Study Protocol and Analytic Plan for the Individualized Response to Vitamin D Treatment Study (INVITe)

NCT02925195

Multi-Ethnic Study of Atherosclerosis
Individual Response to Vitamin D Treatment
(MESA-INVITe)

Study Protocol

Version date: February 2, 2018

GENERAL INFORMATION

Funding Agencies

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PROTOCOL SUMMARY

Study Title	Individual response to vitamin D treatment (INVITe)
Objectives	To determine individual genetic and metabolic characteristics that modify the response to cholecalciferol treatment
Study Design	Double blind randomized clinical trial of 16 weeks duration
Participating Centers	University of Washington, Seattle, WA (Coordinating center) Wake Forest University, Winston-Salem, NC (Field center) Columbia University, New York, NY (Field center) Northwestern University, Evanston, IL (Field center) Johns Hopkins University, Baltimore, MD (Field center)
Study Population	1,600 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) study will be recruited from up to 3 different MESA cohorts: 1. MESA Classic who are returning for their scheduled 6th MESA study visit. 2. MESA Air Family and New Recruit participants 3. Non-MESA Air Family participants Participants will be recruited from five field centers: Wake Forest University, Winston-Salem, NC; Columbia University, New York, NY; Northwestern University, Evanston, IL; University of California Los Angeles, Los Angeles, CA; and Johns Hopkins University, Baltimore, MD. <u>Exclusion criteria</u> 1. Current use of >1,000 international units (IU) of cholecalciferol daily 2. Current use of any activated vitamin D product (calcitriol, paricalcitol, hectorol) 3. Known history of allergy or adverse reaction to vitamin D treatment 4. Known clinical history of primary hyperparathyroidism 5. Known clinical history of kidney stones within the previous 5 years 6. Known clinical history of kidney dialysis or transplant. 7. Known clinical history of sarcoidosis 8. Self-reported history of having elevated serum calcium levels 9. Serum calcium level >11 mg/dl at MESA Exam 1 (2000-2002) 10. Current participation in another interventional study 11. Inability to provide written informed consent
Study Duration	16 weeks
Treatment, Dosage, and Route of Administration	2,000 IU cholecalciferol or inactive placebo taken by mouth once daily
Efficacy Assessments	Change in serum 1,25-dihydroxyvitamin D concentration, serum parathyroid hormone concentration, and blood pressure from baseline to 16-week follow-up visit.

Safety Assessment	Change in urine and serum concentrations of calcium and phosphate from baseline to 16-week follow-up visit. Research staff will assess adverse effects during the study duration by participant self-report.
Modifying Characteristics	The following characteristics will be evaluated for their influence on the biological response to cholecalciferol treatment: genetic polymorphisms, serum concentrations of vitamin D metabolic markers, race, gender, and body mass index.
Statistical Methods	Linear models will be constructed for the follow-up value of each outcome variable. Models will include the initial value of the outcome variable, treatment assignment, and the interaction of treatment with the potential modifying variables.
Date of protocol	February 5, 2018

ABBREVIATIONS

MESA = Multi Ethnic Study of Atherosclerosis

$1,25(OH)_2D$ = 1,25-dihydroxyvitamin D, the activated form of the hormone

25(OH)D = 25-hydroxyvitamin D, the accepted marker of vitamin D storage

Vitamin D_3 = cholecalciferol, the active treatment in the study

PTH = parathyroid hormone

IU = international units

UV = ultraviolet

AE = Adverse event

SAE = Serious adverse event

GFR = glomerular filtration rate

CHSCC = Collaborative Health Studies Coordinating Center

NORC = Nutrition and Obesity Research Center

1. INTRODUCTION

Vitamin D is a vital metabolic hormone that may reduce the risks of cardiovascular diseases, cancer, fractures and other chronic health conditions. Traditionally understood as a calcium regulatory hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$, the potent hormonal form of vitamin D, binds to its target receptor to regulate the transcription of hundreds of genes relevant to health and disease. Vitamin D receptors are present in nearly all nucleated cells in the body, including those in cardiac muscle and the blood vessel wall. In experimental models, $1,25(OH)_2D$ promotes endothelial vasodilation, inhibits osteogenic differentiation of vascular smooth muscle cells, prevents cholesterol uptake in macrophages, reduces the progression of ventricular hypertrophy, and suppress the renin-angiotensin system. Cardioprotective actions of vitamin D are further supported by associations of low serum vitamin D concentrations with hypertension, myocardial infarction, stroke, left ventricular hypertrophy, cancer, and mortality. Large clinical trials are currently testing whether vitamin D treatment will reduce the risks of cardiovascular disease, cancer, and other major health outcomes in the general population.

