CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: Effects of Vitamin D on Cardiovascular Health in Black Women (PART 2)

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KEY INFORMATION

Important aspects of the study you should know about first:

- **Purpose:** The purpose of this study is to evaluate the effect of vitamin D supplementation on 24-hour blood pressure, blood vessel function, and sleep in generally healthy, vitamin D insufficient or deficient 18-30-year-old black women.
- **Procedures**: If you choose to participate in this study, you will be enrolled in an 8-week vitamin D supplementation intervention. At week 4 and week 8 of the intervention you will return to the lab for blood vessel function testing visits (Visit 3 and Visit 4). Within the 2 weeks prior to Visit 3 and Visit 4 you will undergo a 2-week sleep monitoring period with a sleep watch, a 24-hour period of ambulatory blood pressure monitoring, and an overnight urine collection.
- **Duration:** The study will be 8 weeks long, in addition to a ~4 hour in-lab commitment.
- **Risks**: The main risks or discomforts from this research are minor and include risk of infection due to blood sampling, discomfort during flow-mediated dilation (blood vessel function test) due to inflation of a forearm cuff causing obstruction of blood flow to the hand, and discomfort in the arm during 24-hour blood pressure monitoring due to repeated, brief cuff inflations. Potential risks associated with vitamin D supplementation include an allergic reaction from the pills or symptoms such as nausea, vomiting, poor appetite, weight loss, constipation, weakness, confusion and disorientation, heart rhythm problems, kidney stones and kidney damage in the event that they develop vitamin D toxicity. However, the risk of developing vitamin D toxicity during this study will be minimal for participants who consume the vitamin D supplements as directed.
- **Benefits:** Vitamin D supplementation may cause an increase in blood vitamin D concentration to a sufficient level.
- **Alternatives:** There are no known alternatives available to you other than not taking part in this study.
- **Costs and Compensation:** If you decide to participate there will be no additional cost to you and you could be compensated up to $120 for your time.
- **Participation:** Taking part in this research study is your decision. You can decide to participate and then change your mind at any point.

Please carefully read the entire document. You can ask any questions you may have before deciding If you want to participate.

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether you want to participate. This is the informed consent form for Part 2 of this two-part study.

PURPOSE OF THE STUDY

The purpose of this study is to better understand the effects of vitamin D supplementation on various cardiovascular disease (CVD) risk factors in young adult black women. Vitamin D status is determined using a venous blood sample to evaluate serum vitamin D concentration. Concentrations ≥30 ng/ml are deemed sufficient, concentrations between 20.0-29.9 ng/ml are deemed insufficient, concentrations <20 ng/ml are deemed deficient, and concentrations ≤ 7 ng/ml are deemed severely deficient. Vitamin D deficiency has been associated with increased CVD risk, although the link is still unclear. Black women are at increased risk of vitamin D deficiency as well as CVD as compared to women of other races in the U.S. Moreover, black women tend to develop high blood pressure, reduced blood vessel function, and sleep disturbances starting at a young age. Previous research indicates that vitamin D deficiency may be linked to such conditions. Vitamin D deficiency/insufficiency may be corrected with vitamin D supplementation. Therefore, Part 2 of this study aims to evaluate if 8 weeks of vitamin D supplementation in vitamin D insufficient or deficient black women improves vitamin D status to sufficient levels in addition to providing improvements in blood pressure, blood vessel function, and/or sleep in young adult black women.

WHO IS BEING ASKED TO PARTICIPATE?

You will be one of approximately 25 participants in this study.

