Protocol BBC (Blood pressure in Blacks and Calcium) and Vitamin D Study
Protocol Version 2.0

Protocol Date March, 8, 2018

Principal Investigator: Holly Kramer

Research Team: Holly Kramer MD MPH, Sue Penckofer RN PhD, Pauline Camacho MD, Richard Cooper MD and Ramon Durazo-Arvizu PhD

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Abstract:

I. Abstract

Adequate levels of vitamin D are essential for bone health at all ages but low levels of vitamin D may also negatively impact other aspects of health such as blood pressure. We have previously shown that adults with African ancestry living near the equator have much higher levels of vitamin D and higher levels of blood pressure compared to adults with African ancestry living in the Chicago area. Multiple clinical trials have examined vitamin D supplementation for reducing blood pressure levels **but very few studies have focused on adults with African ancestry and low vitamin D levels**. In addition, most previous clinical trials have not addressed calcium intake. While vitamin D may modulate blood pressure via its actions on activation of the renin angiotensin system, it is also possible that vitamin D mediates blood pressure via its effects on gastrointestinal calcium absorption. This pilot study is a one arm study which will assess the safety and feasibility of supplementing 15 young adults with African ancestry and low vitamin D levels with 5,000 IU of Cholecalciferol (vitamin D3) combined with 1000 mg of elemental calcium daily for 12 weeks. Participants will be recruited from the Maywood and surrounding areas with flyers and brochures. We will also contact previous participants of the Modeling the Epidemiologic Study/Vitamin D Ancillary Study by phone and letters. At baseline, all participants will have blood pressure measured and a fasting serum and 24-hour urine specimen will be collected for measurement of calcium, parathyroid hormone and vitamin D levels and urinary calcium excretion. Repeat visits will be completed at 6 and 12 weeks of follow-up to again measure resting blood pressure and serum calcium and vitamin D levels and urinary calcium excretion. The overall goal is to collect pilot data to help design a larger trial of vitamin D and calcium supplementation for lowering blood pressure in young adults with African ancestry.

II. Background and Significance

Adequate levels of vitamin D are essential for bone health at all ages with low levels recognized as a cause of rickets in children over a century ago. We and others have previously demonstrated significantly higher levels of 25-hydroxyvitamin D [25(OH)D] in Africans living near the equator. [1, 2] In fact, total 25(OH)D inadequacy is approximately 50-fold higher among young adults with African ancestry living near Chicago, Illinois compared to young adults living in Ghana.[2] Epidemiological studies have also consistently demonstrated that blood pressure levels are higher among adults with African ancestry living in countries close to the equator compared to African Americans.[3-5] It has previously been hypothesized that vitamin D may impact blood pressure due to its independent role as a regulator of the renin-angiotensin-aldosterone system.[6] However, associations between vitamin D and blood pressure may also be attributed to its actions on gastrointestinal absorption of calcium. If true, supplementation with vitamin D alone, without increasing calcium intake, would have minimal effect on blood pressure levels. This is especially important for U.S. blacks due to the high rate of both vitamin D deficiency and low calcium intake in this population.[7, 8] However, most clinical trials of vitamin D supplementation for cardiovascular risk factors are not focused on individuals with 25(OH)D deficiency or insufficiency and do not address calcium intake.[9] African Americans remain poorly represented in clinical trials of vitamin D supplementation for reduction of cardiovascular risk factors. In approximately 500 U.S. blacks, we have demonstrated a high prevalence of vitamin D deficiency and low calcium intake. In this population, the association of serum 25(OH)D levels with blood pressure shows a plateau effect with higher blood pressures limited to adults with 25(OH)D levels < 20 ng/ml. These findings are consistent with observational studies examining the association of 25(OH)D levels with mortality.[10-12]

As discussed by the Institute of Medicine, 25(OH)D levels > 20 ng/ml are likely sufficient for the majority of U.S. adults, especially if calcium intake is adequate because the main physiologic action of vitamin D is to increase absorption of dietary calcium.[9] While clinical trials have demonstrated that calcium supplementation modestly reduces both systolic (SBP) and diastolic blood pressure (DBP), especially for adults less than 35 years of age,[13] clinical trials of vitamin D supplementation for blood pressure lowering have shown mixed results with most trials showing no association between vitamin D supplementation and blood pressure change.[14] Forman et al completed a 4-arm double blind placebo controlled 3-month trial of 1000, 2000, or 4000 IU of vitamin D3 per day vs. placebo in African-Americans with a median age of 51 years. The difference in SBP between baseline and 12 weeks was 1.7 mmHg for the placebo group, -0.66 mmHg for the 1000 IU/d group, -3.4 mmHg for the 2000 IU/day group, and -4.0 mmHg for the 4000 IU/day group. Each 1 ng/ml increase in plasma 25(OH)D was associated with a 0.2 mmHg decline in SBP levels (P=0.02) if baseline 25(OH)D level was < 20 ng/ml. It should be noted that in this trial, all participants were given 200 mg of calcium daily and baseline calcium intake was not assessed.

