Title:
Personalized Vitamin D Supplementation in Women of European and African Ancestry

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IRB:
University of Wisconsin Health Sciences Institutional Review Board

Monitor:
Institute for Clinical and Translational Research, Study Monitoring Service

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Amendment 1 Summary (V1.2 Dec 2015): Edited to specify that data not included in the OnCore data entry system will be provided to Dr. Engelman via coded Excel files. Additionally, changed to explain that if subject is not eligible for study, their screening serum calcium sample will not be run.

Amendment 2 Summary (V1.3 11Jan2016): Dr. Engelman made updates to the algorithm. Additionally, an editorial inconsistency was corrected.

Amendment 3 Summary (V1.4 01Dec2016): Language regarding subject discontinuation when developing diseases/conditions while on study that are exclusionary was updated per IRB recommendation. Per suggestion of the DSMB to re-evaluate inclusion/exclusion criteria, the investigators determined the "menopausal status" criterion was too conservative for women with surgical menopause. Consequently the associated age cut-off is being changed from 65 to 55 years.

Amendment 4 Summary (V1.5 April 2017): Updated recruitment materials to include past SHOW participants. Clarified inclusion criteria regarding menopausal status. Additional, editorial inconsistencies were corrected.

Amendment 5 Summary (V1.6 November 2017): Removed race stratification by site. Each site can now enroll both white and black participants.

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PROTOCOL OVERVIEW AND INTRODUCTION

Vitamin D deficiency is associated with many adverse health outcomes and is much more prevalent in individuals of African ancestry than in those of European ancestry. Treatment of vitamin D deficiency in both clinical and research settings has focused on the administration of a fixed dose of vitamin D. However, the increase in circulating 25-hydroxyvitamin D (25[OH]D) in response to a fixed dose is highly variable from person to person [Aloia et al., 2008; Binkley et al., 2011], yet little is known about the determinants of this inter-individual variation. Our preliminary data suggest two factors, baseline total 25(OH)D and 24,25$(OH)_2$D (an indicator of 25(OH)D degradation), explain a significant amount of this variation. We have developed an algorithm that uses these factors to tailor the vitamin D_3 dose administered. **The next step and primary goal of this proposal is to validate the algorithm in an independent sample of post-menopausal women of European and African ancestry. Additionally, we will examine the following novel factors that may contribute to the remaining inter-individual variability in response to vitamin D supplementation**: serum levels of cholecalciferol (vitamin D_3; absorption), body composition (storage), and genes coding for the vitamin D binding protein (*GC*; transportation) and 25 hydroxylase (*CYP2R1*; synthesis) [Engelman et al., 2013]. Our rationale for the proposed research is that validation and refinement of our dosing algorithm would optimize vitamin D supplementation and potentially improve downstream health outcomes.

BACKGROUND

In our pilot study of 91 community-dwelling postmenopausal women of European ancestry treated with 2300-2500 IU of vitamin D_3 daily for 4-6 months, we utilized novel liquid chromatography, tandem mass spectroscopy (LC-MS/MS) methodology to evaluate serum levels of cholecalciferol (vitamin D_3) and 24,25$(OH)_2$D at baseline and after 4-6 months. We observed the anticipated relationship with cholecalciferol: a greater increase in circulating vitamin D_3 is associated with a greater increment in 25(OH)D. Additionally, we reproduced the observation of others [Wagner et al., 2011] that a higher ratio of 24,25$(OH)_2$D/25(OH)D after supplementation is associated with a less robust increase in 25(OH)D. Thus, our pilot work supports the premise that smaller increments in 25(OH)D may result from relatively low vitamin D_3 input (presumably from poor absorption) and/or greater degradation. To this end, we performed multiple regression analysis to determine the characteristics that might enable prediction of the 25(OH)D achieved with supplementation. The equation from the final model was: post-treatment 25(OH)D = 8.3 + (1.05*baseline 25(OH)D) – (7.7*baseline 24,25$(OH)_2$D) + (0.53*follow-up cholecalciferol) + (4.2*follow-up 24,25$(OH)_2$D). This model had an adjusted R^2 = 0.55 with p <0.001 for all the parameters, thus accounting for approximately half of the variance in the final 25(OH)D level. The baseline values of cholecalciferol could not be used in this model because the data were highly skewed (virtually all values were zero since the assay is currently not capable of measuring very low concentrations of cholecalciferol). Nonetheless, these preliminary data support the dosing algorithm described below.

HYPOTHESES AND SPECIFIC AIMS

Aim 1:

Determine the efficacy of our dosing algorithm in achieving total 25(OH)D concentrations of 35-50 ng/mL in women of European and African ancestry.