<u>You are being asked to participate because</u>:

- You completed Part 1 of this study within the past 2 weeks
- You are a generally healthy black woman between the ages of 18-30
- You are vitamin D deficient or insufficient (serum vitamin D concentration 8 – 29.9 ng/ml)
- You are not currently taking supplements that contain vitamin D

<u>You may not be able to participate if</u>:

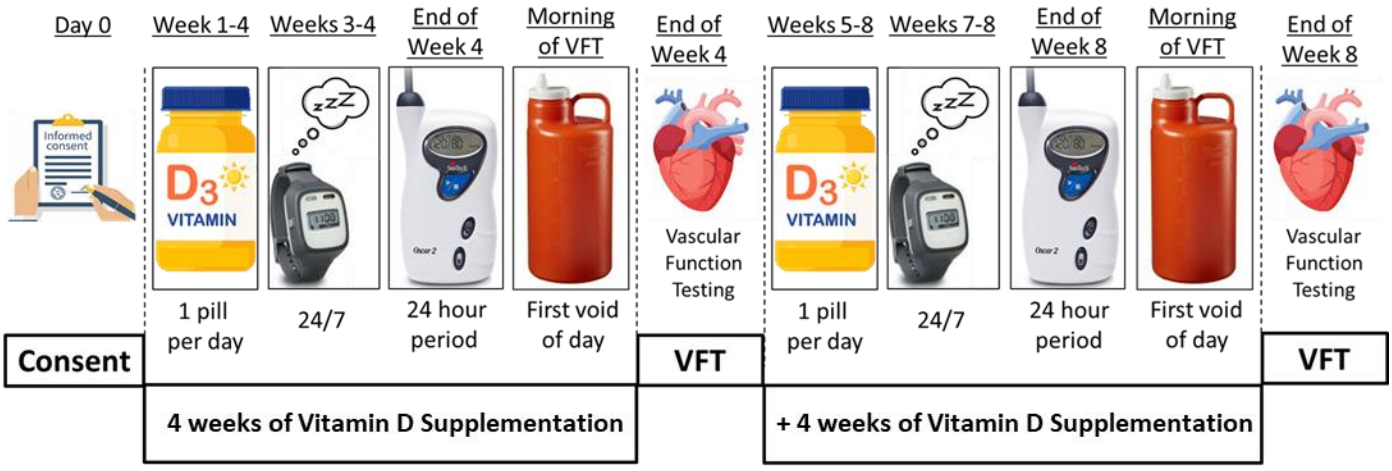
- Your serum vitamin D concentration is ≥ 30 ng/ml or ≤7 ng/ml (determined during Part 1 of the study)
- You are currently taking vitamin D supplements or multivitamins that contain vitamin D
- You are unwilling or unable to give consent
- You are unwilling or unable to undergo a venous blood draw
- You have been diagnosed with any chronic diseases or conditions including cardiometabolic diseases, cardiorespiratory diseases, chronic mental or psychological illness, musculoskeletal diseases/conditions, autoimmune diseases, cancer, gastrointestinal/malabsorption disorders, hyper-/hypocalcemia, hyper-/hypoparathyroidism, hyper-/hypothyroidism, kidney disease, or a history of kidney stones
- You are taking medication that may influence blood pressure or blood vessel function
- You have been diagnosed with a sleep disorder (e.g., insomnia, restless leg syndrome, sleep apnea), or are at high risk for a sleep disorder according to the ISI (score >14) or STOP-bang (score ≥3) questionnaires

- You are currently taking medications or supplements that affect sleep (e.g., Ambien, sedatives, melatonin, etc.)
- You are currently working night-shift work
- You have a resting blood pressure >130 or >80 mmHg
- Have a BMI >30 kg/m^2
- You are currently pregnant, breast feeding, peri-menopausal, or post-menopausal
- You currently use tobacco (≥1 cigarette in the last month)
- You have had COVID-19 in the past 60 days
- You received the COVID-19 vaccine or booster within in the past 14 days

PROCEDURES: WHAT WILL YOU BE ASKED TO DO?

You will be asked to undergo an 8-week supplementation period, during which you consume 1 pill daily for 8 continuous weeks. You will be provided 4 weeks of pills at a time. The vitamin D supplements contain 5,000 IU of vitamin D3 per pill in white powder form. The pill capsules are made of gelatin derived from both cows (bovine) and pigs (porcine).