The other limiting factor for previous clinical trials of vitamin D for blood pressure lowering is the use of total 25(OH)D as the only measure of vitamin D status. Clinically, 25(OH)D remains the key biomarker of vitamin D status in part because 25(OH)D has a substantially longer half-life than the active form of vitamin D called 1, 25-dihyroxyvitamin D (1,25(OH)D), and because 25(OH]D reflects total vitamin D stores obtained from cutaneous synthesis or via foods and supplements.[15] However, total 25[OH]D may not be the optimal measure of vitamin D required for good health, especially if total 25(OH)D levels are low.[9] Over 90% of total 25(OH)D is tightly bound to vitamin D binding protein (DBP) with approximately 1% circulating free. This fraction of 25(OH)D not bound to DBP shows stronger associations with bone density and serum calcium and parathyroid hormone (PTH) levels compared to total 25(OH)D.[16-18] Thus, free 25(OH)D may be a more clinically useful marker of vitamin D status. We hypothesize that a negative association exists with both 25(OH)D and free 25(OH)D and blood pressure in young U.S. blacks with 25(OH)D levels < 20 ng/ml and calcium intake < 1000 mg/day. We further hypothesize that supplementation with both vitamin D 3 and calcium reduces blood pressure levels and increases both 25(OH)D and free 25(OH)D.

III. Study Aims

a. Recruit 15 black U.S. adults ages 25-45 years of age with 25(OH)D levels < 20 ng/ml, estimated calcium intake < 1000 mg/day and no use of anti-hypertensive medications and a resting seated systolic blood pressure ≥ 120 mmHg. Measure baseline levels of total 25(OH)D free vitamin 25(OH)D, active vitamin D [1,25(OH)D], parathyroid hormone, serum calcium levels, 24-hour calcium excretion and estimate calcium intake using an interactive calcium calculator.
b. Supplement study participants with 5000 IU vitamin D3 with 1000 mg elemental calcium daily for 12 weks and measure changes in blood pressure, total 25(OH)D, $1,25(OH)_2D$ and free 25(OH)D, PTH, serum calcium and 24-hour urine calcium excretion over the three month period.

The overall goal is to collect pilot data to determine the feasibility of a larger trial to determine whether vitamin D combined with calcium lowers blood pressure in young black adults with low 25(OH)D levels

IV. Administrative Organization

Participants will be recruited by using flyers enrolled from Loyola outpatient clinics including the primary care clinics in Elmhurst and at the Loyola Outpatient Center. We will also contact persons who previously participated in the Modeling the Epidemiologic Transition Study/Vitamin D Ancillary Study. Contact will be done by phone and by mailed letters. The study will be limited to Loyola only and study recruitment will not include any other universities or medical centers. Data will be collected by the Loyola study investigators and will be kept in a secure password protected Loyola server. Data will be kept in RedCap on a secure and

protected Loyola server and will be analyzed by Loyola investigators. Only investigators for the BBC and Vitamin D Study will have access to the RedCap data.

Participants will provide written informed consent to participate in the screening visit and study. We will also ask participants to provide written informed consent to participate in Loyola's biobank repository. During the screening visit, 15 ml of blood will be obtained and participants will provide a 24-hour urine collection. Study staff will collect information on medical history and medication use to determine study eligibility. Screening laboratory assays including serum calcium, creatinine, and 25-hydroxyvitamin D. These assays will be performed by Quest Diagnostics (Quest). A blood pregnancy test will be done in all women at the screening exam and the pregnancy test will be completed by Quest. We will ask participants to provide written informed consent for their blood and urine specimens to be placed in a biorepository for future studies. To reduce participant burden and cost, we will also bank 5 ml of serum obtained at the screening visit to measure 25(OH)D and $1,25(OH)_2D$ at Quest only if the participant is determined to be eligible for the BBC and Vitamin D study and enrolls. We will discard all banked specimens if the participants are not eligible to enroll in the BBC and Vitamin D study unless the participant provided consent to participate in the biorepository Participants may opt to not enroll in the biorepository regardless of eligibility for the BBC and Vitamin D study. A separate consent form will be used to obtain written informed consent for the biorepository.

