Protocol Synopsis: Vitamin D Insufficiency
HSC Protocol H-2009-0055
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Background

Osteoporosis is a major health problem in postmenopausal women. At age 50, half of women will suffer an osteoporotic fracture in their remaining lifetime [1], causing increased disability and mortality [1, 2]. Vitamin D deficiency, defined as a serum 25(OH)D <15 ng/mL, contributes to osteoporosis via decreased calcium absorption (Ca·Ab), secondary hyperparathyroidism (HPT), increased bone resorption and decreased bone mineral density (BMD) [3]. Thus, experts agree that patients with vitamin D deficiency should receive vitamin D therapy [4-7]. Vitamin D insufficiency (VDI) is a milder form of hypovitaminosis D defined as a 25(OH)D level between 15 and 30 ng/mL regardless of parathyroid hormone (PTH) status [8]. Experts disagree on whether to treat VDI, as the clinical benefits of therapy are uncertain. Some experts [8-11] insist the optimal 25(OH)D level is ≥30 ng/mL. By contrast, both the Food and Nutrition Board [12] and NIH Evidence Report No. 158 [13] state that insufficient evidence exists to declare the optimal serum 25(OH)D for bone health, despite review of ~170 studies. More recently, in late 2010 the Institute of Medicine declared that virtually all individuals were vitamin D replete with a serum 25(OH)D level of ≥20 ng/mL. Despite this comprehensive document and review of the scientific evidence, experts continue to declare that the optimal vitamin D level is still ≥30 ng/mL. Indeed, our own University of Wisconsin laboratory continues to cite a serum 25(OH)D level of ≥30 ng/mL as "optimal." Confusion over the optimal 25(OH)D level results, in part, because previous trials failed to recruit subjects based on initial 25(OH)D levels and/or failed to target or achieve 25(OH)D levels ≥30 ng/mL. Moreover, secondary HPT, the proposed mechanism by which VDI causes bone loss, occurs in only 10% to 33% of people with VDI [14-17]. As such, people with VDI and normal PTH might not experience clinical benefits from vitamin D therapy. VDI is widespread [15, 18, 19], affecting 26% to 39% of postmenopausal American women with [20] and without [21] osteoporosis. Therefore, determining the ideal 25(OH)D level for optimal calcium homeostasis and bone health is of utmost clinical and public health importance. Our overall goal, congruent with Healthy People 2010 objective 2-9, is to evaluate the effect of vitamin D therapy on the risk of osteoporosis in postmenopausal women with VDI, as reflected by changes in Ca·Ab, BMD and muscle fitness. Our second goal is to evaluate whether a high-dose vitamin D regimen, chosen to achieve and maintain a 25(OH)D level ≥30 ng/mL [17], is superior in its effects on study outcomes compared to a low-dose vitamin D regimen that can permit continued VDI [22-25].

We hypothesize that in postmenopausal women with VDI, high-dose vitamin D and subsequently higher serum 25(OH)D levels will facilitate greater increments in Ca·Ab than low-dose vitamin D or placebo therapy. We propose this hypothesis for the following reasons. First, 25(OH)D has a lower affinity for intestinal vitamin D receptors than $1,25(OH)_2D$ [26]. Thus, higher serum 25(OH)D levels might be required to facilitate 25(OH)D receptor binding to increase Ca·Ab. Second, higher 25(OH)D levels might permit greater local $1,25(OH)_2D$ production and higher receptor occupancy, relevant for older women who, due to increased age, have fewer intestinal vitamin D receptors [27-29]. Third, higher levels of vitamin D metabolites appear to up-regulate expression of calcium transport genes [30-32]. Fourth, recent studies suggest that in postmenopausal women, low-dose vitamin D might have no effect on Ca·Ab [25, 33-35], whereas high-dose vitamin D increases Ca·Ab [17]. Finally, a recently published large epidemiologic study associates high 25(OH)D levels with a lower risk of hip fracture in postmenopausal women [36]. We propose a randomized, double-blind, placebo-controlled trial of low-dose and high-dose vitamin D_3 in postmenopausal women with VDI order to investigate the following Specific Aims:

<u>Aim 1</u>: To evaluate the effect of vitamin D_3 therapy on Ca·Ab in postmenopausal women ≤75 years old with VDI. Sub-aims include the investigation of subject variables influencing Ca·Ab and 25(OH)D levels at baseline and 52 weeks, the accuracy of oral isotope plasma levels for

Ca·Ab measurement and the ability of questionnaires to identify patients with low vitamin D status and low calcium intake.

<u>Aim 2</u>: To evaluate the effects of vitamin D_3 therapy on the 12-month change in BMD and bone turnover in the same trial conducted for Aim 1. Sub-aims include the identification of subject variables significantly influencing change in BMD and an evaluation of the relationship between changes in Ca·Ab and changes in BMD.

<u>Aim 3</u>: To evaluate the effect of vitamin D therapy on muscle mass and functional capacity in the same trial conducted for Aim 1. We will measure muscle mass by whole body bone densitometry and assess muscle function using the Timed Up and Go (TUG) Test and the modified Stanford Health Assessment Questionnaire (HAQ) score. Sub-aims include the identification of subject variables significantly influencing muscle outcomes.

Exploratory Aim: We will analyze spine bone mineral density images to determine the trabular bone score. We will assess whether the trabecular bone score is altered by vitamin D therapy.

Methods

We will recruit 250 postmenopausal women with VDI for this study and randomize equal numbers of women into three treatment arms: placebo, low-dose vitamin D (800 IU daily) and high-dose vitamin D (50,000 IU daily x 15 days then twice monthly thereafter). We will evaluate the functional outcomes of low-dose and high-dose vitamin D therapy among these women.