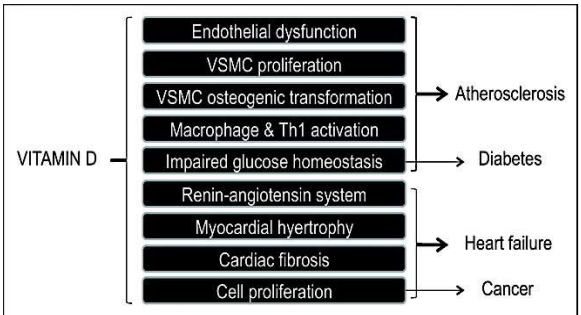


Figure 1. Mechanisms through which vitamin D treatment may prevent selected chronic health conditions. VSMC = vascular smooth muscle cells.

Published studies demonstrate substantial differences in the individual response to vitamin D treatment. Person-level differences in vitamin D treatment response may be caused by polymorphisms in vitamin D metabolism genes, variation in race/ethnicity, and/or heterogeneity in functional vitamin D status. Vitamin D_3 (cholecalciferol) is generated in the skin upon exposure to UV light, consumed in the diet, or taken as supplements. Vitamin D_3 is then converted to $1,25(OH)_2D$ through a series of regulated steps that include transportation, metabolism, receptor binding, and catabolism. These processes are carried out by polymorphic proteins and enzymes that are subject to genetic variation and are closely regulated by internal vitamin D status. Differences in vitamin D metabolism by race and ethnicity have long been suspected due to differences in skin pigmentation. Cutaneous vitamin D_3 synthesis is lowest in African Americans compared to Caucasians; however, $1,25(OH)_2D$ concentrations are actually higher in African Americans, suggesting more efficient vitamin D utilization or slower vitamin D catabolism.

The goal of this clinical trial is to determine individual-level genetic and metabolic characteristics that modify the response to cholecalciferol treatment. The trial will identify and recruit eligible participants from within the Multi-Ethnic Study of Atherosclerosis (MESA), an ongoing observational cohort study of cardiovascular disease. Results of

this trial will identify novel genetic and biomarker characteristics that influence the vitamin D treatment response in humans and inform relevant subgroup analyses for ongoing vitamin D trials. The use of quantitative vitamin D response variables, comprehensive genotyping of common and rare genetic variants, and novel biomarkers of functional vitamin D status fill important gaps of ongoing trials and will enhance the knowledge to be gained.

2. STUDY OBJECTIVES

This primary aim of this study is to identify genetic polymorphisms, clinical characteristics, and biomarkers that modify the biologic response to vitamin D_3 treatment, assessed by changes in

serum concentrations of parathyroid hormone (PTH) and $1,25(OH)_2D$ and urine calcium excretion.

This secondary aims of this study are to develop improved biomarkers of vitamin D sufficiency based on the individualized response to vitamin D_3 treatment and to create a comprehensive model of vitamin D treatment response based on genetic, clinical, and biomarker characteristics.

3. STUDY DESIGN

This study is double blind, parallel design, randomized clinical trial that will compare treatment with 2,000 international units of cholecalciferol daily by mouth *versus* inactive placebo. Eligible participants will be randomly assigned to receive cholecalciferol treatment or placebo in a 3:1 ratio for a total duration of 16-weeks. The planned sample size is 1,600. Study outcomes are changes in serum concentrations 1,25-dihydroxyvitamin D and parathyroid hormone after 16-weeks. Study procedures will take place at five existing MESA field centers.

4. PARTICIPANTS

We will conduct this vitamin D intervention study within MESA, an ethnically diverse, community-based cohort study. Up to three MESA cohorts will be approached for study recruitment. MESA originally recruited 6,814 participants in 2000-2002 from six U.S. communities: Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; St. Paul, MN; Chicago, IL; and Los Angeles County, CA. MESA Family participants are mainly siblings and parents of MESA Classic participants who were recruited in 2004-2006 for genetics-based studies. MESA Air New Recruit participants were recruited in 2006-2007 from three areas in Rockland County, NY; Riverside County, CA; and Coastal Los Angeles, CA. MESA oversampled by race/ethnicity to create a cohort that was approximately 40% White/Caucasian, 30% Black/African-American, 20% Spanish/Hispanic/Latino, and 10% Asian, primarily Chinese. All MESA Classic and Air New Recruit participants were free of clinical cardiovascular disease at the start of the study. Follow-up MESA examinations occurred 1.5, 3, 5, and 10 years after the baseline exam. A 15-year follow-up study visit ("MESA exam 6") is ongoing and serves as the source population for this vitamin D trial.

We will recruit a total of 1,600 MESA study participants from five MESA field centers: Wake Forest University, Winston-Salem, NC; Columbia University, New York, NY; Northwestern University, Evanston, IL; University of California Los Angeles, Los Angeles, CA; and Johns Hopkins University, Baltimore, MD. MESA Classic Participants will be recruited at the time of their 6^{th} MESA study examination, scheduled from September 2016 - March 2018. MESA Air and MESA Family participants will be recruited either during follow-up phone calls, or during a MESA INVITe-specific recruitment phone call. Inclusion criteria are active participation in MESA at one of the five field centers and willingness to participate in a clinical trial.