For participants who do enroll in the BBC and Vitamin D study, a serum specimen (15 ml) will be collected at the 6 –week follow-up visit to measure serum calcium and 25(OH)D. Serum calcium and 25(OH)D will be measured at the Quest lab.

At the 12 week visit, 15 ml of blood will be obtained to again measure 25(OH)D, $1,25(OH)_2D$ and PTH levels and serum calcium. Vitamin D measures, serum calcium and PTH will be measured by Quest. Participants will also be asked to collect a 24-hour urine collection to measure urinary calcium excretion. Urine calcium excretion will be measured by Quest.

Participants will receive a copy of all laboratory test results via letter. A data safety monitoring board will review all adverse events and laboratory data at 6 weeks to ensure safety of all participants. Participants will be provided a copy of all laboratory tests. Participants will be instructed to share these test results with their physician. If needed, Dr. Kramer will identify a primary care physician for the participant if the participant does not have a primary care doctor.

V. Study Design

a. This will be a single arm non-blinded study of 15 participants.

b. The study population will consist of 15 adults ages 25-45 years with self-reported African American race/ethnicity. The study will be conducted during a 4 month window to eliminate the seasonal effects on vitamin D levels. All participants will provide written informed consent after study investigators ensure that potential participants meet all eligibility criteria outlined below.

Inclusion Criteria

1. Age 25-45 years
2. Self-reported race/ethnicity African American
3. BMI ≥ 25 kg/m^2
4. Total 25(OH)D levels < 20 ng/ml (50 nmol/L)
5. No use of vitamin D supplements within past 30 days of the screening visit
6. Able to provide written informed consent
7. Willing to take a vitamin D supplement daily for 12 weeks
8. Willing to return for follow-up visits to measure blood pressure and provide a blood sample to measure vitamin D and serum calcium
9. No current use of blood pressure lowering medications
10. Systolic blood pressure ≥ 120 mmHg and diastolic blood pressure ≥ 60 mmHg
11. Estimated calcium intake < 1000 mg/day

Exclusion Criteria

1. Medical history of chronic disease that affect gastrointestinal absorption of vitamin D: Crohn's disease, cystic fibrosis, and celiac disease
2. Use of medications which may affect total 25(OH)D levels: antiepileptic drugs, steroids, bile acid sequestrants, lipase inhibitors, orlistat
3. Current use of vitamin D supplements or use in past 8 weeks and unwillingness to discontinue for at least 1 month prior to study enrollment
4. History of kidney stones or hypercalciuria (> 250 mg urinary calcium excretion)
5. Fasting serum calcium ≥ 10.2 mg/dl at the screening visit
6. Average systolic blood pressure ≥ 140 mmHg
7. Average diastolic blood pressure ≥ 90 mmHg
8. Presence of active malignancy, active thyroid disease or sarcoidosis
9. Pregnant or planning a pregnancy or breastfeeding
10. Estimated glomerular filtration rate (based on serum creatinine level) is < 60 ml/min/1.73 m^2 based on the Modification of Diet in Renal Disease formula
11. Enrolled in a clinical trial
12. Any history of a heart attack, stroke, angioplasty or coronary artery bypass grafting or physician diagnosis of heart failure

c. Sample size determination

This pilot study will collect data to design a larger randomized clinical trial. The sample size of 15 participants will allow us to detect adverse events which occur at a rate ≥ 20% with 96% probability. We will have a 79% probability of detecting complication rates ≥ 10%. We will have moderate probability to detect adverse events which occur in 5% or less of participants. As a small pilot study, we will determine the tolerability of taking 5,000 IU of Cholecalciferol with 1000 mg elemental calcium daily for 12 weeks and will examine changes in measures of vitamin D status including total 25(OH)D, free 25(OH)D levels and PTH levels.