Inclusion criteria are:

a) Vitamin D insufficiency, defined as a serum 25(OH)D 14 to 27 ng/mL by HPLC assay. We will enroll women with a 25(OH)D ≤27 ng/mL, instead of <30 ng/mL, to allow for patient variability in 25(OH)D levels and laboratory variability in 25(OH)D measurements.
b) Women ≥5 years past the date of last menses or bilateral oophorectomy, or ≥60 years old if they had prior hysterectomy without bilateral oophorectomy [37-39]. We will recruit women ≥5 years past menopause, since the perimenopausal period associates with a rapid decline in Ca·Ab and BMD [40-43].
c) Total dietary and supplemental calcium intake <600 mg or >1400 mg daily, based on a food frequency questionnaire [44]. We will recruit women with a calcium intake typical of postmenopausal American women [45], to ensure applicability of results to most postmenopausal women and because high calcium intake lessens the import of vitamin D-mediated Ca·Ab [46-48]. We will avoid recruiting women with low calcium intake and instead, counsel them to increase intake to the currently recommended range suggested by the National Osteoporosis Foundation and the Institute of Medicine. We will avoid recruiting women with calcium intake >1400 mg as these women can absorb calcium passively and rely less on vitamin D-mediated active calcium absorption.

Exclusion criteria include the following:

a) Women >75 years old, as increasing age associates with intestinal resistance to $1,25(OH)_2D$ therapy and presumably, to vitamin D as well [29, 49].
b) Hypercalcemia (serum calcium corrected for albumin >10.4 mg/dL [50, 51]), as vitamin D therapy may exacerbate hypercalcemia.
c) Nephrolithiasis by medical record or patient report, as vitamin D may increase urine calcium levels and thereby the frequency of nephrolithiasis.
d) Inflammatory bowel disease, malabsorption or chronic diarrhea, as presence of intestinal diseases may interfere with normal absorption of calcium or vitamin D.
e) Chronic Kidney Disease with a GFR <45 mL/minute based on the MDRD formula [52], as physicians might need to prescribe calcitriol to correct patients' low vitamin D status and secondary HPT [53].

f) Use of bone-active medications within the past 6 months including bisphosphonates, estrogen compounds, calcitonin, teriparatide, oral corticosteroids and anticonvulsants, as study outcomes of bone turnover and BMD may be confounded by their co-administration.
g) Allergy or intolerance to orange juice, as the oral isotope is administered in orange juice.
h) Allergy or intolerance to sunscreen, as all subjects must apply this during participation.
i) Prior adult clinical fragility fracture of the hip, spine or wrist or a T-score below -2.5 at the lumbar spine or femur, as these women should receive osteoporosis prescription medication.
j) Diabetes, as the disease [54] and some medications used to treat it can cause fracture [55]
k) Use of high dose vitamin D supplements (>400 IU) or prescription vitamin D including cholecalciferol and ergocalciferol as patients are unlikely to be eligible for the study [20].
l) Active cancer within the past five years, other than skin cancer, as cancer and its treatments often affect nutrition and skeletal health.

Recruitment. We will recruit potential subjects using newspaper advertisements, letters of invitation sent to UW female faculty and to registrants of the UW Institute on Aging Subject Registry, Research Match and our study team's website. The study team website address is https://www2.medicine.wisc.edu/home/rheumatology/hansencurrentstudies. We will describe the study to women who call our center and initially phone-screen interested women. If a woman is taking >400 IU of vitamin D per day without a prescription or suggestion from her primary care provider, and has no clinical evidence of skeletal fragility (such as a fragility fracture or a provider's decision to prescribe bone active medication), we will ask if she is willing to stop vitamin D. If so the study team will call back the prospective participant in 3 months to rescreen over the phone. If at the repeat phone screen it is found that she is has not reduced her vitamin D intake or fails to meet a different eligibility requirement, she would fail the phone screen and a screening visit would not be scheduled. Likewise, if a woman is taking <600 mg of calcium or >1400 mg of calcium per day through the combination of food and supplements, we will ask if she is willing to modify her intake in order to qualify for the study (and also to be closer to the optimal calcium intake suggested by the Institute of Medicine, 1200 mg per day). If she is willing to modify her intake, she would be eligible to attend a screening visit. Unlike the vitamin D, it would not be necessary to wait an extended period of time for her calcium levels to normalize, thus eliminating the need to repeat the phone screen. Obviously, if women are eligible for the study, they will be asked to refrain from ingestion of vitamin D outside of the study pills, during participation in the study.

Women passing our phone screen will come to the Clinical and Translational Research Core (CTRC) for a screening visit. Based on a previous study (HSC Protocol #2005-0159), we predict screening 1,000 women to identify 250 women with VDI. To decrease study costs, we will employ a three-step test result screening process described below.

Screening Visit. At the first visit we will obtain a medical history, collect blood, measure 25(OH)D levels and freeze remaining aliquots of serum for step 2 laboratory tests. Among women with confirmed VDI, we will measure serum calcium, albumin, PTH and creatinine levels (**Table 1**). Women who qualify for participation based on blood tests will undergo a whole body BMD measurement. We will instruct eligible women to complete food diaries in preparation for baseline Ca·Ab measurement.