Exclusion criteria

1. Current use of >1,000 international units (IU) of cholecalciferol daily
2. Current use of any activated vitamin D product (calcitriol, paricalcitol, hectorol)
3. Known history of allergy or adverse reaction to vitamin D treatment
4. Known clinical history of primary hyperparathyroidism
5. Known clinical history of kidney stones within the previous 5 years
6. Current or previous history of maintenance kidney dialysis or kidney transplantation

7. Known clinical history of sarcoidosis
8. Self-reported history of having elevated serum calcium levels
9. Serum calcium level >11 mg/dl at MESA Exam 1 (2000-2002)
10. Current participation in another interventional study
11. Inability to provide written informed consent

Standard over the counter vitamin D supplements and multivitamins typically contain between 400-800 IU of vitamin D_3 and *will be allowed* during this trial. If participants are taking >1000 IU of vitamin D daily but would like to participate in INVITe, they may participate if they reduce their total vitamin D supplement dose (from all vitamin D-containing supplements combined) to 1000 IU or less for at least 12 weeks prior to enrollment. Study staff will request that interested participants ask their health care provider whether it is safe for them to temporarily reduce their vitamin D supplement(s). Participants who temporarily reduce their vitamin D supplement(s) will wait at least 12 weeks from their last higher than 1000 IU vitamin D supplement dose prior to completing their baseline INVITe study visit. They can continue to take a total vitamin D supplement dose of up to 1000 IU daily (including 1000 IU daily) during both the 12-week temporary reduction and the 16-week study drug periods.

5. RANDOMIZATION

Randomization will be performed centrally by the MESA data coordinating center (University of Washington, Seattle) in blocks of variable size (4-12), stratified by MESA study site. Participants will be assigned to active vitamin D_3 or placebo in a 3:1 ratio, because short-term variability in response is expected to be substantially larger among participants who are assigned to active treatment.

6. TREATMENTS

6.1. Study drug and dosage. Participants randomized to active treatment will receive cholecalciferol (vitamin D_3) 2000 IU capsules daily (Carlson Labs, Arlington Heights, Illinois). Cholecalciferol is the most commonly used vitamin D supplement and can be purchased over the counter. The 2000 IU daily dosage meaningfully increases vitamin D stores, lowers circulating PTH concentrations, and can be safely added to pre-existing vitamin D supplements and usual dietary vitamin D intake while remaining safely below the tolerable upper intake (4000 IU daily) defined by the Institute of Medicine. Moreover, the 2000 IU dosage of cholecalciferol is currently used in ongoing vitamin D treatment trials, enhancing the applicability of this study.

6.2. Placebo. Participants randomized to placebo will receive identically appearing softgel capsules, which contain sunflower oil in gelatin/glycerin/water (Carlson Labs).

6.3. Duration. The 16-week treatment duration will reliably raise serum 25(OH)D concentrations, the accepted measure of vitamin D stores, to their steady state concentrations.

6.4. Adherence. Adherence will be assessed by participant return of unused treatment at the end of the 16-week study period.

6.5. Discontinuation. Participants will be with withdrawn from the study drug if they experience a significant adverse reaction related to the study drug or an intolerable adverse reaction, such as a persistent allergy or rash, or if the patient withdraws consent. Premature termination of this clinical trial may also occur due to a regulatory authority decision, drug safety problems as determined by the Data Safety Monitoring Board (DSMB), or at the discretion of the funding agency (NHLBI).

7. OUTCOMES

7.1. Change in serum 1,25(OH)$_2$D concentration. 1,25(OH)$_2$D is the active vitamin D hormone that mediates biological effects through binding to the intracellular vitamin D receptor. The conversion of cholecalciferol to 1,25(OH)$_2$D and catabolism of 1,25(OH)$_2$D proceed through a series of metabolic steps that plausibly vary across individuals. Serum 1,25(OH)$_2$D will be measured at baseline and 16-weeks using our established high performance liquid chromatography-tandem mass spectrometry assay.

7.2. Change in serum PTH concentration. PTH is a classic response marker for vitamin D. PTH gene expression is directly suppressed by 1,25(OH)$_2$D and is variably suppressed by cholecalciferol, providing an ideal opportunity to assess potential genetic, biomarker, and clinical characteristics that explain differences in the response to cholecalciferol treatment. Serum PTH will be measured at baseline and 16-weeks using an automated two-site sandwich immunoassay.

7.3. Change in blood pressure. 1,25(OH)$_2$D directly suppresses the expression of renin and lowers blood pressure in experimental models. However, in humans, cholecalciferol treatment has variable effects on blood pressure, with no net difference observed in a recent clinical trial. We will explore inter-individual differences in blood pressure response to cholecalciferol treatment as a clinically relevant study outcome among a subset of study participants who are either (1) not taking anti-hypertensive medications, or (2) report no changes in anti-hypertensive medications over the 16-week study period. Three systolic and diastolic blood pressures will be obtained five minutes apart using an automated cuff with the participant seated. The average of the last two measurements will be used for analysis.