To validate our vitamin D_3 dosing algorithm, we will conduct a randomized, double-blind, controlled, six-month duration clinical trial of daily oral vitamin D_3 to determine the efficacy of dosing following our algorithm compared to 2500 IU/day (control arm) in achieving the target total 25(OH)D concentration of 35-50 ng/mL. Participants will be community-dwelling post-menopausal women of European and African ancestry, who have a baseline total serum 25(OH)D of ≥10 but <30 ng/mL.

H1a: The percent of individuals with total 25(OH)D of 35-50 ng/mL will be significantly higher in the dosing algorithm arm than in the control arm.

H1b (exploratory aim): The percent of African ancestry women with total 25(OH)D of 35-50 ng/mL will be similar to (i.e., not significantly different than) that in women of European ancestry.

Aim 2:
Determine additional factors that predict response to vitamin D supplementation.
We will expand our predictive model to include baseline serum cholecalciferol and body composition, measured by dual-energy x-ray absorptiometry (DXA), and genotypes in the *GC* and *CYP2R1* genes. In exploratory analyses we will also determine the effect of self-reported ancestry on our dosing algorithm.

H2a: Baseline body mass, but not total body fat, will be negatively associated with total 25(OH)D after supplementation, indicating that individuals with larger body size, not just body fat, will require a higher dose of vitamin D_3 to attain the target total 25(OH)D range.

H2b: Baseline cholecalciferol will be positively associated with 25(OH)D after supplementation, where individuals with higher values will require a lower dose.

H2c: There will be a negative association between the number of risk alleles in the two genes and total 25(OH)D after supplementation, where individuals with more risk alleles will require a higher dose.

Completion of these aims will allow us to validate and refine our vitamin D dosing algorithm. This predictive tool will allow clinical estimation of the dose needed to achieve a target 25(OH)D level, advancing clinical care by allowing personalization of vitamin D supplementation. Moreover, the insight gained from the proposed study will enable clinical trials of other vitamin D-related outcomes to achieve more effective vitamin D supplementation by using our dosing algorithm. This will enhance future research proposals by radically improving the study design.

STUDY DESIGN
Study Design and Population.
The proposed study is a randomized, double-blind, controlled, multi-center clinical trial of six months of daily oral vitamin D_3 (cholecalciferol). This study will randomize 334 community-dwelling post-menopausal women of European and African ancestry (~167 from each ancestry) in a 1:1 ratio between the control arm and the dosing algorithm arm using stratified block randomization with a block size of six and stratification by site (ancestry). The sample size of 334 includes 10% over-recruitment to allow for loss to follow-up. The proposed study will focus on post-menopausal women because this is the subset of the population that both Dr. Engelman's and Dr. Binkley's preliminary data are drawn from. Moreover, 25(OH)D concentrations are typically lower in women and in older individuals, since production of vitamin D in the skin following sun exposure decreases with age. Therefore, this group of individuals is likely to benefit the most from vitamin D supplementation, especially when personalized based on biology using our dosing algorithm.

Vitamin D Preparation and Dosing.
All vitamin D_3 preparations will be produced by Tishcon Corp (Westbury, NY). Vitamin D_3 content of these preparations will be independently validated in the laboratory of Dr. H. DeLuca prior to study initiation and annually thereafter to assure stability. The control group will receive 2500 IU of vitamin D_3 daily while the dosing algorithm group will initially receive 1000, 2500, or 4000 IU daily. The rationale for these dosing arms is based on our pilot data. First, we aim to reduce the variation in achieved 25(OH)D following vitamin D supplementation, bringing more individuals into the target range of 35-50 ng/mL. In our pilot study, participants received 2300-2500 IU/day, a dose similar to many other vitamin D supplementation trials. Therefore, to test the alternate hypothesis that our dosing algorithm will improve vitamin D supplementation beyond that typically given in clinical trials, the appropriate control group should receive 2500 IU/day. Second, in our multiple regression analysis to determine characteristics that predict 25(OH)D achieved after supplementation, the following equation had an adjusted R^2 of 0.55 (p<0.001): post-treatment 25(OH)D = 8.3 + (1.05*baseline 25(OH)D) –