Table 1: Summary of Study Visits

Visit	Day	Study Procedures
Screen	- 30	Obtain consent & medical history, estimate calcium intake [44], complete vitamin D and sun exposure questionnaire, measure 25(OH)D and, if VDI confirmed, measure PTH, calcium, albumin and creatinine
Visit 1	- 25	Perform DXA, teach eligible subjects how to complete food diary, dispense food scales
	- 14	Bionutritionist analyzes food diary and determines subjects' diet during Visits 2 & 3
Visit 2	0	1st Ca·Ab study; measure 24-hour urine calcium/sodium/magnesium and serum 25(OH)D, $1,25(OH)_2D$, PTH, calcium, phosphorus, magnesium, FGF-23, estradiol,

		BSAP, CTX; score TUG, STS-5, HAQ, PASE; **collect blood at 1, 3 and 5 hours post-isotope dosing**
	1	UW PRC randomizes subjects into treatment arms with stratification based on PTH/calcium and dispenses first month of vitamin D/placebo capsules
Visit 3	30	Measure 25(OH)D, calcium, magnesium, PTH, FGF-23, BSAP, CTX, count pills, score TUG, STS-5, HAQ, PASE
Visit 4	60	Measure 24-hour urine calcium and magnesium, serum 25(OH)D, calcium, magnesium, PTH, FGF-23, BSAP, CTX; score TUG, STS-5, HAQ, PASE
Visit 5	120	Measure 24-hour urine calcium and magnesium, serum 25(OH)D, calcium, magnesium, PTH, FGF-23, BSAP, CTX, count pills, score TUG, STS-5, HAQ, PASE
Visit 6	240	Measure 25(OH)D, calcium, magnesium, PTH, FGF-23, count pills, score TUG, STS-5, HAQ, PASE complete diet diary
Visit 7	365	2nd Ca·Ab study; measure 24-hour urine calcium/sodium/magnesium and serum 25(OH)D, 1,25$(OH)_2$D, PTH, calcium, phosphorus, magnesium, estradiol, FGF-23, BSAP, CTX; score TUG, STS-5, HAQ, PASE; **Perform DXA**

"DXA" denotes dual energy x-ray absorptiometry; TUG denotes the Timed Up and Go test; STS-5 denotes the sit-to-stand test with five repetitions. Questionnaires include Health Assessment Questionnaire (HAQ) and Physical Activity for the Elderly (PASE) score. Cells in grey indicate the study visit occurs at the Osteoporosis Clinical Research Center. All other study visits occur on the CTRC.

Dietary Assessment. We will teach eligible women to complete 4-day food diaries [56], providing food scales to weigh portions. A nutritionist will review and analyze nutrient intake and replicate each woman's nutrient intake (except alcohol) during her inpatient Ca·Ab studies.

Calcium Intake. We will assess subjects' dietary and supplemental calcium intake at 0 and 8 months by food diaries. We will use calcium intake as a covariate when modeling study outcomes.

Calcium Absorption Studies. We will admit women to the UW CTRC for Ca·Ab studies at baseline and ~365 days following vitamin D or placebo therapy. Women will fast after midnight the night before admission. Testing will begin after each woman empties her bladder. Nurses will place an intravenous catheter and draw blood for measurement of study labs (**Table 1**). With breakfast, women will drink a glass of calcium-fortified orange juice containing ~8 mg of ^{44}Ca. The nurse will fill the glass with de-ionized water and the subject will drink the water, ensuring she receives the entire oral isotope dose. Simultaneous to oral isotope ingestion, the nurse will infuse ~3 mg of ^{42}Ca over 5 minutes and flush the intravenous line with 25-50 mL normal saline for 15 minutes. The nurse will weigh the calcium isotope syringes before and after use to record the administered doses of ^{42}Ca and ^{44}Ca. During the first Ca·Ab study, nurses will collect blood samples 1, 3 and 5 hours after isotope dosing, allowing us to compare single plasma isotope estimates of calcium absorption to gold-standard estimates based on dual isotope levels in a 24-hour urine collection.

Ca·Ab measurements in the placebo arm will preserve blinding and confirm the low monthly variation in Ca·Ab reported by others [43, 57]. Importantly, each woman's baseline Ca·Ab measurements will provide a comparison for Ca·Ab measurements following vitamin D. Such pairing of data will control for individual characteristics, including dietary intake of calcium, fat and fiber [43, 58, 59], age [43] and race [60], that might independently affect Ca·Ab.

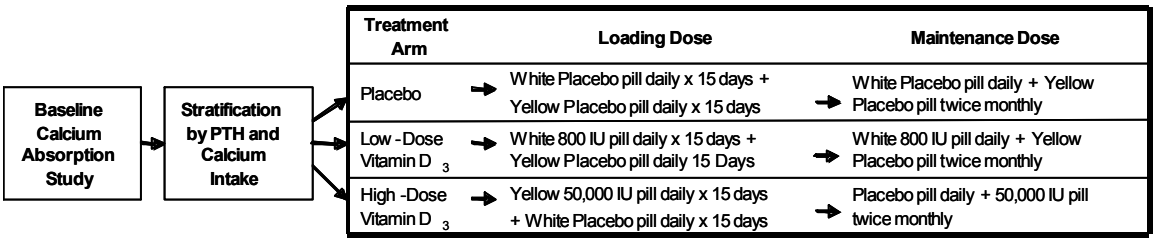
Calcium Isotopes. We use dual stable calcium isotope tracers to measure Ca·Ab. The method is the "gold standard" because the intravenous isotope tracks renal re-absorption and endogenous fecal calcium excretion [61, 62]. Stable calcium isotopes are not radioactive and have no known toxicity [61]. We purchase isotopes as calcium carbonate powder and confirm their purity and enrichment by mass spectrometry. UW Waisman Clinical Biomanufacturing Facility personnel reconstitute the calcium powders as previously described [17]. They test solutions for sterility and pyrogenicity, using procedures described in the United States Pharmacopoeia [63] and in our pilot study [17]. Once the

solutions demonstrate sterility and non-pyrogenicity, they are released to the PRC for human use. Before use in research subjects, Dr. Hansen will test the isotopes on herself. Dr. Shafer (Wisconsin State Laboratory of Hygiene) quantifies concentrations and ratios of calcium isotopes in clinical specimens by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) [64, 65] as described in greater detail in our pilot study [17].