7.4. Change in urine and serum calcium concentrations. Vitamin D increases gastrointestinal calcium absorption. Some of the absorbed calcium deposits within bones and extraosseous tissues, but the majority is excreted in the urine. We will evaluate changes in urinary calcium excretion and serum calcium concentrations as safety outcomes and we will investigate genetic, biomarker, and clinical characteristics that may modify the effect of cholecalciferol treatment on these outcomes. Spot urine calcium to creatinine ratios, a marker of urinary calcium excretion, and serum calcium concentrations will be measured at baseline and 16-weeks using a Beckman-Coulter clinical chemistry analyzer.

8. STUDY PROCEDURES

8.1. MESA Exam 6. For MESA Classic participants, baseline data and biosamples for this clinical trial will be obtained from existing procedures that are performed at MESA Exam 6. MESA-INVITe will use some of the MESA Exam 6 baseline data and biosamples, in accordance with the scientific aims and informed consent of the overall MESA study. Types of data that are collected as part of MESA exam 6 that may be used for MESA-INVITe include:

- Demographic information
- Medical and surgery histories
- Social habits (smoking and alcohol use)
- Prescription and non-prescription medication use
- Anthropomorphic measurements (height and weight)
- Blood pressure measurements
- Blood and urine collection

- DNA and RNA collection for epigenomic and transcriptomic analysis

Site investigators at each included MESA field center are co-investigators on this trial. The MESA exam 6 study visits are scheduled from September 2016 - March 2018. Study personnel will mail materials explaining the goals and procedures of this trial to MESA participants and will call participants prior to their Exam 6 visit to review eligibility. At the time of Exam 6, or soon after Exam 6, study personnel will approach potentially eligible subjects, confirm eligibility criteria, explain the details of the trial, and answer relevant questions. The MESA coordinating center will match all medications and supplements reported in the inventory to a known list of vitamin D containing medications in real time to determine the amount of current vitamin D use. For participants who are found to be suitable and interested in this study, MESA personnel will obtain informed consent, consult the MESA Coordinating Center for randomization, and distribute the study medication.

Participants who are unable to use their regularly scheduled Exam 6 as the baseline visit for MESA-INVITe (for example because study staff were unable to start MESA-INVITe at the scheduled Exam 6, or the participant elected to reduce their current vitamin D supplement use [Section 4]) may complete a separate "Exam 6-plus" to collect baseline MESA-INVITe measurements and dispense study medication. All MESA Family and Air participants will use Exam 6-plus visits for basement measurements. In these cases, "Exam 6-plus" will include informed consent, a medical history focused on MESA-INVITe eligibility criteria, vital signs measurement, phlebotomy, urine collection, and study drug dispensing.

Participants in MESA INVITe will be contacted by telephone approximately two weeks after Exam 6 (or 6-plus) to address any participant questions regarding the study, encourage adherence to the study drug, and confirm scheduling for MESA Exam 6a.

8.2. MESA Exam 6A. We will ask enrolled trial participants to return to their respective MESA field centers for a specialized examination specific to this trial approximately 16-weeks after their Exam 6 (or 6-plus) visit. This follow-up visit is denoted as "MESA exam 6A." Study personnel will encourage participants to return as close to their 16-week follow-up time as possible, within +/- 4 weeks. Study medication bottles include 20 weeks of medication and participants will be asked to continue to take their medication up until the time of their exam 6A visit.

The trial will make every effort to follow the intention-to-treat principle of clinical trials, in which all participants who enter a trial are included in the analysis. Therefore, there are no time limitations for the exam 6A visit- it is better to bring a participant back after 30 weeks than not at all, even if the participant has stopped taking the study medication.

The Exam 6A study visit is expected to last approximately 60 minutes and will include the following components:

- Return of unused study medication to assess compliance during the trial
- Measurement of end-of-study systolic and diastolic blood pressures
- Completion of an end-of-study medication inventory, including the use of vitamin D-containing medications (both prescribed and over the counter)
- Completion of brief questionnaire to assess sunlight exposure during the trial
- Collection of end-of-study blood and spot urine specimens

- Collection of end-of-study DNA and RNA for future assessment of changes in gene expression with vitamin D treatment.

MESA study coordinators will count and record all unused study medication. Measurements of systolic and diastolic blood pressures, completion of the medication inventory, and collection of blood and spot urine specimens will be performed using identical procedures to those used for MESA Exam 6.

The primary outcomes of this trial are changes in serum concentrations of parathyroid hormone (PTH), calcitriol, and urinary calcium.

9. ADVERSE EVENTS

Cholecalciferol is a commonly used over-the-counter supplement that has been evaluated in many published clinical trials using identical or similar dosing strategies to that proposed in this trial (eg, 50,000 IU monthly and 2400 IU daily). Previous trials have found no differences in the frequency of adverse events comparing cholecalciferol to placebo. Some studies have noted a small increase in serum and urine calcium concentrations, prompting the measurement of these characteristics in this study. One study found a small increase in the frequency of nephrolithiasis, presumably due to urinary calcium excretion, prompting our decision to exclude MESA participants who have a history of kidney stones.