(7.7*baseline 24,25$(OH)_2D$) + (0.53*follow-up cholecalciferol) + (4.2*follow-up 24,25$(OH)_2D$). Using only the baseline values, which would be available at an initial clinical visit, including baseline cholecalciferol, post-treatment 25(OH)D = 18.19385 + (0.65967*baseline 25(OH)D) + (1.02393*baseline 24,25$(OH)_2D$) + (0.13564*baseline cholecalciferol). For the current study, the dosing at both baseline and the 3-month follow-up visit will be determined using this equation to predict post-treatment 25(OH)D (at the 3-month follow-up visit, the 3-month values will be substituted for the baseline values in the equation). Specifically, for those whose predicted 25(OH)D is ≥35 ng/mL and <50 ng/mL, we will administer a dose of 2500 IU daily. For those whose predicted 25(OH)D is <35 ng/mL, we will administer 4000 IU/day and for those whose predicted 25(OH)D is >50 ng/mL we will administer 1000 IU/day. The target 25(OH)D range was selected as nearly all experts agree that 25(OH)D levels above ~30-32 ng/mL are adequate; hence we have chosen a target level ≥35 ng/mL to account for assay variability, thereby assuring that all have concentrations >30-32 ng/mL. An upper limit of 50 ng/mL was selected based on evidence suggesting that 25(OH)D levels above this value might have adverse effects [Sempos et al., 2013; Stolzenberg-Solomon et al., 2010]. All three of the daily doses are within the range considered to be safe by a recent Institute of Medicine report [Ross 2011].

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria:

- Healthy, community-dwelling postmenopausal woman (natural menopause, 12 consecutive months without menstruation, or surgical menopause and ≥ age 55, or surgical menopause and postmenopausal symptoms ≥ 12 months ago)
- Self-reported > 50% European or > 50% African ancestry
- Able and willing to sign informed consent as affirmed by research staff person obtaining consent
- Screening serum 25(OH)D concentration of 10.0-29.9 ng/mL
- Willing to not alter the amount of their baseline vitamin D supplementation during the course of this study
- Willing to use sunscreen (SPF ≥15) when sun exposure of >15 minutes is expected during the months of May through September

Exclusion criteria:

- Diagnosis of chronic kidney or liver disease requiring care by a nephrologist or hepatologist respectively. Note this includes active kidney stone disease within the past 12 months
- Current hypercalcemia (serum calcium ≥ 11.0 mg/dL) or other disorders that may affect vitamin D metabolism and predispose to hypercalcemia, i.e., sarcoidosis, active tuberculosis or other granulomatous disease
- Other chronic diseases or conditions potentially affecting vitamin D metabolism or absorption (inflammatory bowel disease, cystic fibrosis, ulcerative colitis, and malabsorptive surgery)
- History of nephrolithiasis
- Current use of medications determined by study investigators to interfere with vitamin D metabolism (for example: glucocorticoids, anticonvulsants, antifungals, and HIV/AIDS medications)
- History of any form of cancer within the past two years with the exception of basal or squamous cell skin lesions, *in situ* tumors or thyroid cancer
- Terminal illness/on hospice
- Severe end-organ disease (e.g., cardiovascular, pulmonary, etc.), which may limit the ability to complete the study in the opinion of the clinical investigators
- Treatment with high dose vitamin D (≥50,000 IU weekly) or any active metabolites of vitamin D, e.g., calcitriol, within six months of screening; current use of multiple vitamins and other vitamin D supplements will be allowed

- Use of tanning beds or salons or unwillingness to utilize sunscreen during periods of sun exposure of 15 minutes or longer from May through September
- Planned trips/vacations likely to be associated with substantial amounts of sun exposure during the course of the study (i.e., more than 500 miles south of Madison/Milwaukee)
- Not suitable for study participation due to other reasons at the discretion of the PI

STUDY VISITS AND EVENT FLOWCHART

Potential volunteers will be screened by telephone. Those meeting all inclusion and no exclusion criteria will be invited to a screening study visit. At screening, informed consent will be obtained. We will then collect the following to determine study eligibility: basic demographic information (age, ancestry, and education); medical history; medication and supplement use; and blood for screening 25(OH)D and calcium tests. If a subject is not eligible for study participation based on 25(OH)D value, her screening serum calcium sample will not be run, and the sample will be destroyed. Additionally, these subjects will be notified if their ineligibility was due to their 25(OH)D value being too high or too low for study requirements. This will occur starting with subjects who are screened from the time of Amendment 1's IRB approval. All subjects with screening 25(OH)D within ± 5 ng/mL of the range noted above, and offer permission, may be contacted to re-screen at a future time point. Volunteers meeting all inclusion and no exclusion criteria will be invited to participate and complete a study baseline visit, at which point they will be considered enrolled. At baseline, participants will be randomly assigned to the control or dosing algorithm group. Both participants and study staff who have contact with the participants will be blinded to group assignment. Follow-up visits will occur at three and six months. At baseline and follow-up visits, height and weight will be measured, and blood will be drawn for the vitamin D panel, calcium, and PTH. Blood for DNA extraction and body composition will only be obtained at the baseline visit. Participants will be asked to return all unused study supplements and compliance will be assessed at each follow-up visit by pill count. Safety will be monitored through adverse event reporting and review of calcium results. Study visits are summarized in the flow chart below.