Vitamin D Repletion. At the end of the first Ca·Ab study, an unblinded member of the University of Wisconsin (UW) Pharmaceutical Research Center (PRC) will randomize women into treatment arms (**Fig. 1**). The pharmacist will stratify subjects such that equal numbers of women with a) high PTH (above upper limit of normal) at the screening visit and b) calcium intake 600-1,000 mg daily and >1,000 mg daily based on four day diet diary data enter each treatment arm. Approximately 365 days after onset of therapy [66], women will undergo a second Ca·Ab study visit identical to the first.

We summarize the randomization strategy in **Fig. 1**. In the high-dose vitamin D treatment arm, women will ingest vitamin D_3 50,000 IU daily for 15 days and daily placebo. The high-dose regimen is both safe [17, 67] and effective [17, 68] at achieving 25(OH)D levels ≥30 ng/mL. Women randomized to low-dose vitamin D will receive 800 IU of vitamin D_3 daily and placebo for 15 days. Women randomized to placebo will receive two placebo capsules daily for 15 days. After the first 15 days of therapy, women will immediately begin maintenance doses of vitamin D or placebo and continue these for the remaining 350 days of participation. Subsequent 25(OH)D measurements will occur just before scheduled ingestion of yellow capsules, representing nadir 25(OH)D levels for subjects receiving high-dose vitamin D.

Figure 1: Summary of Treatment Arms



We will obtain low and high-dose vitamin D and matching placebo capsules from Tischon, Inc. (Westbury, NY). We will collect blood to measure participants' nadir 25(OH)D levels at visits 3, 4, 5 and 6 (**Table 1**). An unblinded member of PRC will review these 25(OH)D results. If a woman in the high-dose treatment arm develops recurrent VDI (25(OH)D <30 ng/mL), the PRC will adjust vitamin D capsules and we will subsequently re-measure 25(OH)D to confirm vitamin D repletion. For example, a woman whose 25(OH)D level is 25 ng/mL will receive vitamin D_3 50,000 IU daily for 7 days to achieve repletion, followed by 50,000 IU once weekly for the remainder of the study to maintain repletion (Figure 2). To maintain blinding, ~8% of women in each of the placebo and low-dose vitamin D treatment arms will receive similar sham capsule adjustments.

Our specific instructions to the Pharmacy Research Center regarding adjustment of high-dose vitamin D capsules are shown in the figure below. Redosing will occur in the following cases:

1. After visits 3, 4, 5, and 6, an unblinded member of the PRC will review 25(OH)D results, and if a woman in the high-dose treatment arm develops recurrent VDI (25(OH)D < 30 ng/mL), the PRC will adjust vitamin D tablets.
2. To maintain blinding, up to ~8% of women in each of the placebo and low-dose vitamin D treatment arms will receive similar sham capsule adjustments.

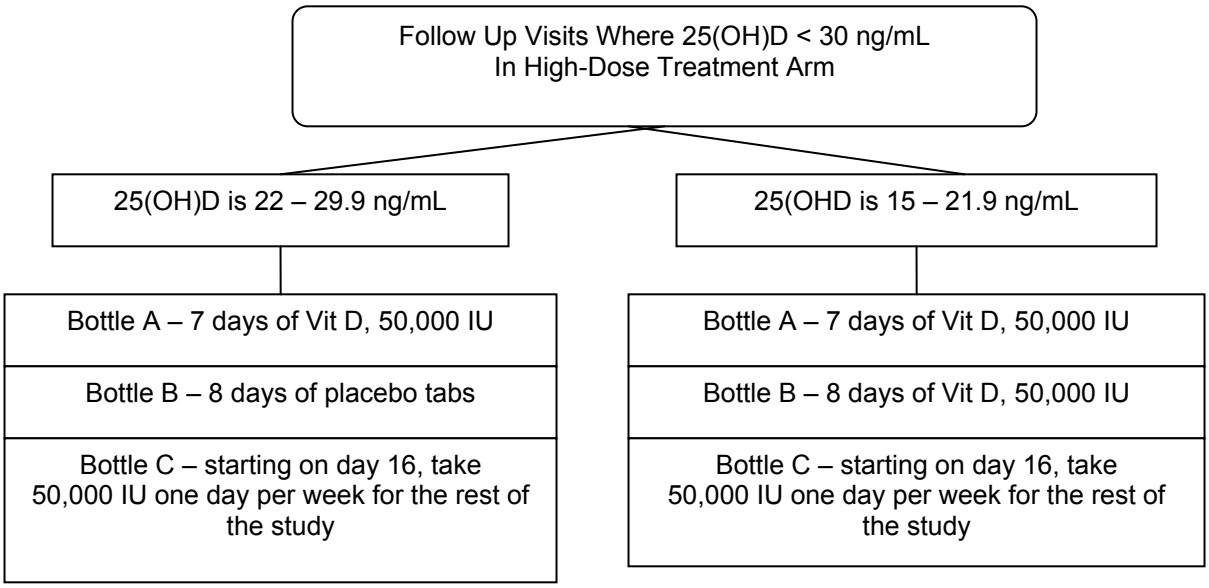
For the redosing, the Pharmacy will dispense the following study medication as shown in the figure below. PRC will dispense daily white pills (containing either low-dose vitamin D or placebo) plus Bottle A, Bottle B and Bottle C yellow capsules (containing either high-dose vitamin D or placebo).