9.1. Definitions. An adverse event (AE) is any untoward medical occurrence in a study participant regardless of its relationship to study treatment. A serious adverse Event (SAE) is any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of an existing hospitalization, results in persistent or significant disability, or is a congenital anomaly/birth defect. Important medical events that do not fall into the above categories may also be considered an SAE when, based on medical judgment, such events may jeopardize the patient's safety and require medical/surgical intervention to prevent one of the outcomes listed in the SAE definition. The term SAE is not intended as a measure of severity or intensity. All AE's/SAE's that occur after the time of informed consent will be reported.

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the event. "Reasonable possibility" is typically defined as evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. An unexpected adverse event or unexpected suspected adverse reaction is an adverse event or suspected adverse reaction that is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

9.2. Adverse Events Reporting. All AEs will be reported on the Adverse Events Form that will be completed by MESA field center staff during the study. Pre-existing conditions (any condition that was known to be present prior to signing of informed consent or identified during the screening procedures) will not be considered or recorded as AEs unless the condition worsens in intensity or frequency. The investigators will be responsible for reporting all AE's to the IRB and DSMB in a timely fashion.

9.3. Assessment of Causality and Severity. The seriousness of adverse events will be ascertained by the study staff and the need for further evaluation, follow-up, or referral. The relationship between study participation and AEs will be determined according to standard criteria:

A. Not related – temporal relationship of the onset of the event, relative to study participation, is not reasonable or another cause can by itself explain the occurrence of the event.

B. Possibly related – temporal relationship of the onset of the event, relative to study participation, is reasonable but the event could have been due to another, equally likely cause.

C. Probably related – temporal relationship of the onset of the event, relative to study participation, is reasonable and the event is more likely explained by the study treatment than by another cause.

D. Definitely related – temporal relationship of the onset of the event, relative to study participation, is reasonable and there is no other cause to explain the event.

10. ANALYTIC PLAN

We will describe MESA participants screened, enrolled, and completing this ancillary study according to CONSORT guidelines and compare characteristics of participants who do and do not pass through each of these stages. Primary analyses will include participants who complete baseline and 16-week follow-up regardless of adherence. Secondary analyses will include all consented participants using multiple imputation to address data missing from the 16-week follow-up visit.

We will construct linear models for the follow-up value of each outcome variable to determine whether common and rare polymorphisms in six candidate genes modify vitamin D_3 treatment effects: *CYP2R1, CYP24A1, CYP27B1, CYP3A4, SULT2A1, and VDR*. Independent variables in the model will include treatment group, number of minor alleles at a given locus, the interaction between treatment group and number of minor alleles, the baseline value of the outcome variable, and the following preselected precision variables: age, gender, BMI, season, and the estimated glomerular filtration rate (GFR). Given sample size limitations we will conduct primary genetic analyses among only the white and Black subgroups with potential associations carried to the Chinese and Hispanic groups. Power to detect genetic associations of common variants are shown in Table 1. Secondary genetic analyses are planned to explore all measured and imputed genetic variants with >5% minor allele frequency derived from the Affymetrix 6.0 chip and 1000 Genomes imputation using the regression framework described above.

Table 1. Power for analyses of common variants.

Minor allele frequency	All	White	Black
0.1	0.013	0.001	0.001
0.2	0.141	0.013	0.006
0.3	0.357	0.045	0.021
0.4	0.521	0.083	0.041

We will test whether race/ethnicity, gender, body mass, index, estimated GFR, and serum concentrations of 25(OH)D, bioavailable 25(OH)D, and $24,25(OH)_2D_3$ modify the response to vitamin D_3 treatment using analogous regression models to those described above.

11. DATA COLLECTION AND QUALITY ASSURANCE

MESA Exam 6 and Exam 6A study data will be collected at the individual MESA field centers and transmitted electronically to the Collaborative Health Studies Coordinating Center (CHSCC). The CHSCC is an established coordinating center for many multi-center NIH studies. Methods are in place to transmit encrypted data electronically from the MESA field centers to the CHSCC. The CHSCC database server allows for the maintenance of a large and complex database that may be used to retrieve data and generate reports, or to extract subsets for statistical analysis. The CHSCC will format all data into accessible files that exclude any unique identifier fields for analyses

Laboratory measurements will be performed in the Nutrition and Obesity Research Center (NORC) at the University of Washington, a reference laboratory for several multi-center NIH studies. Dr. Andy Hoofnagle, who directs the NORC analytical core and developed the mass-spectroscopic vitamin D assays used in this trial will oversee all laboratory measurements. Reference materials are run with each batch of vitamin D measurements for evaluation of analytical drift over time. External proficiency testing is performed three times each year in association with the College of American Pathologists to ensure concordance with other clinical laboratories nationwide.

