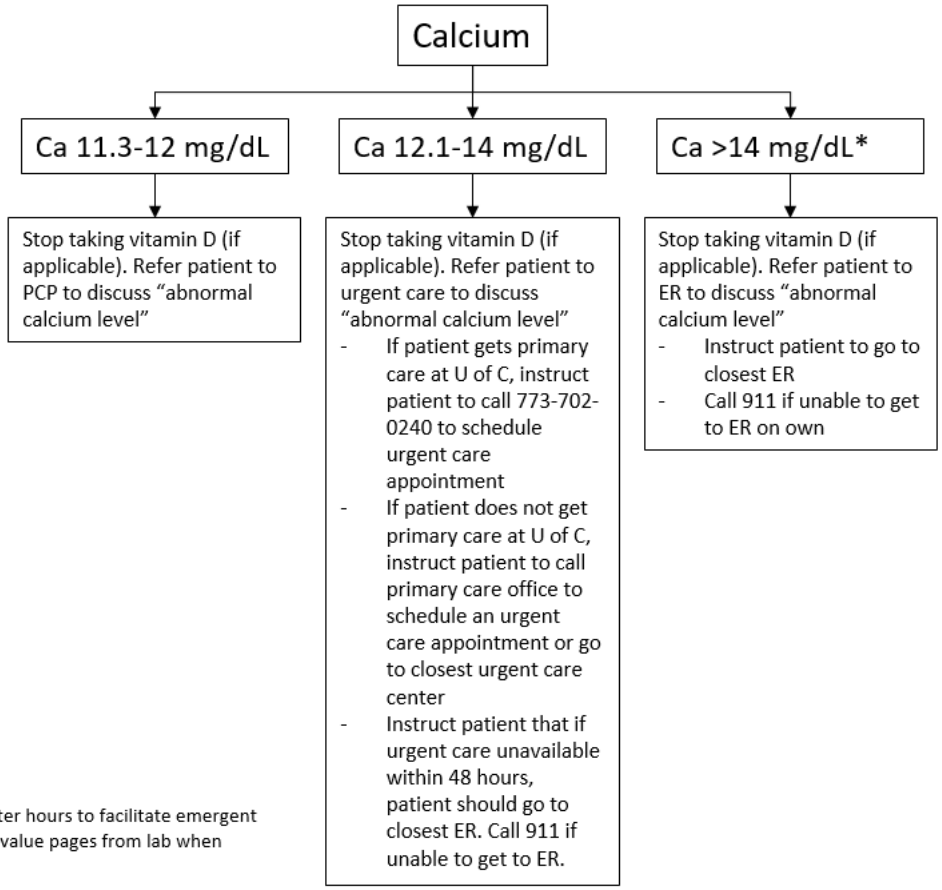
Abnormal results emailed / paged to research coordination team for follow-up:



*Study investigator on call after hours to facilitate emergent ER referrals. Will take critical value pages from lab when research team unavailable.

Rush Laboratory Abnormality Referral Algorithm

Abnormal results emailed / paged to research coordination team for follow-up:

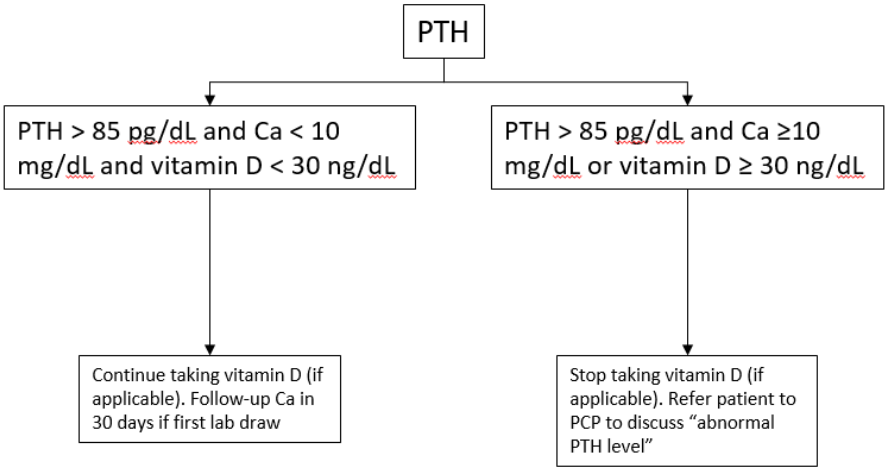


*Study investigator on call after hours to facilitate emergent ER referrals. Will take critical value pages from lab when research team unavailable.

PTH Referral Algorithm- UCM and Rush

Laboratory Abnormality Referral Algorithm

Abnormal results emailed / paged to research coordination team for follow-up:



XII. Data and Safety Monitoring Board (DSMB) Charter

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. In addition to these quarterly meetings, Board members, along with the PI and Co-Investigators, will receive regular data reports from phlebotomy with all subjects' laboratory values, with higher values indicated. Board members will keep in close contact with the PI and Co-Investigators to refer individual patients to seek outside care for high laboratory values, in addition to the resources provided to subjects to seek care, such as an on-call pager for primary care.

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, Co-PI, and Co-Investigators at the direction of the DSMB and UChicago Medicine Biological Sciences Division Institutional Review Board.

XIII. Payment to subjects

Participants will not receive payment for participation.

XIV. Procedures to obtain and record informed consent

Consent will be sought by our Clinical Research Coordinators over the phone using a virtual consent procedure (APPENDIX A). In the event a potential subject has difficulty with the virtual consent method, we will meet the subject in our research offices to sign the consent form in-person by providing them the RedCap eConsent form using an iPad. Since we are seeking healthy volunteers from the community, we do not believe any individual will require determination for capacity to consent.

After a potential subject expresses interest via email, in-person, or phone, potential subjects will complete the preliminary screening of self-reported underlying health conditions as described in the exclusion criteria via a screening phone call from the CRC/RA and consent will be sought virtually. In rare instances when subjects have difficulty signing the consent form vitually, the RedCap eConsent will be provided to them in-person by research staff using an iPad and they will sign and date digitally. Both subjects who sign the consent form virtually and in-person will receive a signed copy of the consent form to the email address provided at the start of the RedCap eConsent form. For those signing in-person, signed consents will be printed by request.

At the beginning of the consent process, subjects will be asked to verify their identity by emailing or texting the study email or study cell phone a photo of their diver's license, state ID, passport or employee ID. They will be asked for their email address where a signed copy of the consent form will be sent for their records. In the consent form, subjects will be informed about the nature of the intervention, that they will be receiving a daily dose of vitamin D, either low or moderate/high, and must self-administer this dose each day. They will be informed about the nature of the intervention, that they are blinded to the dose they receive. Subjects will be reminded that participation is entirely voluntary, and they may withdraw at any time without consequence. Subjects will also be informed about the data collection methods, including the survey and testing they will undergo at each stage, and that they will be informed about test results for COVID-19 antibodies only during the study period. They will be informed that their PTH, calcium and vitamin D results will be withheld until completion of study participation unless they suggest a safety concern. They will be educated about the risks of the study as described above in "Potential Risks". Subjects will be informed about what will be done if they are found to be hypercalcemic: First, they will be told to cease vitamin D supplementation. Subjects at UCM with a calcium level 10.5-12 mg/dL and at Rush with a calcium level 11.3-12mg/dL will be instructed to consult with their primary care physician. All subjects with a calcium level of 12.1-14 mg/dL will be referred to urgent care. All subjects with a calcium level greater than 14 mg/dL will be referred to the emergency room. These participants will continue to undergo monitoring blood draws and surveys and may resume vitamin D supplementation per study protocol with a physician note.