Table 2. Probability of Observing an Adverse Event[19]

Sample Size	Complication Rate		
	20%	10%	5%
5	0.67	0.41	0.23
10	0.89	0.65	0.40
15	0.96	0.79	0.54
20	0.99	0.88	0.64
25	1.0	0.93	0.72
30	1.0	0.96	0.79
35	1.0	1.0	0.80

d. Study outcomes/endpoints:

a. Blood pressure

Seated blood pressure and pulse are measured at the screening visit, enrollment visit and at 6 and 12 week follow-up visits in the clinic using an appropriate sized cuff after a rest period using an automated device (Omron). Arm circumference will be measured using a standard flexible tape measure. The cuff size will be determined based on arm circumference. Participants will be instructed to not smoke for 30 minutes prior to the exam and to not drink any caffeinated beverages for one hour prior to the exam. All participants will be instructed to fast for 4 hours prior to the exam. Participants will be instructed to empty their bladder prior to the exam. Participants will be seated for 5 minutes prior to blood pressure measurement. Blood pressure will be measured three times in one minute intervals using an Omron

automatic device with the study staff not in the room. The average of these three recordings will be recorded as the mean blood pressure.

b. Total 25(OH)D and 1,25$(OH)_2$D levels

Total 25[OH]D and 1,25$[OH]_2$D: Total 25[OH]D and 1,25$[OH]_2$D will be measured with the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS)(inter-assay coefficient of variation for 25[OH]D3 = 4.8% and for 1,25$[OH]_2$D = 8.8%). The assay will measure total 25[OH]D, including 25[OH]D3 and 25[OH]D2, calibrated to standards provided by the National Institute of Standards and Technology.[20] We will provide all potential participants who are screened or enrolled with the results of their vitamin D results. For screening purposes, 25(OH)D will be measured in specimens collected at the screening visit and testing will be done by Quest. Then 25(OH)D will be measured at Quest in banked specimens from the screening visit and from visits 6 and 12 weeks after the enrollment visit. 1,25(OH)2D will be measured in banked specimens obtained at the screening visit and in blood specimens obtained at the visit 12 weeks after enrollment.

c. Free 25(OH)D
Free 25(OH)D levels-will be measured using kits from Quest. The free 25(OH)D ELISA is based on a two-step immunoassay procedure performed in a microtiter plate. During the first incubation step, free 25(OH)D (including both 25(OH)D2 and D3) is bound to the antivitamin D antibody coated on the wall of the microtiter plate. The in vivo equilibrium between free and bound 25(OH)D is minimally disturbed. After washing, a fixed amount of biotinylated 25(OH)D is added to each well. The nonbound biotinylated 25(OH)D is removed by washing, and a streptavidin peroxidase conjugate is added. In a next step, a 3', 5, 5'-tetramethylbenzidine chromogenic substrate is added. Finally, the reaction is stopped by the addition of a stop reagent and the absorbance (A450 nm) is measured using a plate spectrophotometer. The concentration of free vitamin D in the sample is inversely proportional to the absorbance in each sample well. Free vitamin D will be measured in banked specimens obtained at the screening visit and in blood specimens obtained at 12 weeks after enrollment.

d. Parathyroid hormone (PTH) levels
Intact PTH levels will be measured with the use of the Elecsys Parathyroid Hormone Immunoassay (Modular Analytics E170, Roche Diagnostics) (inter-assay coefficient of variation, 2.5%). PTH levels will be measured by Quest. PTH levels will be measured in banked specimens obtained at the screening visit and in blood specimens obtained at the visit 12 weeks after enrollment.

e. Calcium and creatinine
Serum and urine calcium and creatinine will be measured using colorimetric assays from Roche Diagnostics by Quest. Creatinine will be measured in serum collected at the screening visit. Serum calcium will be measured from specimens collected at the screening visit and at 6 and 12 weeks of follow-up after starting the enrollment visit.

f. Calcium intake-calcium intake will be estimated at baseline only using an online interactive calcium calculator from the International Osteoporosis Foundation http://www.iofbonehealth.org/calcium

VI. Study Procedures

a. Sampling plan
Individuals who are known to have low vitamin D levels due to their enrollment in the Modeling the Epidemiologic Study/Vitamin D Ancillary Study (METS/VIDA) will be asked to participate in a screening visit. Thus, we anticipate that at least 25% of recruited participants will meet study eligibility. We will provide a small incentive to participants cover travel and time spent at the clinic. Participants will be paid $50 for each completed visit including the screening visit so the maximum amount a participant could be paid is $200 for completing all four visits. We will also provide parking passes for each clinic.

b. Recruitment procedures
We will recruit potential participants by contacting previous METS/VIDA participants with low vitamin D levels via phone. We will also place flyers in clinics and will provide flyers to family practice physicians, internal medicine physicians and endocrinologists at Loyola outpatient clinics. We will also work with the Loyola marketing team to market the study.