12. PROTECTION OF HUMAN SUBJECTS

12.1. Risks to the subjects

A. Human subjects involvement and characteristics

The purpose of this study is to determine individual level characteristics that modify the biological response to vitamin D_3 treatment. We will conduct a 16-week clinical trial of oral vitamin D_3 (cholecalciferol) therapy *versus* placebo among 1,600 participants from the MESA study, an ongoing NHLBI funded cohort study of cardiovascular disease. Participants will be recruited for this trial from five MESA field centers: Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; Los Angeles, CA; and Chicago, IL. MESA investigators from these field centers are co-investigators in this clinical trial. MESA originally recruited 6,814 adults who identified their race/ethnicity as White/Caucasian, Black/African- American, Chinese, or Spanish/Hispanic/Latino from six U.S. communities between July 2000 and August 2002. MESA excluded individuals who had any previous diagnosis of cardiovascular disease. All MESA participants gave informed consent and Institutional Review Board approval was obtained for each study site.

A total of 3,884 participants attended the 5th MESA examination in 2010 at one of the five field centers described above and are scheduled to return for the 6th MESA exam. The average age of these returning participants is 70.3 years; 44% are Caucasian, 36% are African American, and 55% are female. We are proposing to recruit our ancillary study population of 1,600 MESA participants from the 6th MESA exam.

Exclusion criteria specific to this ancillary study are the use of high dose vitamin D supplements (>1000 IU per day), use of any activated vitamin D analog (i.e. calcitriol), a clinical diagnosis of hyperparathyroidism, kidney dialysis or transplantation, a personal history of kidney stones within the previous 5 years, known clinical history of sarcoidosis, self-reported history of elevated serum calcium levels, or serum calcium greater than 11mg/dl at MESA Exam 1 (2000-2002). Each MESA field center will mail ancillary study materials to eligible participants in advance of Exam 6. These materials will explain the goals and procedures of this ancillary study.

At the time of Exam 6, MESA study personnel will approach potentially eligible subjects, confirm eligibility criteria, explain ancillary study details, and answer relevant questions. MESA personnel will then obtain informed consent for those participants who are found to be suitable and interested in this study. All hands-on recruitment procedures will be performed at the MESA field centers described above within MESA Exam 6.

B. Sources of material

As part of MESA Exam 6, participants will complete a series of interviews and questionnaires regarding their medical and surgical histories, medication use, family history, symptomotology, dietary habits, and socioeconomic status. Exam 6 participants will undergo a brief physical examination, which includes measurements of height, weight, and blood pressure, and will provide fasting blood and urine specimens. DNA was collected at the baseline MESA examination in 2000-2002 among participants who consented to genetic testing. Gentotyping was performed from participant DNA in 2009 on the Affymetrix Genome-Wide Human SNP Assay 6.0 (Affymetrix, Inc., Santa Clara, CA) and in 2012 on the HumanExome BeadChip v1.0 (Illumina, Inc., San Diego, CA).

For this trial we will ask participants to complete a brief questionnaire regarding the amount of time spent outdoors and the amount of body surface that was exposed to sunlight during the previous month. A total of 1.0 ml of collected serum and 1.0 ml of collected urine from Exam 6 will be shipped to the University of Washington Department of Laboratory Medicine for measurement of biomarkers relevant to this ancillary study: serum 25-hydoxyvitamin D, 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitmain D, vitamin D binding globulin, parathyroid hormone, albumin, and creatinine, and urine calcium and creatinine. Study medication will be dispensed to all enrolled participants at Exam 6 (section C, below).

Enrolled participants will return approximately 16 weeks after Exam 6 visit for a single follow-up examination (Exam 6A), which will include (1) return of unused study medication to assess adherence, (2) determination of end-of-study blood pressures, (3) completion of an end-of-study medication inventory, (4) completion of a questionnaire to assess sun exposure during the study, (5) collection of blood and spot urine samples. (6) DNA and RNA will be collected to complete transcriptomic analysis to explore epigenetic modifications that modify the biologic response to vitamin D_3 supplementation. Subsequently, we will mail results of serum 25-hydroxyvitamin D and calcium testing before and after vitamin D_3 treatment. Participants will then continue to be followed within the parent MESA study.

C. Study intervention

Pharmacies at each MESA field center will dispense the vitamin D_3 study medication (cholecalciferol; 2,000 IU per day) or matching placebo in a 3:1 ratio at MESA Exam 6. Cholecalciferol and matching placebo will be purchased from Carlson labs (JR Carlson Laboratories, Arlington Heights IL). All study participants will be treated for 16 weeks.

Cholecalciferol is the most widely available and commonly used vitamin D supplement and can be purchased over the counter. The dose of 2000 IU daily is consistent with that used by a number of ongoing, large-scale clinical trials of vitamin D_3. This dose effectively raises vitamin D stores and can be added to pre- existing low-dose vitamin D supplements, including those

contained in multivitamins, while maintaining total daily vitamin D dose within the Tolerable Upper Intake Level (4000 IU daily), defined by the Institute of Medicine as the highest level of nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

D. Potential risks

The short-term, oral vitamin D_3 treatment regimen proposed has been well studied and has minimal safety concerns. Vitamin D_3 can modestly raise urinary calcium levels and some (but not all) studies have observed a small increase in the long-term risk of kidney stones, prompting our exclusion of individuals who have a prior history of stones. Vitamin D_3 supplementation at the doses proposed does not generally affect serum calcium levels.