During your participation, you will be asked to make a total of 2 visits to the lab in STAR (Visit #3 and #4). Visit 3 will take place 4 weeks following the start of the intervention supplementation period and Visit 4 will take place 8 weeks following the start of the intervention. You will also undergo two 2-week sleep monitoring periods, 24-hour ambulatory blood pressure monitoring periods, and overnight urine collections prior to each visit. Monitoring will be performed during the two weeks immediately prior to Visit #3 and again during the two weeks immediately prior to Visit #4. Refer to the schematic below for an overview of the study:



Informed Consent/Equipment Set-up

Consenting procedures will take place following your completion of Part 1-Visit 2. Procedures will be completed under the direction and supervision of a research team member and will include:

Pregnancy testing:

Following consenting, you will be asked to provide a urine sample. The sample will be used to administer a pregnancy test. If you are being consented immediately following completion of Part 1, we will utilize the overnight urine collection sample to administer the test, thus an additional sample will not need to be provided. A positive test will result in exclusion from the study. The research staff will notify you of your results at the time of testing.

Supplementation procedures:

You will be asked to take 1 vitamin D pill daily, for 8 continuous weeks. Each pill should be taken in the morning with food (e.g., with breakfast or a snack). If missed at the first meal, you may consume the pill with your next meal of the day. You will be given 4 weeks of pills that you will take leading up to Visit #3. At Visit 3 you will be provided an additional 4 weeks of pills that you will take leading up to Visit #4. You will be provided the pills in a 30-day pill organizer. The primary researcher will check in weekly via text message, email, or phone call to confirm that you are compliant to the protocol and are not experiencing any side effects from the pills.

Sleep monitoring:

You will receive a wrist-worn sleep monitor as well as detailed directions for use. You will be asked to wear the monitor on your non-dominant wrist for a 2-week period leading up to Visit #3 and again leading up to Visit #4. You will also be asked to complete a daily sleep diary which includes details about your sleep while you were wearing the monitor, such as the time you fell asleep, the time you woke up, and other details about your sleep behaviors. Sleep monitors and sleep diaries will be returned to research staff at Visit 3 and again at Visit 4.

24-hour blood pressure monitoring:

You will receive an ambulatory blood pressure cuff during this visit and will be instructed to wear it on the upper-part of your non-dominant arm during the day and overnight for a full 24-hour period. The 24-hour wear period for the blood pressure monitoring will be initiated during the morning hours. This cuff will be set to automatically take blood pressure measurements every 20 minutes during the day and every 30 minutes during the night. The monitor records and saves each blood pressure measurement automatically. If possible, you are encouraged to sit and relax when you feel the blood pressure cuff begin to inflate during the 24-hour wear-time period. Specific directions for use of the monitor will be described in detail during this visit, and you will also be provided with a directions sheet and log sheet for you to reference and record the time that you put the cuff on and took the cuff off. You will have the option to wear the blood pressure monitor starting on day 10, 11, or 12 of each 14-day sleep monitoring period. The blood pressure cuff and log sheet will be returned to the research staff at Visit 3 and again at Visit 4.

Overnight Urine Collection:

You will also receive a urine specimen collection container and will be instructed to collect your urine during a scheduled overnight period, which is specifically scheduled the night before/morning of Visit 3 and again at Visit 4. You will be instructed to discard your last void of the night at bedtime into the toilet, and then to collect all voids which occur from bedtime through the first void when you get out of bed on the following day (i.e., all voids after ~12:00 AM, including the first void when you get out of bed for the day). You will also be given a log to document when the samples were collected during this timeframe. You will be instructed to return the urine collection container and the log to the research staff at Visit 3 and again at Visit 4. Urine samples will be frozen and stored for later analysis of melatonin and other sleep hormones.