Subjects will then be instructed to select their randomization preference on the consent form. If a potential subject says they are already taking a supplement or supplements that contains 2,000IUs or less of vitamin D in total, they may continue to take those supplements while part of the study, however, they will only be allowed to enroll in the arm that randomized them between 400 and 4,000IUs.

If a potential subject says they are already taking a supplement or supplements that contains more than 2,000IU vitamin D in total, we will ask the subject to discontinue supplements in excess of 2,000IU during their time on the study supplement to keep the guidelines straightforward for participants and avoid any potential for overdose.

After consenting, subjects will be asked to visit the laboratory to obtain the initial blood test to screen for abnormal PTH and calcium levels, obtain baseline 25(OH) vitamin D levels, and determine the presence of COVID-19 antibodies. After the initial blood tests, to improve compliance with vitamin D supplementation and screen for patients who may only be interested in joining the study to obtain baseline labs / antibody levels, subjects will be called and offered the opportunity to withdraw from the study after initial lab results are available, prior to randomization. We will also tell subjects about their other lab results if they suggest a safety concern and are outside normal ranges outlined in the exclusion criteria. If potential subjects have declined to withdraw at this initial stage, we will proceed with randomization.

Summary of Consent Frorm Versions

For those consented March 31 2022-December 31, 2022: We will use consent form Version 10 (UCMC) or Version 11 (Ingalls), both in English and Spanish. This is because these consent forms are written for those who participate in the study for 6-9 months. Subjects who consent before October 1, 2022 will be in the study for 9 months. Subjects who consent on or after October 1, 2022 will be in the study for 6 months. This information is also clarified in these consent forms and in Table 3 of Section IV above. Version 10, like Version 8, has language for opt-in labs while Version 11 does not. We will use consent form Version 4 for Rush subjects.

For those who consented using UCM Consent Version 8 or 9, or Rush Consent Version 3 (thus who consented on or after January 1, 2022 and in follow up for less than 6 months), we wish to allow for the option to re-consent these subjects on UCM Consent Version 10 or 11, or Rush Consent Version 4, to allow these subjects to participate in the study for a full 9-month period (and would thus receive 4 total blood tests and 4 follow-up

surveys). This re-consent is entirely voluntary. If these subjects do not wish to re-consent, they will end their time in the study June 30, 2022.

For those consented January 2023-March 31, 2023: We will use consent form Version 12 (UCMC) or Version 13 (Ingalls), both in English and Spanish. With the removal of the final PTH lab approved in Amendment 54, we will then use consent form Version 14 (UCMA) or Version 15 (Ingalls), both in English in spanish. This is because these consent forms are written for those who participate in the study for less than 6 months. This information is also clarified in these consent forms and in Table 4 of Section IV above. Version 12 and 14, like Versions 8 and 10, have language for opt-in labs while Version 13 and 15 do not.

XV. Procedures to maintaining confidentiality

Electronic study data will be maintained on password-protected, encrypted devices in locked offices at UCM and RUMC, including internally using the standard of minimum necessary PHI, assigning subject IDs to each subject and using that as the mode of identification rather than name and/or MRN. All study staff will have up-to-date HIPAA training. Consent forms will be stored in HIPPA-compliant lab share drives maintained by the UCM Center for Research Informatics. Paper forms as related to the study will be stored in locked filing cabinets at UCM and RUMC in the site PI's office suites.

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XVII. Description of recruiting methods:

Given the current risk of COVID-19, we will use virtual recruiting as much as possible. We will recruit using a variety of mechanisms, including emails, flyers, presentations, and social media posts.

We plan to recruit at UCMC and Ingalls Memorial, and at Rush University near west side, South Loop and Oak Park locations.