Subjects will take the new study medications after they have finished taking previously dispensed study medications. Study medication from Bottle A will be taken for 7 days. After that, study medication from Bottle B will be taken for 8 days. Starting on day 16, subjects will take one pill from Bottle C once a week for the rest of the study.

The study team will contact the subject to discuss the redosing. Each redosing event should be examined to determine whether a redosing visit is necessary. Redosing visits will likely be needed if the redosing is identified after visit 5 or visit 6. The study team will document the date of the redosing in the database.

All subjects, regardless of whether they undergo adjustments in yellow capsules, will continue to have 25(OH)D measured throughout the study, as the study is focused on whether the benefits of vitamin D therapy require a serum level >30 ng/mL.

Figure 2: Instructions to Adjust High-Dose Vitamin D Capsules for Subjects with Recurrent Vitamin D Insufficiency



If a subject continues to have vitamin D insufficiency despite an increase in high-dose vitamin D tablets, the PI will decide whether this relates to poor adherence to therapy, poor absorption of vitamin D capsules, or other factors. The PI will then decide whether to withdraw the subject from the study or permit continued participation. The intent to treat principle favors the plan to allow each subject to complete the study, but mitigating circumstances might influence this decision.

To avoid vitamin D repletion in the placebo arm, we will ask *all participants* to apply Total Block® sunscreen between April and October when at Wisconsin's latitude, sun exposure promotes cutaneous vitamin D synthesis [69]. Such a strategy prevented vitamin D repletion among subjects randomized to placebo in a recent study conducted by the PI (HSC Protocol 2004-0011).

At the time of randomization, subjects will receive verbal and written instructions regarding study medication and use of sunscreen. With each subject's permission, we will send a letter to her primary care provider regarding her participation in the study.

Physical Activity Score. Physical activity may interact with nutritional factors to enhance BMD [70]. At visits 2 through 7, we will assess activity using the Physical Activity Scale for the Elderly (PASE) questionnaire to assess subjects' physical activity, a 5-minute, easily scored, reliable and validated instrument [71-73] commonly used to assess physical activity in older adults.

Timed Up and Go Test. The TUG score is the number of seconds required to stand from a standard arm chair, walk three meters, turn, walk back to the chair and sit down again [74]. The final score is the best score of two attempts [74]. The test typically takes less than one minute to complete [75, 76] and demonstrates a high intra-and inter-rater reliability [74].

Sit-To-Stand 5 (STS-5) Test. The STS-5 test is the time it takes for a person to stand from a a 43 cm chair without armrests, with his or her arms folded, five times in a row. The test is a validated non-invasive measure of function and predicts the risk of falls [77].

Health Assessment Questionnaire. We will administer the modified HAQ to subjects. The modified HAQ asks patients to note difficulty performing everyday activities and to indicate the need for aids, devices or assistance. The HAQ is sensitive to change in functional status and predicts relevant outcomes such as unemployment and medical costs [78, 79].

VIDSUN Sun Exposure Index. At screening, we will assess sun exposure using a questionnaire [14] we modified by the addition of a Fitzpatrick score for sun sensitivity and questions we previously found to predict vitamin D status [80].

Bone Mineral Density and Muscle Mass. Technologist Mary Chechovich will measure each woman's total body BMD and BMC, lumbar spine and femur BMD and muscle mass at screening and twelve months following randomization, using a 2006 iDXA bone densitometer (GE Healthcare, Madison, WI). The subject lays supine on a padded table for ~15 minutes. The radiation dose is approximately 15% [81] of that associated with a chest X-ray [82]. We analyze images using the software graphics display image and manufacturer instructions.

We will also analyze lumbar spine bone mineral density images to determine the trabecular bone score. If our initial statistical analysis reveals that vitamin D has no effect on spine, hip or whole body BMD or BMC, we will perform post-hoc analyses to assess the effect of vitamin D on the trabecular bone score.

Assessing and Encouraging Adherence. We will assess adherence by measuring 25(OH)D_3 levels and counting remaining capsules at study visits 3-7. We will encourage adherence verbally and through use of calendars that indicate the timing of white and yellow study capsule ingestion. Additionally, we will contact participants by phone approximately one week after randomization to encourage adherence to study tablets.

Indications for Unblinding and Withdrawal. Throughout the study, an unblinded UW PRC member will monitor subjects' safety by reviewing their levels for serum calcium (visits 2 through 7) and 24-hour urine calcium (visits 2, 3 & 6). If a woman develops hypercalcemia, defined as a serum calcium ≥10.4 mg/dL, she will undergo a second serum calcium measurement. If hypercalcemia persists, the woman will be un-blinded and withdrawn from the study.

If a woman develops >400 mg of urinary calcium loss in 24 hours, we will repeat the test and if urine calcium remains high, counsel the woman to reduce her calcium intake. As hypercalciuria occurs in 15% to 25% of adults and most often remains asymptomatic [77], we will not withdraw women with hypercalciuria from the study.

Finally, we will unblind and withdraw any woman reporting nephrolithiasis or fragility fracture. We will follow CONSORT guidelines [83], tracking all adverse events and reasons for study withdrawal.