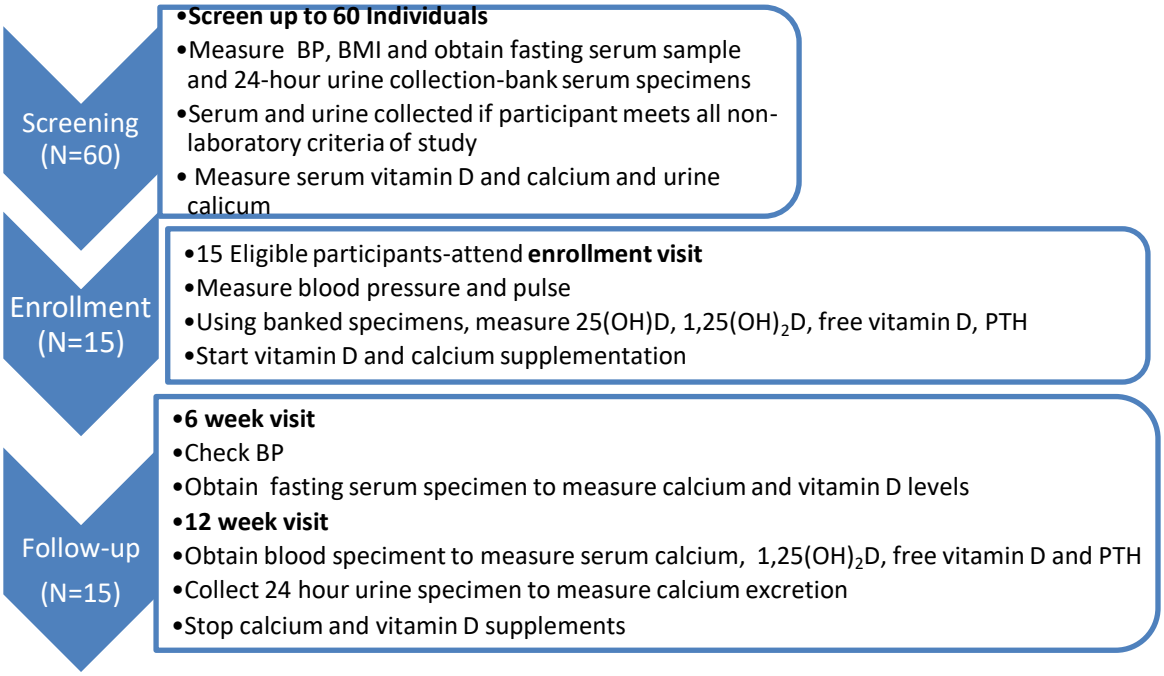
c. Screening procedures
Screening visits will occur in the CTRE clinic on the lst floor and written informed consent for the screening visit and study will be obtained by research staff or investigators. At the screening visit, participants will provide written informed consent. Participants will also be asked to provide written informed consent for their serum and urine specimens to be placed in a biorepository for future studies. Participating in the biorepository is optional and will not affect their ability to participate in the screening exam or their overall eligibility to participate in the BBC and Vitamin D study. The screening visit will take ninety minutes to complete. Potential participants will be instructed to fast for at least four hours prior to the screening exam. All laboratory tests taken during the screening visit will be for research purposes and will not be part of standard care. During a screening visit, a research investigator will review the past medical history and any medication use to ensure the participant meets all inclusion and exclusion criteria. Resting blood pressure will be taken per protocol and height and weight will be measured. A fasting blood sample (15 ml) will be collected from the potential participant. Blood will be sent to Quest for measurement of serum creatinine and calcium and vitamin D. Participants will be asked to collect a 24-hour urine collection to measure urinary calcium excretion. Urine calcium will be measured by Quest. We anticipate that we will need to screen 60 adults to identify 15 individuals who are eligible for the study. Data collected from any individual who is found not be eligible for the study will be kept in a secure database (REDCAP) on a secure Loyola drive. For participants who do not enroll in the BBC and Vitamin D study, all banked specimens will be discarded unless they provided written informed consent to participate in Loyola's biorepository.