Study Visit Event Flow Chart

Time	**-1**	**0**	**3**	**6**
Study visit	Screening	Baseline	3-month	6-month
Informed consent	X			
Demographic information	X			
Medical history	X			
Medication and supplement use	X	X	X	X
Serum 25(OH)D	X			
Serum calcium	X	X	X	X
Height and weight measurement		X	X (weight only)	X (weight only)
Body composition measurement		X		
Whole blood for DNA extraction		X		
Serum vitamin D panel		X	X	X
Serum free 25(OH)D and $1,25(OH)_2D$		X	X	X
Plasma calcium and PTH		X	X	X
Randomization		X		
Supplement dispensation and accountability		X	X	
Adverse event recording		X	X	X
Assess compliance			X	X

VITAMIN D PREPARATION
Capsules containing 1000, 2500, and 4000 IU of vitamin D_3 will be obtained from Tischon corporation (Westbury, NY). The capsules will be protected from light and not stored above 25° C. Participants will be instructed to take their vitamin D_3 capsules with supper daily. Compliance with study supplementation will be documented by capsule count at the time of each study visits.

VITAMIN D ACCOUNTABILITY
Subjects will be mailed 110 capsules following the Baseline visit. The subjects will be asked to bring any unused supplement to the 3-month visit to be counted and re-dispensed. An additional 110 capsules will be mailed to the subjects following the receipt of their 3-month vitamin D panel lab values. All unused supplement will be returned at their 6-month visit to be counted and retained by the study team.

BLOOD COLLECTION, LABORATORY ANALYSES, AND BODY COMPOSITION
Blood collection.
Blood will be collected via venipuncture into vacutainer tubes. For subjects who agree to optional banking, serum aliquots will be obtained and banked for future study of markers relevant to vitamin D assessment. Banked samples will be stored in a locked -80 freezer. Stored samples will be labeled with subject ID number, initials and collection date.

All blood specimens will be stored and managed at the University of Wisconsin. Calcium, vitamin D and PTH tests will be conducted at the UW Health Clinical Research Laboratory, WNPRC or Dr. Binkley's research lab. The whole blood for DNA extraction will be frozen at -80°C on the day of collection until transportation in batches to the University of Wisconsin DNA Sequencing Facility for DNA extraction and genotyping

Vitamin D Panel.
We will obtain serum concentrations of vitamin D_3 (cholecalciferol; an indicator of vitamin D_3 absorption), 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2 [25(OH)D_2], the C3-epimer of 25(OH)D_3 [3-epi-25(OH)D_3] (a potentially inactive or less active metabolite), and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] (an indicator of 25(OH)D degradation). The vitamin D panel will be run in the UW WIMR laboratory by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Additional Laboratory Analyses.
An assay to directly measure free serum 25(OH)D is currently nearing completion of development in Dr. Wiebe's lab and will be used for the proposed study in year 2 or 3. Evidence on the importance of free 25(OH)D is evolving; it is important to measure this to enable us to incorporate it into our analytic plan. Serum 1,25$(OH)_2$D will also be measured in Dr. Wiebe's lab by LC-MS/MS to fully characterize vitamin D status. Serum (at screening) and plasma (all other visits) calcium will be measured in the UW Clinical Laboratory to assess potential vitamin D toxicity. Plasma PTH will be measured using commercially available kits in Dr. Binkley's laboratory as a surrogate of the physiologic effect of vitamin D.

Genotyping of Vitamin D-related Genetic Variants.
Genotyping will be performed at the using the University of Wisconsin DNA Sequencing Facility using the KASPar® assay system from KBiosciences, Ltd (Hoddesdon, UK). We will genotype a non-synonymous (coding) SNP, rs4588, in *GC* that results in an amino acid change in the vitamin D binding protein, affecting its metabolism, [Kawakami et al., 1981] blood concentration [Lauridsen et al., 2001], and affinity for 25(OH)D [Arnaud and Constans, 1993]. This SNP, or a SNP in strong linkage disequilibrium (correlation) with this SNP, is associated with 25(OH)D in multiple studies [Ahn et al., 2010; Engelman et al., 2008; Engelman et al., 2013; Lasky-Su et al., 2012; Lu et al., 2012; Robien et al., 2013; Wang et al., 2010; Zhang et al., 2012]. We will also genotype a SNP, rs2060793, in *CYP2R1*.