The ancillary study will utilize previously collected MESA data and stored specimens. The principal risk is the potential loss of confidentiality, which is addressed by procedures at the MESA coordinating center (see section 2, below).

12.2. Adequacy of protection against risks

The parent MESA study has Institutional Review Board (IRB) approval from each field site and from the University of Washington, which is the Data Coordinating Center. All MESA participants provided informed consent at the time of initial enrollment in 2000-2002. At Exam 6, study personnel will approach MESA participants for potential inclusion into this ancillary study. Interested and eligible participants will be provided with adequate time and privacy to undergo the informed consent process.

Data management and security will be handled by the MESA Data Coordinating Center at the Center for Collaborative Studies (CHSCC) at the University of Washington. Only CHSCC staff has access to the Coordinating Center's personal computers, simplifying security arrangements. Access is protected by individual unit keys to prevent tampering or unauthorized access to the units. Use of a Local Area Network (LAN) provides two additional levels of security in the unlikely event of an unauthorized user finding a machine unattended or unlocked. The LAN restricts access to the Database files via passwords, and the Database itself requires a password before files may be opened. All data transmissions occur without accessing unique identifiers. A master file that matches identification numbers with information that identifies an individual is maintained at the CHSCC, and is not accessible to study investigators.

Potential benefits of the proposed research to study participants and others

Vitamin D may help reduce the risks of cardiovascular disease and other chronic medical conditions. However, substantial variability in the biological response to vitamin D among individuals is suspected from previous studies and understanding of vitamin D metabolism. Defining individual-level determinants of vitamin D treatment response may facilitate a "personalized medicine" approach to vitamin D treatment, in which long-term therapy is effectively targeted to patients who are most likely to derive clinical benefit. We believe that the overall benefits of this trial outweigh the risks to participants, because the risk to participants is minimal, and because the potential benefits of lowering cardiovascular risk in the general population are significant. We believe that a placebo group is justified because the clinical benefits of vitamin D supplementation are still undergoing evaluation in clinical trials that will not report results for several years and the proposed duration of treatment is short (16 weeks).

After completing participation in MESA INVITe, each participant will receive his or her serum 25-hydroxyvitamin D concentrations, measured from serum drawn at both Exam 6 and Exam 6a. They will also receive their treatment assignment (placebo or 2000 IU vitamin D_3 daily) to help interpret their laboratory values. The 25-hydroxyvitamin D measurements will be performed in a CLIA-supervised laboratory supervised by Dr. Hoofnagle and calibrated to NIST standards. The 25-hydroxyvitamin D measurements will thus be suitable for use by participants and their healthcare providers to make future decisions regarding non-study vitamin D supplementation.

Importance of the knowledge to be gained

Cardiovascular disease is a leading cause of death in the United States, and is responsible for more than 16 million annual deaths worldwide. This trial focuses on a promising therapy that may help to reduce the risk of cardiovascular disease. We anticipate this trial will help identify characteristics that predict the biological response to vitamin D treatment, which is currently undergoing evaluation in trials and increasingly used in clinical practice. This trial is intended to generate new knowledge regarding how current and future vitamin D therapies could be used.

ANALYTIC PLAN

Changes to recruitment, analysis, and sample size in response to the low accrual of research participants for R01 HL096875-07: Individual Response to Vitamin D Treatment (INVITe)

Brief statement of the problem

The Individualized Response to Vitamin D Treatment (INVITE) trial was designed to identify clinical, genetic, and biomarker differences across individuals in response to cholecalciferol therapy. The trial was originally conceived to address two emerging research directions within the National Heart Lung and Blood Institute (NHLBI):

1. Innovative use of existing observational cohort studies to include nested clinical trials
2. A personalized medicine approach that includes testing individualized responses to treatments, including clinical and genetic characteristics.

The INVITE trial is nested within the NHLBI sponsored Multi-Ethnic Study of Atherosclerosis (MESA). Participants are recruited from four MESA clinical sites at the time of their 6th examination. Recruitment for the INVITE trial has been lower than anticipated due to:

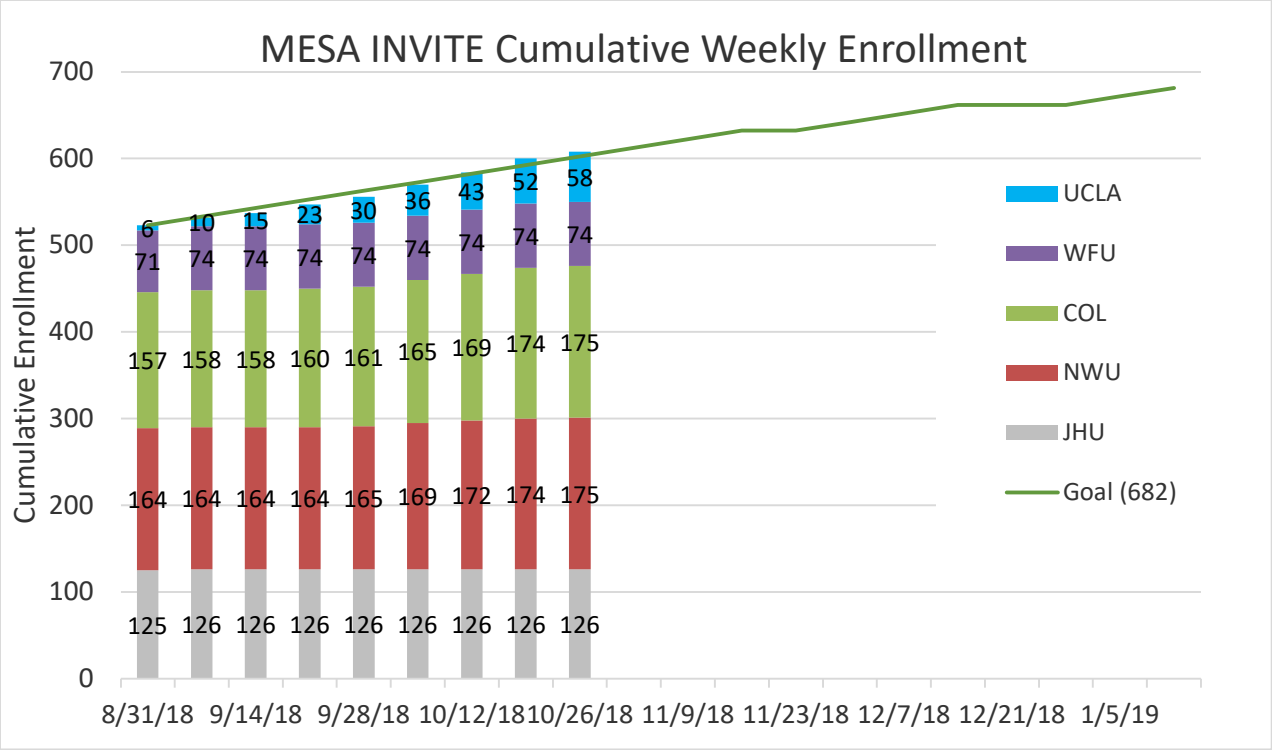
1. Overall turnout for MESA Exam 6 was considerably lower than expected, particularly at the Wake Forest site, which is a primary INVITE recruitment site.
2. The INVITE grant was funded after MESA Exam 6 was already underway.
3. The use of high-dose cholecalciferol supplementation was greater than expected.

We have implemented several procedures to increase recruitment, including the creation of specialized visits for participants who already completed MESA Exam 6, the inclusion of an optional washout period for pre-existing vitamin D users, and the addition of a 5th MESA recruitment site. Each of these procedures have increased enrollment; however, the final anticipated sample size will be 682.

Changes to enrollment

As of September 1, 2018, the MESA INVITe study had enrolled 523 participants. We now plan to enroll 682 participants by January 18, 2019, at which time we will close enrollment. We are currently on track to meet this goal based on the current rate of recruitment (below). With 16 weeks of scheduled follow-up for each participant, we plan to complete all study visits by May 30, 2019.

Figure 1. Enrollment in MESA INVITe



Changes to the analytic plan

The overall design of the study has not changed. INVITE remains a randomized two-arm, double-blind clinical trial with a 3:1 ratio of assignment to active vitamin D treatment versus placebo. We discussed the possibility of removing the placebo group to create a treatment only study; however, implementing such a change after recruitment of greater than 75% of participants would be difficult to explain to the DSMB, the MESA study sites, and the participants themselves. Moreover, we believe that such a change in design would diminish the impact of manuscripts resulting from this study.

Unlike typical event driven trials, INVITE seeks to identify characteristics that modify the response to treatment. To increase study power to detect such associations, we will start by evaluating only participants who are treated with active vitamin D to assess main effects of genetic polymorphisms and clinical characteristics on the changes in study outcomes. In parallel we will present analytic results from our original approach, in which we analyze all participants and test the interaction of relevant exposures with treatment assignment.

Changes to sample size and study power

Study power for the treatment only analytic approach is considerably greater than that of the original interaction-based analyses. Differences in the effect of vitamin D treatment on study outcomes of change in parathyroid hormone, 1,25-dihydroxyvitamin D, and urine calcium to creatinine ratios according to a modifying genetic polymorphism are presented below. Under the anticipated sample size of 682, the INVITE study will maintain power to detect clinically relevant associations. The INVITe DSMB, which includes a faculty biostatistician, met on September 13, 2018, at which time it reviewed and approved these changes to the analytic plan and power.

Figure2. Minimal detectable associations for genetic polymorphisms under a treatment-only analysis.