Once the lab values for the potential participant have been reported to the investigators, then study eligibility may be determined. Individuals will be contacted by phone or via mailed letter regarding study eligibility. For those individuals who are eligible to participate in the study, an enrollment visit will be scheduled within 28 days of the screening visit. The enrollment visit and all subsequent visits will be held at Loyola University Chicago Health Sciences Campus in the CTRE clinic. In order to reduce participant burden, we will bank serum specimens from the screening visit. For those participants who provide written informed consent to enroll in the BBC and Vitamin D study, we will use this banked serum to measure 25(OH)D and $1,25(OH)_2D$ using an LC/MS method at Quest. During the enrollment visit, participants will receive education about the study and the potential risks of participating and will provide written informed consent. The Loyola pharmacy will dispense the cholecalciferol and the calcium citrate to the enrolled participants.

Participants will then return at 6 and 12 weeks after the enrollment visit and participants will be instructed to fast at least four hours before the next visits. At the 6 and 12 week visit, a fasting blood specimen (15 ml) will be collected. Study staff will query participants about adverse effects and symptoms of vitamin D intoxication. Pill counts will be done at the 6 and 12 week visits. Participants will also collect their urine for 24 hours at the 12 week visit for measurement of urinary calcium excretion.

d. Randomization procedures-not applicable
This is a single arm non-blinded study.

Figure 1. Flowchart of study procedures



e. Optional Biobanking. Participants may *optionally* agree to reposit blood and urine samples into CRO-BIOREP (LU# 204853) for future research purposes. Participants will sign a second informed consent document should they agree to reposit their samples. The research team members and Biorepository staff will each retain a copy of the signed repository informed consent document. It is important to point out that participants do not need to agree to reposit samples in order to participate in the study.

All patients who agree to reposit specimens into CRO-BIOREP (LU# 204853) will be assigned a unique code using the SMART-ID system. This number will be used to connect coded specimens and coded data to other studies conducted by the Clinical Research Office in which they may participate. To receive this number, we will ask participants for sex at birth, month of birth, day of birth, year of birth, and social security number. This information is entered into a secure website where it is hashed into a unique ID using a java script. The participant and research team will receive the unique ID number. Once the number is created, the information that was entered is immediately deleted. This means the website will not retain any of the information that is entered to create the SMART-ID. However, should anyone need to retrieve a SMART-ID number, you will be able to get it again at a later date by going to the website and entering the same information (i.e., sex, date of birth, and social security number) that was entered before.

Freezerworks, the biospecimen tracking software used in the repository, will also automatically assign specimen numbers to incoming specimens. These numbers will identify all donated specimens. The list that links these codes with patients' identifying information will be kept separate from all research specimens and clinical data. Information that may readily identify patients will never be shared with anyone.

Blood and urine samples will be stored according to the Clinical Research Office Biobank protocols at the following location:
Clinical Research Office Biobank
CTRE, Bldg. 115
2160 S 1st AVE
Maywood, IL 60153

Repository Phone: 708-216-8002
Repository Fax: 708-216-2059
Repository Director: Susan L. Uprichard, suprichard@luc.edu
Repository Coordinator: Sarah Rahman, srahman7@luc.edu

Study Intervention

1. This study has only one arm and all participants will receive Cholecalciferol (vitamin D3) 5,000 IU and calcium citrate 1000 mg daily for 12 weeks. There will be no placebo and no blinding of the agent. Participants will receive a 3 month supply of drugs administered by the Loyola pharmacy. Serum vitamin D and calcium levels will be measured at study initiation and at 6 and 12 weeks (study end). We will stop both vitamin D and calcium supplementation in participants with serum calcium levels ≥ 10.6 meq/L and/or a 25(OH)D level ≥ 80 ng/dl at the 6 week visit. These participants will continue to be monitored in the study but they will not receive any drugs. The vitamin D will be obtained from Biotech Pharmacal, Inc. Calcium citrate 1000 mg tablets will be obtained from Walgreens. The Loyola pharmacy will dispense all supplements.

Early withdrawal of subjects

A subject may withdraw from the study anytime he/she wishes. Any participant who withdraws from the study along with their reasons for withdrawing from the study will be reported to the Loyola Institutional Review Board.

G2. Schedule of visits and measurements

If the subject meets all of the eligibility criteria after screening measures described above are completed, he/she will be consented by the research investigator or designee. Screening Visits/Baseline Visit:

	Prior to enrollment	+Enrollment Visit	Six weeks	12 weeks
Verify interest in study and eligibility	x	x		
Inclusion/exclusion form	x			
Obtain informed consent	x			
Obtain consent for biorepository	x			
Obtain HIPAA authorization for study (part of consent form)	x			
Measure sitting BP	x	x	x	x
Assess dietary calcium intake	x			
Measure serum calcium	x		x	x
Measure total 25(OH)D	x		x	x
Measure parathyroid hormone levels		x		x
Measure free 25(OH)D		x		x
Measure 1,25(OH)2D		x		x
Measure 24-hour urine calcium excretion	x			X

Pill counts			X	X
Adverse event check list			X	x

+All laboratory tests for the enrollment visit will be performed in blood collected during the screening visit to reduce participant burden. A fasting blood sample will be collected during the screening visit and any unused sample will be stored in a -80° freezer. All blood samples collected from persons who do not meet study eligibility will be discarded. Participants will be asked to provide written informed consent during the screening visit to allow their urine and serum specimens to be placed into the Loyola CRO biorepository.