This SNP, or a SNP in strong linkage disequilibrium with this SNP, has also been associated with 25(OH)D in multiple racial/ethnic groups [Ahn et al., 2010; Engelman et al., 2013; Lasky-Su et al., 2012; Robien et al., 2013; Wang et al., 2010; Zhang et al., 2012]. Dr. Engelman is co-leading the TRANS-ethniC EvaluatioN of vitamin D (TRANSCEND) consortium, a multi-study collaborative effort to combine existing genome-wide association data from ethnic/racial minority populations with measured 25(OH)D. This consortium currently includes 6,765 individuals of African ancestry. Through this collaborative effort, Dr. Engelman may become aware of additional SNPs that are relevant in individuals of African ancestry. If this occurs, these additional SNPs will be genotyped.

Preliminary data support our hypothesis (H2c) that variability in 25(OH)D response to supplementation is determined, in part, by the number of risk alleles at these two SNPs (rs4588 and rs2060793). In a 3-month RCT of vitamin D supplementation in Asians, the *GC* variant, rs4588, was genotyped to investigate the change in serum 25(OH)D concentrations according to genotype [Nimitphong et al., 2013]. With vitamin D_3 supplementation, subjects with 1 or 2 copies of the rs4588 risk allele had significantly less increase in 25(OH)D after 3 months of dosing than those with 0 risk alleles. In a study led by Dr. Engelman, rs4588 and rs2060793, in *CYP2R1*, were examined and a genetic risk score was formed by summing the number of risk alleles for these two genetic variants [Engelman et al., 2013]. In 1,204 post-menopausal women from the Women's Health Initiative, 29% with 0 risk alleles and 30% with 1 risk allele whose vitamin D intake was in the highest quartile (≥670 IU/day) had 25(OH)D ≥35 ng/mL; this fell to 11% and 9% for individuals consuming at least 670 IU/day, but who had 2 or 3-4 risk alleles, respectively [Engelman et al., 2013]. This suggests that individuals with multiple genetic risk factors may need higher amounts of vitamin D to achieve our target 25(OH)D range of 35-50 ng/mL.

Genotype data will be evaluated for quality by computing the call rate and testing for departure from Hardy Weinberg equilibrium (HWE). In the unlikely event that the call rate is <95% or the genotype frequencies are not in HWE, the genotyping for the single nucleotide polymorphism (SNP) will be redone. The concordance rate from 15 blind duplicates will be calculated and any discordant genotypes will be set to missing.

Body Composition.
Total body dual energy x-ray absorptiometry (DXA) will be used to assess body composition, specifically percent body fat and lean mass. Body composition will be obtained using a GE Lunar densitometer. Both study sites' DXA technologists will follow standard operating procedures to assure uniform scan acquisition [Libber et al., 2012]. One technologist at UW, blinded to treatment arm, will review all scans for quality and analyze all scans, manually correcting any auto-analysis errors. The densitometers will be compared with a body composition phantom prior to study initiation and annually.

SAFETY
This study will be conducted in accordance with the International Conference of Harmonization (ICH) Good Clinical Practice (GCP) Guidelines and FDA regulations related to the use of investigational devices.

Adverse Events
Adverse events defined as any untoward medical occurrence in a subject that receives the study intervention. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study intervention. All adverse events will be documented, regardless of the relationship or attribution to the study intervention.

Clinical PIs will review all laboratory reports. Only laboratory values outside of the normal range that are deemed clinically significant will be recorded as adverse events Changes in medical history at month 3 and 6 new reported conditions, etc.

According to the ICH GCP Guidelines, a Serious Adverse Event is any untoward medical occurrence that, at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Adverse events will be elicited from subjects from the time of enrollment (Baseline visit) and followed until the subject's last visit.

Adverse events, including Serious Adverse Events, will be reported to the lead study site. The clinical PIs will evaluate all adverse events and determine relationship to study activities. SAE reports will be shared with the data and safety monitoring board, the IRB and additional appropriate institutional and federal authorities according to the applicable reporting requirements and timeframes.

The risks of participation in this study are extremely low. Specific risks due to venipuncture include pain, bleeding, bruising, infection and inflammation at the site. These risks will be minimized by using trained staff and aseptic techniques to obtain samples, reducing the likelihood of infection.

Safety concerns regarding the potential development of acute vitamin D toxicity manifesting as hypercalcemia could potentially be raised. However, the risk of acute vitamin D toxicity with the doses utilized in this study is extremely remote. Specifically, virtually all high-quality reports of vitamin D toxicity involve the long-term intake of >40,000 IU daily, significantly higher than the maximum daily vitamin D dose administered in the present study of 4000 IU.

In addition to the wide safety margin, we have taken additional steps to assure participant safety by use of inclusion/exclusion criteria selected to exclude those with existing hypercalcemia or renal failure, and those with other disorders or on medications that may affect vitamin D metabolism or absorption to minimize the potential of physical harm from vitamin D supplementation. Adverse events will be collected and recorded on case report forms for completeness, reviewed every six months by a Data Safety Monitoring Board, and reported at time of study publication.