Testing Visits (Visit #3 and Visit #4) Equipment Return/Blood Vessel Function Testing (2 hours)

Visit #3 will be scheduled 4 weeks following the start of the supplementation period. Visit #4 will be scheduled 8 weeks following the start of the supplementation period.

Each testing visit will last approximately 2 hours and will require you to return the sleep monitor and associated written sleep log, the blood pressure monitor and log, and the urine collection container and log. The testing visits will consist of a series of blood vessel structure and function assessments.

Testing visits will be scheduled in the morning as you will be asked to abstain from food for approximately 6 hours, from caffeine for approximately 12 hours, and from alcohol and exercise for 24 hours prior to each visit. In addition, if appropriate, you will be asked to withhold any over-the-counter medications, supplements, vitamins, and anti-inflammatory drugs ≥3 days prior to this visit, as well as any prescribed medications on the morning of the study visit - *Please note*: This does not include the supplement pills that you have been assigned to take for the study, although on the day of your in-lab testing visit you will be asked to refrain from taking your assigned pill until after your testing visit is over. You will be asked to rest laying down on your back on a testing bed in the lab where all tests will take place. Assessments will be performed as follows:

Blood sampling:
A fasted blood sample of approximately 2 tablespoons will be taken by venipuncture by a research team member trained to draw blood via insertion of a needle into a superficial vein in the arm. This blood sample will be assessed for a basic metabolic panel and serum vitamin D concentration through LabCorp. In addition, blood samples will also be stored for later analysis of sleep, stress, and metabolic hormones, as well as markers of oxidative stress and inflammation. You will be provided copies of your blood work from each visit.

Pulse Wave Analysis (PWA):
Using a standard blood pressure cuff and specialized computer software, a pressure waveform (i.e., “shape”) will be created from measurement of brachial artery blood pressure, the blood vessel in your upper arm. These blood pressure waves are simple to measure and are an estimate of the stiffness of the blood vessels in the heart.

Pulse Wave Velocity (PWV):
Carotid to femoral PWV will be measured by simultaneously recording carotid artery and femoral artery pressure waves using a similar technique described above. A small wand-like probe will be placed on the neck at the carotid artery while a blood pressure cuff inflates around your upper leg. PWV is an estimation of how stiff the arteries are throughout the body.

Carotid Artery Wall Thickness and Pulsatility:
This assessment uses ultrasound to measure the thickness of the inner layer of the carotid artery wall, a blood vessel located in your neck. We will record three 20-second video clips of your carotid artery. Measures will be averaged and used as an evaluation of blood vessel structure. Carotid pulsatility index will also be recorded using ultrasound to evaluate the velocity of the blood traveling through the blood vessel.

Flow-Mediated Dilation (FMD):

The FMD technique assesses the diameter and blood velocity of the blood vessel located in the upper arm via ultrasonography. Following a 20-minute rest period, the blood vessel in your upper arm near your bicep will be located and one minute of baseline images will be recorded. A cuff located right below the elbow joint will then rapidly inflate to a high pressure of 250 mmHg for five minutes. This pressure value is used to reduce blood flow through the blood vessels in your forearm and hand, which may cause some tingling in the hand like that of your hand "falling asleep". Following deflation of the forearm cuff, images will continue to be collected for two minutes to assess changes in blood vessel diameter and blood velocity. FMD will be used as a measure of blood vessel function determined by changes in blood flow and blood vessel size throughout the test.

Passive Leg Movement (PLM):

Blood vessel function will also be measured via a PLM technique. Following a 10-minute rest period, we will use ultrasound to record one minute of baseline diameter and blood velocity of a blood vessel in your leg imaged directly below your stomach and at the level of your hip, on the front side of your upper right thigh. During baseline testing you will be sitting with your back upright, both legs supported, and knees fully extended straight (i.e., 180 degrees). Passive movement will then be initiated by a second team member who will continuously move your right leg through a 90-degree range of motion at the knee joint (i.e., passive movement of your lower leg up and down while your upper leg remains relaxed) for two minutes. You will be urged to remain passive and to avoid any assistance with limb motion. Blood velocity of the blood vessel will be continuously recorded during the two minutes of passive leg movement and analyzed for changes. Heart rate and blood pressure will be continuously monitored during the protocol by using chest sticker electrodes and a small finger cuff.