In order to quickly capture serious adverse events, we will ask participants to notify us as soon as possible regarding hospitalization, regardless of the cause. If a woman's hospitalization is deemed related to a side effect from the study, we will withdraw the woman from the study.

Aim 1 Primary Outcome and Sample Size Calculation. We will evaluate **c**hanges in Ca·Ab within and between treatment arms. In our pilot study [17], the SD for absolute change in Ca·Ab with high-dose vitamin D therapy was 1%. In another study [25], the SD for the change in Ca·Ab with low-dose vitamin D was 7%. The SD for the monthly change in Ca·Ab is 1% with no intervention, based on data from an ongoing study on the effect of omeprazole on Ca·Ab in postmenopausal women (clinicaltrials.gov, NCT00582972). Thus, recruitment of 70 women into each treatment arm provides ~90% power to detect a 3% difference in the change in Ca·Ab between high-dose and placebo arms, and ~80% power to detect a 3% difference in between high-dose and low-dose vitamin D arms with a two sided alpha of 0.05. We increased the sample size by ~10% to account for potential attrition during the study. We will recruit women of all racial backgrounds. However, we recognize that race may influence the effect of vitamin D on Ca·Ab, especially in African American women [84]. Rather than limit recruitment to Caucasian women, we will increase the sample size by ~10% (n=250, all subjects), as non-Caucasians represent ~10% of adults living in Dane County, WI.

Statistical Analysis. We will use R (The R Project for Statistical Computing, http://www.r-project.org) to analyze study data and a two-sided alpha of 5% for statistical significance. If the data are parametric, we will use the independent sample t-test to compare changes in Ca·Ab between treatment arms and the paired t-test to evaluate changes in Ca·Ab within treatment arms. If data are non-parametric, we will use the Wilcoxon signed-rank test (paired data) or the Mann-Whitney-U test (data for independent groups). Further details on our analysis plan are found in the R01 application.

Anticipated Outcomes to Subjects and Society. We hypothesize that postmenopausal women with VDI will enjoy higher Ca·Ab, increased BMD and possibly improved functional status and muscle mass from vitamin D therapy. We expect to clarify whether a 25(OH)D level ≥30 ng/mL, achieved using a high-dose vitamin D regimen, is necessary to achieve these functional outcomes. Our study might confirm vitamin D as an inexpensive treatment to lower osteoporosis risk for postmenopausal women with VDI, congruent with Healthy People 2010 objective 2-9. If vitamin D therapy does not significantly influence Ca·Ab or BMD in women with VDI, a significant cost-savings will occur with fewer 25(OH)D tests and fewer prescriptions for vitamin D therapy nation-wide.

Data Management. We will use Oncore Enterprise Research Management (Oncore-ERM) to securely manage and protect study data and R (The R Project for Statistical Computing, http://www.r-project.org) to analyze data. The NIH-sponsored UW Institute for Clinical and Translational Research recently signed an agreement with PercipEnz Technologies, Inc. (Madison, WI) to implement the Oncore-ERM system in clinical and translational research projects. Twenty cancer centers across the country currently use the Oncore system to handle clinical trials data. The Oncore-ERM system will allow us to securely handle data collected from this clinical trial.

Project Time Table. Our proposal requires five years of effort. Within the first two months of grant support, we will purchase and reconstitute calcium isotopes and secure IRB approval for the study. Subsequently, we estimate ~4 years of effort to identify 250 eligible subjects, each participating for one year. In the final year of the study we will enter data, calculate Ca·Ab, analyze data and compose multiple manuscripts.

PROTECTION OF HUMAN SUBJECTS

We expect no toxicity from the vitamin D doses planned for use in this study. Vieth's review found no

cases of hypercalcemia from vitamin D doses <40,000 IU daily for ≤12 consecutive *weeks* [67] (**See Table 2**). Furthermore, we will exclude women with preexisting conditions increasing the risk of vitamin D-induced hypercalcemia. If a woman develops hypercalcemia or nephrolithiasis during the study, we will re-evaluate the protocol to determine if we should use lower doses of vitamin D therapy to achieve and maintain vitamin D repletion.

A. Human Subjects Involvement and Characteristics: We will recruit 250 postmenopausal women with VDI for this study. We describe the inclusion and exclusion criteria in the proposal itself. Several criteria exclude women in whom vitamin D may be harmful (e.g. preexisting hypercalcemia). Based on inclusion/exclusion criteria, we anticipate that participants will be at least five years past menopause and in good health without preexisting osteoporosis.

Working with the legally blind population: If subjects who cannot read the consent materials due to blindness, or the subject's legally authorized representative (LAR) is legally blind, the following is consent process will be followed:

a) An impartial witness will observe the consent process, such as a subject advocate or someone not affiliated with the research team. Family members are not recommended to serve as witnesses. The current CTRC nursing staff are considered an impartial witness.
b) The consent and HIPAA authorization forms will be presented to potential subjects orally.
c) If potential subjects have access to equipment that can read the consent document for them, study staff will provide sufficient time for them to review the consent document independently prior to the screening visit. This may include emailing the consent documents ahead of time.
d) The consent and HIPAA documents will include a statement that the forms were read to the subject by a member of the research team designated to obtain informed consent. This will be satisfied by an added label that says, "this consent form was read orally to patient by the study team with an impartial witness".
e) The subject will sign and date the consent form and HIPAA, if capable of doing so. In the case that informed consent is being obtained from the subject's LAR, the LAR will instead sign and date the form.
f) The witness will sign and date the consent form and HIPAA, and will write a short statement that attests that the consent information was accurately explained and that the subject apparently understood the information, and informed consent was given freely.
g) In accordance with the current protocol, the person obtaining consent will sign and date both the consent form and HIPAA, and signed copies of the consent and HIPAA forms will be provided to the subject or subject's LAR.
h) As would be expected for any consent process, it is the investigator's responsibility to judge the subject's comprehension of the consent information including the understanding that participation is voluntary and that the subject has the right to withdraw at any time during the study. If Dr. Hansen or the study team doubts the subject's consent comprehension, the subject will not be enrolled in the study.