An additional potential risk of study participation is breach of confidentiality. To minimize this risk, all data will be coded using unique 4-digit ID numbers with site-specific identifiers. Subjects will be assigned subject ID numbers sequentially at the time informed consent is obtained. Data for the proposed study will be recorded on paper case report forms labeled with subject name and ID number. The data from these forms will then be entered directly onto a secure server. Hard copies of the case report forms will be maintained in locked file cabinets within a locked office with access given only to the investigative staff. Access to individually identifiable private information about the study participants will be limited to staff involved in recruitment and study visits and this data will not be entered into the database. Datasets will be securely transferred to Dr. Engelman via a direct download from OnCore to Dr. Engelman's password protected space on one of the Social Sciences Computing Cooperative (SSCC) servers or coded excel spreadsheets will be emailed to Dr. Engelman. No data files will be placed on laptops or other portable devices. Participants will be made aware of the fact that their data will be used for publication and that individual identities will not be revealed. Data will be reported in aggregate so no individual person will be identifiable in publications. The IRB will be contacted in the event of any breach of confidentiality.

Data Safety and Monitoring Board

The Midwest Area Research Consortium for Health (MARCH) has established a Data and Safety Monitoring Board (DSMB) to provide a key resource for MARCH investigators conducting clinical

research. This DSMB will provide investigators services to ensure appropriate measures are in place to promote subject safety, research integrity and compliance with federal regulations and local policies for individual clinical research protocols in need of DSMB review (as determined by the Principal Investigator (PI), the funding agency, the local Scientific Review Committee, or the local IRB, and for which no DSMB exists). For these studies, the MARCH DSMB will be the primary data and safety advisory group for the Principal Investigator.

The DSMB is supported in its mission of safety and compliance by experienced MARCH staff to provide administrative assistance and experienced members representing a diversity of backgrounds, skills and knowledge. In addition to the core DSMB members, an ad hoc member with expertise in vitamin D metabolism and measurement will also serve on the DSMB. The DSMB will meet at least twice annually to review all adverse events. Additional meetings may be scheduled as determined by the DSMB. At these meetings, the DSMB members will review protocol-specific reports created by statisticians that serve a non-voting member role on the DSMB using data pulled from the OnCore clinical research management system. These standard reports will include an overview of study objectives, a review of actual and projected accrual rates, an evaluation of patient demographics for balance of randomization, and a summary of the number and seriousness of adverse events. An interim analysis of study results will be performed after 50% of randomized subjects have completed the study. Source documents may be reviewed to allow the DSMB to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. The DSMB will make recommendations to the Principal Investigator that could include actions of continuation, modification, suspension, or termination. The Principal Investigator will forward the communication of the DSMB recommendations and all pertinent regulatory information to the FDA, appropriate Institutional Review Board(s), and the Sponsor when applicable.

SUBJECT WITHDRAWAL, EARLY TERMINATION

Subjects may withdraw from the study, or the PI may terminate subject's participation at any time. Potential reasons for subject termination include the development of exclusionary conditions, including high blood calcium (>11.0 mg/dL), the use of exclusionary medications for greater than 4 weeks and reported travel >500 south miles from the study site for more than 2 weeks. When a subject deviates from inclusion/exclusion criteria after being enrolled in the study, the PIs will evaluate the situation on a case by case basis and determine if the subject can remain on study or if she should be terminated. Reasons to terminate study participation will involve concern for subject safety or impact on study outcomes. All study data collected from subjects prior to being withdrawn or terminated will be retained and included in the data analysis as applicable. If a subject is withdrawn or terminated from the study after initiating the study supplement, the subject will be asked to return to the study site in a timely manner for an early withdrawal visit. During this visit, the study team will make every effort to identify and document potential reasons for withdrawal or termination, all remaining supplement will be collected, and all procedures conducted at the 6-month visit will be performed.

Subjects withdrawn from the study after initiating the supplement will not be replaced.

DATA MANAGEMENT, STATISTICAL METHODS AND SAMPLE SIZE JUSTIFICATION

Data Management.

This study will utilize the clinical research management software, Online Collaborative Research Environment (OnCore), adopted by the School of Medicine and Public Health (SMPH) and available to all researchers conducting clinical research studies. The instance of OnCore that will be utilized for this study is supported and managed by the UW Institute for Clinical and Translational Research (ICTR). OnCore has been, and continues to be deployed nationally at multiple academic medical centers, some of which are NIH Clinical and Translational Science Award (CTSA) sites including UW ICTR, and is an extension of technology currently used in Comprehensive Cancer Centers around the country.