Blood Pressure, Heart Rate, and Respiration measurements:

Resting blood pressure will be determined in the upper arm using a standard automatic blood pressure cuff. In addition, blood pressure will be measured continuously on a single finger with a small finger cuff. You will also have six sticker electrodes placed on your chest to monitor your heart rate. An elastic-like band will be placed around your lower chest/upper stomach to monitor your breathing rate. Once you have rested quietly on your back for 10 minutes on the bed, these measurements will begin to be recorded continuously for 5 minutes. During this time, you will be asked to remain awake while quietly lying still on the bed.

Sleep Quality Questionnaire:

During Visits 3 and 4 you will be asked to complete the sleep quality questionnaire again to assess any changes in your perceived quality of sleep.

Sun Exposure Questionnaire:

During Visit 4 you will be asked to complete the sun exposure questionnaire again to assess any changes in sun exposure during your enrollment in the study.

WHAT ARE POSSIBLE RISKS AND DISCOMFORTS?

Possible risks of participating in this research study include:

Blood Sampling: You may have some pain and bruising in your arm where blood is drawn. Some people may faint during or shortly after blood is drawn. When you pierce the skin there is a chance of infection. This risk will be minimized by always using sterile equipment and supplies. A research team member trained to draw blood (under

previously trained lab personnel or approved by a nurse practitioner employed by the UD STAR campus Nurse Managed Health Center) will perform the blood draw to minimize any discomfort.

Flow-Mediated Dilation: The inflation of the forearm cuff during this procedure could result in some discomfort due to obstruction of blood flow to the hand. During the test you may experience some numbness in the hand, a sensation like having your arm "fall asleep." A trained technician will perform the procedure to improve ease of testing and minimize discomfort. If you request to stop the test due to discomfort or pain, we will do so. After a short break we may ask you to try the test again. If you decline re-testing, we will continue with the remaining blood vessel assessments.

24-Hour Blood Pressure Monitoring: You may feel brief, mild discomfort on your arm while the cuff inflates during this 24-hour period. The cuff may also cause minor disruptions of normal daily living due to the measurements which occur periodically throughout the day and night. You may remove the blood pressure monitor for brief periods of time or you may switch the arm that you are wearing it on as a means of reducing discomfort while wearing the monitor.

Vitamin D supplementation: Daily oral vitamin D supplements are safe to consume and are offered over the counter in the amount of 5,000 IU, which is the amount we are providing in this study. The main risk associated with vitamin D supplementation includes risk of developing vitamin D toxicity (high blood levels of vitamin D) because of consuming too much vitamin D. The risk of developing vitamin D toxicity within the 8-week supplementation period in this study is extremely low if you are consuming the vitamin D supplements as directed. However, to minimize risk, we will be checking in regularly with you to ensure you are taking the pills as directed, in addition to evaluating serum vitamin D status at both week 4 (Visit 3) and week 8 (Visit 4) of the study. If there is any indication that you are at risk of vitamin D intoxication, you will be asked to stop taking the supplements and we will refer you to your primary care physician. The potential side effects experienced by someone with vitamin D toxicity include nausea, vomiting, poor appetite, weight loss, constipation, weakness, confusion and disorientation, heart rhythm problems, kidney stones and kidney damage. There is also a potential risk of allergic reaction to taking vitamin D supplements which may present as a rash.

WHAT ARE POTENTIAL BENEFITS FROM THE STUDY?