For employee recruitment: We will begin by sending emails to all eligible employees via existing institutional listservs using the Employee Listerv email scripts and by engaging leaders in varying units to seek permission to present at meetings of their units, which typically now occur virtually by Zoom. While we will recruit in diverse settings across both sites, we will prioritize recruitment of health care workers in outpatient ambulatory

settings given the higher likelihood of exposure to outpatients and given greater COVID-19 testing of inpatients. At both sites we will prioritize front line clinicians, research and other staff (e.g., clinical research coordinators, cafeteria and custodial workers) with the greatest likelihood of exposure based on contact with patients or other colleagues. We expect these criteria to result in a population that significantly over represents African American persons, addressing a high-risk population since African Americans and HCWs are at increased risk of COVID-19, increasing our statistical power. Our outreach efforts will also prioritize participants who are older, where infection risk and adverse outcomes are more likely. As vaccines are rolled out to healthcare workers, we will also prioritize those less likely to have access to the vaccine right away.

For community recruitment: We will begin by leveraging our existing relationships with individuals already enrolled in the study and those in our broader UCM and Rush communities.

- For individuals newly enrolled in the study, we will send them a welcome email using either “Welcome and Share Email” scripts to ask them to share with their networks information about the study just after they consent to the study.
- For individuals who have been enrolled in the study for more than one month, we will reach out them individually via email using the Existing Subject email scripts to inform them that in light of vaccine prioritization to HCWs, we are expanding our recruitment to adults in Chicagoland communities to give them access to the possible of benefits of COVID-19 prevention with vitamin D. We will ask them if they would be willing to forward the FAQ sheet to their family members, friends, or others in their network who they think may be interested.
- For groups of HCWs who we have reached already with recruitment materials, we will follow up with those groups using the Community Listserv scripts and inform them that in light of vaccine prioritization to HCWs, we are expanding our recruitment to adults in Chicagoland communities to give them access to the possible of benefits of COVID-19 prevention with vitamin D. We will re-attach the FAQ sheet and ask them to forward it those in their networks who they think might be interested in participating.
- For HCWs who we have not yet reached out to about participating in the study, we will use the Community listserv scripts to both ask them if they are interested and ask if they could share the information with those who may be interested in their networks.
- For individuals who have previously been disqualified at enrollment due to ever having a self-reported positive PCR test or a positive baseline antibody test, we will contact these individuals and ask if they are interested in enrolling. We will have these individuals enroll using the same processes for new subjects.

For community recruitment other than HCWs and the social networks of study participants, we will begin by leveraging existing community relationships to reach out to primary contacts at a variety of community organizations via phone or email. We will also recruit in Chicagoland neighborhoods, handing out brochures and/or flyers to local businesses.

For community recruitment, we use the "Joint Interest Form- RedCap" on iPads or laptops when we are recruiting. RCs, RAs and Americorps members, when a potential participant indicates interest in learning more about the study, will enter potential participant information into this form so they can be later followed up with by study teams. This will allow the study team to answer questions and, as applicable, conduct screening, in a private setting at a time convenient for the potential participant.

We will ask relevant community organizations’ leaders to disseminate study flyers, FAQ sheets, and listserv announcements to their organization and will seek permission to present at meetings. We will allow community organization leaders to decide what is the most appropriate mechanism to reach out their group, and we will provide them with the relevant recruitment materials accordingly. We will also leverage existing community relationships to recruit using social media. These include study staff tweeting recruitment announcements about the study and asking for help in re-tweeting the message, and our group providing social media posts to organizations to post themselves via Twitter, Instagram (via InstaStory or on the feed), Snapchat, Facebook (via Story or their wall), or NextDoor.

We will run also paid advertising campaigns on Facebook and Instagram. The Harris School of Public Policy social media team will manage these campaigns, and ads will be posted from the Harris social media accounts. We will target the ads to people over age 18 in the Chicago area who have broad Chicago-related interests, such as Chicago Public Schools or the White Sox. This targeting will increase effectiveness and efficiency of the ad campaign.

We will also be using a recruitment website, for which we provide the text (see Vitamin D joint study text). This website will recruit for both this study and for protocol 20-0847 (same PI) due to their overlapping study populations and overlapping study interventions and instruments. If participants are interested in this study (study option 2), they will be taken to a RedCap form (see RedCap form text) where they will provide contact information.