ICTR OnCore is a sophisticated, web-based data management system that: a) ensures secure, easy data entry at multiple sites; b) integrates multiple data sources such as individual studies and patient registries; c) provides controlled, secure access to sensitive data using role-based access control; d) provides workflow automation; and e) allows export and reporting of data for Data and Safety Monitoring Boards and biostatisticians.

This software provides protocol management functions (e.g. subject scheduling; screening, data organization), maintains updated forms, addresses budget development, billing, and fiscal management, generates summary reports, and provides essential links with other research administration and electronic medical records systems. ICTR OnCore eases the burden of the individual researcher and unifies protocol management within research programs and including researchers at multiple sites, enhancing protocol integrity and regulatory compliance efforts.

The members of the study team interacting with subjects, collecting and entering study data will have access only to the subjects enrolled at their study site. The UW-Madison study site will be serving the data coordinating center and central randomization role, allowing only those study team member(s) access to subject identifiable information from all study sites.

Source Documents.

The investigators must maintain primary, original source of data and corresponding documents supporting each data element collected and retained. These documents, which are considered 'source data", will include:

- Demographic information
- General information supporting the subject's consent to participate in the study (i.e. the Informed Consent Process)
- General medical history and physical findings
- Hospitalization or Emergency Room records (if applicable)
- Each study visit by date, including any relevant findings/notes by the investigator(s), occurrence (or lack) of adverse events, and changes in treatment including the date the study treatment commenced and completed.
- Any additional visits during the study
- Any relevant telephone conversations with the subject regarding study procedures or possible adverse experiences
- Original, signed informed consent forms for study participation.
- The investigator must also retain all subject specific printouts/reports of tests/procedures performed as a requirement of the study (e.g., laboratory reports).

This documentation, together with the subject's hospital/site medical records, is the subject's 'source data' for the study. During monitoring visits the Study Monitor will need to validate data captured electronically against these source data.

The source documents and Case Report Forms (CRFs) will be developed with the assistance of the ICTR OnCore support team, the Data and Safety Monitoring Board support staff and the ICTR Study Monitoring Service. For this study, all data will be originally recorded on the paper CRFs, thus also serving the role of source documents. All data will be entered into the OnCore electronic Case Report Forms (eCRFs).

It is the Investigator's responsibility to ensure that all CRFs/source documents are properly, legibly and fully completed and signed when appropriate.

Electronic Case Report Forms (eCRFs)

Data will be entered into electronic Case Report Forms (eCRFs) and monitored through OnCore secure web application. All data management will follow standard procedures in place at the UW Osteoporosis Clinical Research Program. Data will be entered into the eCRF within __ business days of a subject's visit. Additional data quality measures will be taken, including the use of minimum and maximum values to be applied to a number data field and the use of constraints to require or omit fields based on data entered in preceding fields.

Additional data monitoring will be conducted by the Institute for Clinical and Translational Research (ICTR) Study Monitoring Service (SMS) program described below.

Statistical Methods.

An analysis of variance test for continuous variables and a χ^2 test for categorical variables, will compare baseline characteristics of the study population by randomized treatment assignment, stratified by site (ancestry), to ensure that balance was achieved by the randomization. All analyses will be conducted on all randomized subjects on an intent-to-treat basis.

In the primary analyses for Aim 1 (H1a), treatment group (control versus dosing algorithm) will be the independent variable; for H1b, ancestral group (European versus African ancestry) will be the independent variable and only individuals in the dosing algorithm group will be included. For both H1a and H1b, the dependent variable will be the percent of individuals with a 25(OH)D concentration of 35-50 ng/mL at the three- and six-month follow-up visits. A two-sample test for binomial proportions will be used. We will also determine the effect of treatment group and ancestry on free (bioavailable) 25(OH)D concentration using an analysis of variance.

The control group will be used as an unbiased external validation of our dosing algorithm, in which we will repeat the multiple regression analysis in the control sample and compare the coefficients to those in our original equation. In Aim 2 (H2a-c), we will further develop our dosing algorithm by determining if baseline serum cholecalciferol, baseline body composition, and genotypes in the *GC* and *CYP2R1* genes are associated with response to vitamin D_3 supplementation. In exploratory analyses we will include two-way interaction terms between ancestry and each of our primary predictors to determine if the effect of any of these biologic predictors varies by ancestral group.

Sample Size Justification.