Adverse event check list will be completed at six and twelve week visits by study investigators.

Visit Frequency:

Potential study eligibility will be determined at a screening visit. For those persons who meet study eligibility, an enrollment visit will be scheduled within 4 weeks of the screening visit. During the enrollment visit, participants will be provided the vitamin D and calcium supplements. Participants will return at 6 and 12 weeks for blood pressure measurement and for collection of a fasting serum specimen. A 24-hour urine collection will be obtained at the enrollment visit and at the week 12 visit.

Study forms:

Consent form
Inclusion/Exclusion Summary Form -completed by study staff
Consent form for the biorepository
Blood pressure form- enrollment visit, 6 and 12 week visits -completed by study staff
Adverse event form-completed at 6 and 12 weeks
Pill count form-completed at 6 and 12 weeks

Measurements:
Seated Blood Pressure and Pulse
Body mass index
Fasting Serum calcium
Fasting Serum creatinine
24-hour urine calcium
Estimated calcium intake
Total 25(OH)D level
Free 25(OH)D level
1,25(OH)2D level
Parathyroid hormone level

All study participants including participants who completed the screening visit but did not enroll in the study will be provided a copy of all their laboratory reports. This report will be in the form of a letter from Dr. Kramer and will be sent directly to the study participants.

G6. SAFETY AND ADVERSE EVENTS

a. Safety Monitoring

We will query any hospitalizations or emergency room visits over the past month and reasons for those visits at the baseline, 6 and 12 week follow-up visits. Serum calcium and vitamin D levels will be measured at 6 and 12 weeks and anyone with serum calcium levels ≥ 10.6 meq/L and/or a 25(OH)D level ≥ 80 ng/ml or symptoms of vitamin D intoxication (e.g. bone pain, nausea, vomiting) will stop treatment. All adverse events will be reported to the Loyola University Chicago Health Sciences Institutional Review Board. The dose of vitamin D3

(5,000IU) is 1000 IU lower than the recommended dose for treating adults with 25(OH)D levels < 20 ng/ml.[21] We will also exclude individuals with calcium intake that is at or above the current recommended daily calcium intake (1000 mg).

Stopping plans

Any participant who develops a serum calcium levels ≥ 10.6 meq/L and/or a 25(OH)D level ≥ 80 ng/ml or symptoms of vitamin D intoxication (e.g. bone pain, nausea or vomiting) will stop treatment but they will continue to be monitored in the study through week 12 when serum calcium and 25(OH)D levels will be measured at the end of the study. The study will continue until participants have been followed for 12 weeks.

VIII. Analysis Plan

This feasibility study will examine multiple aspects of the pilot data including the total number of persons enrolled vs. number of total persons identified as eligible. Pill counts will be done at the 6 and 12 week visit to assess compliance with vitamin D and calcium supplementation. The percentage of participants taking 80% or more of the vitamin D3 supplement and the calcium supplement will be determined. The number of participants reporting symptoms and all adverse events will be quantified. Repeated measures analysis of variance models will be used to assess change in the outcome measures including systolic and diastolic blood pressure, vitamin D measures, parathyroid hormone levels and 24-hour urine calcium excretion values.

References

References

1. Prentice A, Schoenmakers I, Jones KS, Jarjou LMA, Goldberg GR:

Vitamin D deficiency and its health consequences in africa. *Clinic Rev Bone Miner Metab* 7: 94-106, 2009

2. Durazo-Arvizu RA, Camacho P, Bovet P, Forrester T, Lambert EV, Plange-Rhule J, Hoofnagle AN, Aloia J, Tayo B, Dugas L, Cooper RS, Luke A: 25-hydroxyvitamin D in african-origin populations at varying latitudes challenges the construct of a physiologic norm. *Am J Clin Nutr* 100: 908-914, 2014