Below we provide a list of specific community organizations that we plan to reach out to for recruitment. We will update this list with new organizations in an amendment as we identify them.

- The University of Chicago community listservs
- Rush University community listservs
- Stroger Hospital of Cook County
- Friend Family Health
- Howard Brown Health
- Oak Street Health
- Bain & Company
- Ernst & Young
- PWC
- Polsinelli
- Sidley Austin LLP
- Winston Straun LLP
- The Chicago White Sox
- The Chicago Bulls
- Abbott
- McDonald’s Corporation
- Chicago Fire Department
- Chicago Police Department
- The Chicago Sun-Times
- Chicago Defender
- Chicago Tribune
- Jewel-Osco
- Mariano’s
- Walgreens
- CVS
- Trader Joe’s
- Whole Foods
- Uber
- Lyft
- The City of Chicago
- Local area congregations
- Chicago Housing Authority
- Subway
- Jimmy John’s
- Chicago Atheltic Clubs

- LA Fitness
- Fitness Formula Clubs
- Orange Theory
- Core Power Yoga
- Altura

Interested subjects will be directed to a recruitment line staffed by our research staff or, if recruited from an in-person setting, will complete the Joint Study Interest form in RedCap and be contacted by research staff at a later time to review the study and eligibility requirements in a private setting. Once potential subjects are screened for eligibility and express interest in the Intake Form in RedCap, we use the methods described below in Appendix A. We will send them a RedCap eConsent form via text or email, depending on preference, and telephone them to walk them through the informed consent process. Our research team has already developed and successfully implemented protocols for virtual recruitment and consent for other studies using RedCap eConsent in which consent forms are either texted or emailed to all signors and signed digitally. 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 They can then either complete electronic consent via RedCap eConsent or have a paper consent form prepared for them to sign physically, which will be dropped off at a location convenient for the subject by a CRC. We will not exclude potential participants currently working virtually because we expect this to change for many workers over the study period. Study recruitment will take place through March 31, 2022, or, if we receive a second no-cost extension in Spring 2022, through March 31, 2023 (see above timelines). For HCW recruitment, though our primary recruitment approaches will be virtual, the research team will also hang posters throughout UCM/Ingalls campuses and Rush University, including showing these posters electronically with approval from UCM/Rush marketing teams in areas with screens providing employee announcements, and place flyers in departmental mailboxes. For community recruitment, we will similarly follow primarily virtual recruitment approaches and distribute flyers for dissemination at the physical location as appropriate.

To maximize retention rates, our CRCs and study staff will monitor completed drop-in laboratory visits according to the 3-month schedule and follow-up with subjects via telephone and email reminders at each wave to each subject until the lab visit is completed. CRCs and analysts will also monitor survey completion at each wave and send out email and telephone reminders to subjects who have not completed the survey. Laboratory visits will occur for all tests at the same time during study sites' laboratory hours to minimize inconvenience to subjects.

XVIII. 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.

XIX. 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.

XX. 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.

XXI. 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.

APPENDIX A: Virtual consent method

1. Once interest has been expressed and the individual has been screened for eligibility in the RedCap intake form, RC/RA will call procced to the eConsent form.

2. RC/RA will ask permission to send the consent form electronically to the potential subject to review the components of the study over the phone.

3. After a subject has opened the electronic consent form, the RC/RA will verify their identify with their driver's license, state ID, passport or employee badge, review the consent form with them, answer any questions to ensure they understand the study adequately enough to proceed with their participation.

3. Subject will sign electronically and the signed consent form will deliver back to the RedCap consent project. Subject will have an option to download a .pdf version of the consent form at this time.

4. RC/RA will review to make sure consent form is valid. Then, RC/RA will sign & date the consent form under "Person Obtaining Consent". RC/RA will also obtain the PI signature on consent form. RC/RA will keep a copy of this consent form in the study files.

5. The RC/RA will send the intake survey to the subject for completion.