The information gained from your participation could contribute to our understanding of how vitamin D status influences various cardiovascular disease risk factors including blood pressure, blood vessel function, and sleep in black women. Moreover, findings from this study may also inform clinicians about the benefits of vitamin D supplementation in black women. *Please note*: Your vitamin D levels may beneficially increase to normal levels. However, participation in this study does not serve as a treatment or cure for vitamin D deficiency/insufficiency and thus should not be treated as such. The researchers recommend that all participants refer to their primary care physician following completion of this study.

NEW FINDINGS THAT COULD AFFECT YOUR PARTICIPATION

During this study, we may learn new important information. This may include information that could cause you to change your mind about participating in the study. If any new important information becomes available while you are a participant, we will let you know.

CONFIDENTIALITY: WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?

Information obtained from this study will be kept strictly confidential. You will not be individually identified, except by a participant code known only to the investigators.

Any identifiable documents of yours will be stored until closure of the study with the exception of informed consent documents which will be stored for 3 years following closure of the study. Upon closure of this study, these documents will be destroyed in a manner which protects your identity. We will shred paper documents and permanently remove identifiable electronic participant data.

All de-identifiable data will be stored in a locked cabinet or password-protected computer indefinitely.

Your study data will be handled as confidentially as possible. If results of this study are published or presented, individual names and other personally identifiable information will not be used.

The confidentiality of your records will be protected to the extent permitted by law. We will keep your study data confidential and only those with permission in the research team will have access to information that identifies you. We may have to report certain information for legal or ethical reasons, such as intent to hurt yourself or others. If required, your records may be inspected by authorized personnel in the University of Delaware Institutional Review Board, which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans.

COSTS AND COMPENSATION

There are no costs associated with participating in this study.

You will be compensated $120 upon completion of the study. You will receive $40 for the first 4-week supplementation period (including sleep monitoring/blood pressure monitoring/urine collection), $20 for Visit #3, $40 for the final 4-week supplementation period (including sleep monitoring/blood pressure monitoring/urine collection), and $20 for Visit #4.

WHAT IF YOU ARE INJURED DURING PARTICIPATION IN THE STUDY?

If you are injured during your participation in the study, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of a third-party payer (for example, your health insurance). By signing this document, you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

Taking part in this research study is your decision. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide later not to participate, or if you decide to stop taking part in the research, there will be no penalty or loss of benefits to which you are otherwise entitled.

Your decision to stop participation, or not to participate, will not influence current or future relationships with the University of Delaware.

If, at any time during your enrolment in the study, you develop any of the exclusion criteria mentioned, your participation may be terminated by the investigator.

If, at any time, you decide to end your participation on this research study please inform our research team by telling the study investigators. If you do not complete all procedures listed in this form, you will only receive compensation for the tasks you finish.

INSTITUTIONAL REVIEW BOARD

This research study has been reviewed and approved by the University of Delaware Institutional Review Board (UD IRB), which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans. If you have any questions or concerns about your rights as a research participant, you may contact the UD IRB at hsrb-research@udel.edu or (302) 831-2137.

CONTACT INFORMATION

If you have any questions about the purpose, procedures, or any other issues related to this research study you may contact the Principal Investigator, Melissa Witman, at (302) 831-6256 or mwitman@udel.edu.

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY:

I have read and understood the information in this form, and I agree to participate in the study. I am 18 years of age or older. I have been given the opportunity to ask any questions I had, and those questions have been answered to my satisfaction. I understand that I will be given a copy of this form for my records.

_____	_____	_____
Printed Name of Participant (PRINTED NAME)	Signature of Participant (SIGNATURE)	Date
_____	_____	_____
Person Obtaining Consent (PRINTED NAME)	Person Obtaining Consent (SIGNATURE)	Date

<u>OPTIONAL</u> CONSENT TO BE CONTACTED FOR FUTURE STUDIES:

Do we have your permission to contact you regarding participation in future studies? If you agree to being contacted in the future, we will keep your contact information. Please write your initials next to your preferred choice.

_____ YES _____ NO