3. Cooper RS, Forrester TE, Plange-Rhule J, Bovet P, Lambert EV, Dugas LR, Cargill KE, Durazo-Arvizu RA, Shoham DA, Tong L, Cao G, Luke A: Elevated hypertension risk for african-origin populations in biracial societies: Modeling the epidemiologic transition study. *J Hypertens* 33: 473-80; discussion 480-1, 2015

4. Cooper R, Rotimi C, Ataman S, McGee D, Osotimehin B, Kadiri S, Muna W, Kingue S, Fraser H, Forrester T, Bennett F, Wilks R: The prevalence of hypertension in seven populations of west african origin. *Am J Public Health* 87: 160-168, 1997

5. Kaufman JS, Durazo-Arvizu RA, Rotimi CN, McGee DL, Cooper RS: Obesity and hypertension prevalence in populations of african origin. the investigators of the international collaborative study on hypertension in blacks. *Epidemiology* 7: 398-405, 1996

6. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP: 1,25-dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 110: 229-238, 2002

7. Yuen SN, Kramer H, Luke A, Bovet P, Plange-Rhule J, Forrester T, Wolf M, Camacho P, Harders R, Dugas L, Cooper R, Durazo-Arvizu R: Fibroblast growth factor (FGF-23) levels differ across populations by degree of industrialization. *J Clin Endocrinology and Metabolism* In Press2016

8. Alaimo K, McDowell Briefel RR, Bischof AM, Caughman CR, Loria CM, Johnson CL: Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the united states: Third national health and nutrition examination survey, phase 1, 1988-91. *Adv Data* (258)1-28, 1994 Nov 14

9. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, & Institute of Medicine: *Dietary reference intakes for calcium and vitamin D.* National Academies Press, 2011,

10. Kramer HJ, Sempos C, Cao G, Luke A, Shoham D, Cooper R, Durazo-Arvizu R: Mortality rates across 25-Hydroxyvitamin D (25[OH]D) levels among adults with and without estimated glomerular filtration rate < 60 ml/min/1.73 m²: The third national health and nutrition examination survey. *PLoS ONE [Electronic Resource]* 2012

11. Wang L, Song Y, Manson JE, Pilz S, Marz W, Michaelsson K, Lundqvist A, Jassal SK, Barrett-Connor E, Zhang C, Eaton CB, May HT, Anderson JL, Sesso HD: Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: A meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes* 5: 819-829, 2012

12. Durazo-Arvizu RA, Dawson-Hughes B, Kramer H, Cao G, Merkel J, Coates PM, Sempos CT: The "reverse J-shaped" association between serum total 25-hydroxyvitamin D and all-cause mortality: The impact of assay standardization. *American Journal of Epidemiology* 2016 In Press

13. Cormick G, Ciapponi A, Cafferata ML, Belizan JM: Calcium supplementation for prevention of primary hypertension. *Cochrane Database Syst Rev* 6: CD010037, 2015

14. Beveridge LA, Struthers AD, Khan F, Jorde R, Scragg R, Macdonald HM, Alvarez JA, Boxer RS, Dalbeni A, Gepner AD, Isbel NM, Larsen T, Nagpal J, Petchey WG, Stricker H, Strobel F, Tangpricha V, Toxqui L, Vaquero MP, Wamberg L, Zittermann A, Witham DPRESSURE C: Effect of vitamin D supplementation on blood pressure: A systematic review and meta-analysis incorporating individual patient data. *JAMA Internal Medicine* 175: 745-754, 2015

15. Holick MF: Vitamin D deficiency. *N Engl J Med* 357: 266-281, 2007

16. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R: Vitamin D-binding protein and vitamin D status of black americans and white americans. *N Engl J Med* 369: 1991-2000, 2013

17. Bhan I, Powe CE, Berg AH, Ankers E, Wenger JB, Karumanchi SA, Thadhani RI: Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients. *Kidney Int* 82: 84-89, 2012

18. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, Wenger J, Karumanchi SA, Thadhani R, Bhan I: Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. *Journal of Bone & Mineral Research* 26: 1609-1616, 2011

19. Schoenfeld D: Statistical considerations for pilot studies. *Int J Radiat Oncol Biol Phys* 6: 371-374, 1980

20. Phinney KW: Development of a standard reference material for vitamin D in serum. *Am J Clin Nutr* 88: 511S-512S, 2008

21. Aloia JF, Patel M, Dimaano R, Li-Ng M, Talwar SA, Mikhail M, Pollack S, Yeh JK: Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr* 87: 1952-1958, 2008