For H1a, the outcome variable will be the percent of individuals with a 25(OH)D concentration of 35-50 ng/mL at the three- and six-month visits. Based on our preliminary data, we expect 40% of the individuals receiving 2500 IU/day to achieve 25(OH)D of 35-50 ng/mL. We expect our dosing algorithm to increase this to at least 55%, a conservative estimate. We calculated the sample size required in each arm to detect this difference with power of 80% and a one-sided significance level (α) of 0.05. A sample of 167 participants in each arm, decremented to 150 by a 10% loss to follow-up will be required.

In the multiple regression models for H2a-c, with a total sample size of 150 from the control arm, an α of 0.05, and power of 80% we would be able to detect a factor that accounts for as little as 8% of the variation in 25(OH)D concentration after vitamin D supplementation.

Multisite Conduct Plan

The UW-Madison will be serving as the data coordinating center. The UW Osteoporosis Clinical Research Program (OCRP) study staff will be in regular communication with study staff at the Medical College of Wisconsin. This will be either through conference calls or site visits, and will include information regarding changes in protocol and general study conduct.

The OCRP staff will manage all regulatory activities and data cleaning/ housing. The primary MCW coordinators will be responsible for all aspects of study conduct to include recruitment, consent, scheduling and coordinating visits. Informed consent will be obtained per OCRP SOP and determination of ability to consent will be assessed by the person obtaining consent based on their interaction with a subject. These people will also be in close contact with the staff at the OCRP to ensure appropriate conduct of this trial. All subjects will be recruited at MCW through flyers, referrals from another MCW doctor and community talks.

Staff at MCW will register adverse events and report directly to the local PI for causality assessment. All serious adverse events will be reported to OCRP staff through OnCore within 24 hours of MCW staff learning of the event.

Data and source documents will be stored at the UW in locked cabinets and on password protected computers only accessible by OCRP staff. Similarly, MCW staff will securely store source documents in an area limited to research staff. Consequently, UW and MCW staff will have access to non-anonymized data, however, stored master data files will be coded with ID numbers, initials and birthdates.

The protocol will only be amended by the OCRP. When approval is obtained by UW HS-IRB, updated protocols and consent forms will be provided to study staff at MCW and changes will be discussed via conference call or live meeting prior to initiation.

QUALITY ASSURANCE PROCEDURES

The UW Institute for Clinical and Translational Research (ICTR) Study Monitoring Service (SMS) program, a robust academic equivalent to the industry Contract Research Organization (CRO) standards for ongoing study monitoring, will be contracted for the proposed study. The ICTR Study Monitoring Service staff will work very closely with the Medical College of Wisconsin (MCW) Study Monitoring Service staff to conduct monitoring visits throughout the life cycle of the study (e.g., Site Initiation Visit, Interim Monitoring Visits, and Close-Out Visit). Study monitoring visits will occur off-site (remotely) and/or on-site at a frequency necessitated by the protocol risk and complexity.

For this study, the UW ICTR SMS will conduct a Site Initiation Visit (SIV) at each study site, coordinating efforts with MCW SMS staff at their study site. The SIV will be conducted with the site research personnel in person after IRB approval is obtained and prior to subject enrollment. The SIV will include a detailed review of the protocol, Good Clinical Practice guidelines, and data management expectations of the research team at the study site and the SMS personnel.

Following the Site Initiation Visit (SIV), SMS personnel will conduct ongoing Interim Monitoring Visits (IMVs) for all data collection sites, either on-site, remotely or a combination of both, following enrollment of the first subject(s) and throughout the duration of the study. During IMVs, the monitors will review study materials, including but not limited to: regulatory files, consent forms, case report forms, and device accountability logs.

For this study, UW ICTR and MCW SMS personnel plan to review study-related subject records for 10-20% of the enrolled subjects. Monitoring could consist of full or partial review of study records, depending on risk level and observed compliance. The first subjects enrolled will be monitored in their entirety, with the additional number to be randomly selected from each of the study sites. SMS personnel could increase the percentage of study or subject records to be reviewed if warranted.

Within ICTR OnCore, ICTR SMS personnel will verify the accuracy of and validate entered data by comparing the data entered with the information provided on the source document. All modifications made to the data are tracked and logged from the point of initial entry until the data is locked. The study monitor(s) will work closely with the MARCH DSMB statistician and the study statistician to conduct

periodic central data reviews, with follow-up conducted by the study monitors for any data discrepancies identified.

UW ICTR SMS personnel will conduct a Close-Out Visit (COV) upon completion of the study at the study site, working closely with MCW SMS personnel at the MCW study site.

STUDY TIMELINE

This project will occur over three years, with the clinical trial occurring in the first two years and statistical analysis and manuscript preparation in year three. Each site will recruit ~167 participants. Prior to initiation of the study, we will finalize the protocol, obtain IRB approval, and hold a start-up meeting to train the study staff.

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