B. Sources of Material: We will collect blood and urine from study subjects for research purposes only. At the conclusion of the study, we will destroy any residual samples, unless the subject has authorized that her samples can be banked. We will destroy coded samples via autoclave. Among patients who agree to serum, urine and/or DNA banking, we will keep samples indefinitely or until such time as samples are used for other studies. Twice during the study, each woman will complete a four-day diet diary to assess calcium and other nutritional intake. We will collect the following data from each woman enrolled in this study: age, ethnicity, race, weight, height, tobacco use, past medical history, current medications and supplements, BMD, nutritional intake, results of blood and urine tests and calculations of Ca·Ab. For each subject, we will use questionnaires to assess sun exposure, physical activity and functional status.

Table 2: Reported Cases of Vitamin D Toxicity, Excerpted from [67]

Reference	Dose of Vitamin D	Duration of Therapy	Total Cumulative Dose
Mason	50,000 IU	Daily for > 52 weeks	> 18,200,000 IU
Haddock, 1982	75,000 IU	Daily for > 100 weeks	> 52,500,000 IU
Gertner	20,000 IU 40,000 IU 55,000 IU 80,000 IU	Daily for 12-52 weeks	>1,680,000 IU >3,360,000 IU >4,620,000 IU >6,720,000 IU
Counts	100,000 IU	Daily for 12 weeks	8,400,000 IU
Hughes	250,000 IU 150,000 IU 100,000 IU	Daily for > 52 weeks	91,000,000 IU 54,600,000 IU 36,400,000 IU
Streck	100,000 IU	Daily for 200 weeks	140,000,000 IU
Davies	150,000 IU 100,000 IU	Daily for 364 weeks Daily for 520 weeks	382,200,000 IU 364,000,000 IU
Mawer	75,000 IU 200,000 IU 100,000 IU 50,000 IU 171,429 IU 100,000 IU 100,000 IU 50,000 IU	Daily for 520 weeks Daily for 520 weeks Daily for 520 weeks Daily for 1248 weeks Daily for 26 weeks Daily for 520 weeks Daily for 312 weeks Daily for 1040 weeks	273,000,000 IU 728,000,000 IU 364,000,000 IU 436,800,000 IU 31,200,078 IU 364,000,000 IU 218,400,000 IU 364,000,000 IU
Allen	75,000 IU	Daily for 19 years	520,125,000 IU
Rizzoli	600,000 IU 300,000 IU 300,000 IU 43,000 IU 300,000 IU 300,000 IU 10,000 IU	Daily for 96 weeks Daily for 3 weeks Daily for 74 weeks Daily for 12 weeks Daily for 4 weeks Daily for 4 weeks Daily for 390 weeks	403,200,000 IU 6,300,000 IU 155,400,000 IU 3,612,000 IU 8,400,000 IU 8,400,000 IU 27,300,000 IU
Current study	**50,000 IU**	**Daily for 15 days, then 2 per month**	**1,300,000 IU initially, then 100,000 IU per month**
	800 IU	**Daily for one year**	**24,000 IU per month**

C. Potential Risks.

Physical Risks: Phlebotomy is a routine procedure that may cause minor pain or bruising at the site of needle entry. The serious complications reported with phlebotomy (e.g. dizziness, fainting, or local infection) are uncommon. The cardinal sign of vitamin D toxicity is hypercalcemia, a side effect not reported at the vitamin D doses used for this study [67] (**Table 2**). According to Vieth's exhaustive review of vitamin D safety, there are no documented cases of hypercalcemia from the doses of vitamin D used for this study. In my own search of the literature, I can find no additional citations that disagree with Vieth's statement. Hypercalcemia is unlikely to develop in participants, as we exclude women with risk factors for, or the baseline presence of, hypercalcemia. Additionally, nephrolithiasis is unlikely based on results of the Women's Health Initiative Study and another study of high-dose vitamin D [85]. We will exclude women with baseline osteoporosis to minimize the probability of fragility fracture during study participation. Calcium isotopes have no known toxicity including radioactivity. The isotope doses are extremely small compared to an over-the-counter antacid tablet. Before human use, we will test calcium isotopes to confirm sterility and absence of pyrogenicity. The bone density studies involve minimal radiation exposure, estimated at about 13% of that received during a typical airplane ride [82].

Social Risks: The main social risk of study participation is loss of confidentiality. We will label all

study specimens with study codes to maintain participant confidentiality during sample processing. All subjects will sign a HIPAA form documenting the parties involved in the study and the procedures to maintain confidentiality. We will not release study data to participants' employers, banks, insurance companies or other parties. Therefore, participation in this study will not affect insurability, employment or financial health. We will keep all study data in locked cabinets in the PI's office.

Psychological, Legal and Other Risks: During each 24-hour study, subjects may become bored or feel restricted in activities. We will discuss this possible side effect verbally and in written form during the consent process. As we protect confidentiality using study codes, and there is no social stigma associated with low vitamin D levels or changes in intestinal Ca·Ab, we anticipate no additional psychological or legal consequence from study participation.

Adequacy of Protection Against Risks

A. Recruitment and Informed Consent: We will recruit potential subjects through newspaper advertisements and letters of invitation to UW female faculty and registrants of the UW Institute of Aging Study Registry. At the first encounter, subjects will receive a verbal description of the study and read the consent form and HIPAA document. We will answer all questions regarding the study. Consenting volunteers will receive a copy of the consent form and HIPAA document and will then undergo study procedures. Based on results of screening tests, we will invite eligible subjects into the treatment study. At any time prior to, during or after study participation, we will answer all subjects' questions to our best ability.

B. Protection against Risk: As noted above, the risk of harm from participation is low. We carefully selected inclusion and exclusion criteria to minimize risk to participants. Additionally, PRC will regularly monitor serum and urine calcium throughout the study to verify the safety of vitamin D therapy. Hypercalcemia and hypercalciuria are unlikely among women who participate in this study. First, patient risk factors for, or the baseline presence of hypercalcemia at screening is cause for exclusion. Second, the doses of vitamin D we will use in this study have not caused hypercalcemia based on a comprehensive literature review [67]. In addition, the risks of harm from receiving intravenous and oral calcium are extremely low, as the doses of calcium used in this study are less than that within one single over-the-counter calcium tablet. Stable calcium isotopes occur naturally in the environment and have no reported toxicity or radioactivity. Before use, we will test calcium isotopes to confirm sterility and absence of pyrogenicity. The bone density studies involve minimal radiation exposure, estimated at about 13% of that received during a typical airplane ride [82]. Loss of confidentiality is a potential risk that we will minimize by labeling study samples with a code, rather than patient identifiers.

We will organize a formal data safety monitoring board to provide oversight for this study, with predefined endpoints (described below) for excess rates of hypercalcemia, nephrolithiasis or fracture. We will withdraw from the study individuals who develop hypercalcemia, nephrolithiasis or fragility fracture.

We will record all adverse events for this study and assess whether they are related to study participation. We will determine the severity of adverse events as follows:

"mild"	transient and easily tolerated by the subject
"moderate"	causes discomfort and interferes with activities of daily living
"severe"	causes considerable interference with the subject's usual activities

Potential Benefits of the Proposed Research to the Subjects and Others: Potential benefits to participants are limited to vitamin D therapy. Potential risks are minimized, as subjects likely to experience harm from vitamin D therapy are excluded from participation. Additionally, a comprehensive review of vitamin D toxicity found no harm from the vitamin D doses proposed in this study [67]. Thus, the risks of participation are low whereas the import of the knowledge gained upon

the completion of this study is substantial.

Importance of the Knowledge to be Gained: Our overall goal, congruent with Healthy People 2010 objective 2-9, is to evaluate the effect of vitamin D therapy on the risk of osteoporosis in postmenopausal women with VDI, as reflected by changes in Ca·Ab, BMD and muscle fitness. Our second goal is to evaluate whether a high-dose vitamin D regimen, chosen to achieve and maintain a 25(OH)D level ≥30 ng/mL [17], is superior in its effects on study outcomes compared to a low-dose vitamin D regimen that can permit continued VDI [22-25]. If we show vitamin D increases Ca·Ab and subsequent BMD and/or muscle fitness among postmenopausal women or even a subset, such knowledge will widely influence the care of older women, in whom both VDI and osteoporotic fracture are extremely common. If we show that vitamin D therapy does not improve study outcomes, then providers can use health care dollars for other proven osteoporosis remedies. Results will assist experts in their attempts to define the optimal 25(OH)D level for bone health in postmenopausal women. In summary, the results of this study will clarify the clinical benefits of vitamin D for postmenopausal women with VDI, and at what dose these benefits occur.

Data Safety Monitoring Plan: The NIH has assembled a data safety monitoring board (DSMB) for this study. Members include Dr. J. Christopher Gallagher (Professor of Medicine and Chief, Bone Metabolism Section, Creighton University, Omaha, NE) and Kristine Ensrud (Professor of Medicine, and Director of Epidemiology, Clinical Research Center, U of MN) along with statistician Yvette Schuster (Professor of Statistics, Rutgers University). The DSMB will provide oversight for the conduct of this study and will meet at 25%, 50%, 75% and 100% of recruitment to review all adverse events. The predefined stopping points for this study will include excess rates of hypercalcemia (defined as serum calcium > 1 mg/dL above the normal range), hypercalciuria (>400 mg of calcium in a 24-hour urine collection), nephrolithiasis and fragility fracture of the wrist, spine or hip. As required of all CTRC research, the CTRC DSMB will provide additional study oversight. The boundary for excess harm will equal a Z value > -3.0, or an observed excess harm which in the judgment of the DSMB, is excessive. The Peto-Haybittle sequential statistical boundary for identifying excess harm during a clinical trial is described elsewhere [86, 87]. We will report all serious adverse events to the DSMB, and we will review all adverse events at the regular DSMB meetings.

An unblinded member of PRC will review all test results as they become available. Our study has predefined parameters for unblinding due to hypercalcemia, hypercalciuria, nephrolithiasis or fragility fracture. We will notify collaborators, the Human Subjects Committee and the DSMB committees if unexpected adverse events occur including reactions to calcium isotopes, phlebotomy or vitamin D therapy. If we detect excess adverse events, we will modify or halt the study, depending on the severity and frequency of such events. We will minimize loss of confidentiality by labeling all study samples and records with the patient's study code.

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