A Pilot Study of the Effect of Dietary Sodium Intake on Assessments of Vascular Endothelium
A Sub-study of "Dietary Salt in Postural Tachycardia Syndrome"

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1.0 BACKGROUND

1.1 Endothelium

The endothelium is the thin layer of cells that lines the interior surface of blood vessels. It functions as an inert barrier between the vascular system and all other body tissues. Additionally, it aids in modulating hemostasis, growth, tone and inflammation throughout the circulatory system. The endothelium maintains the vasculature in a steady dilated state. Endothelial dysfunction has been identified in several pathologic states such as diabetes, hypertension, age, cigarette smoking, obesity and several cardiovascular diseases [1, 2]. The pathophysiology of endothelial dysfunction is not fully understood. Studies have demonstrated impairment in nitric oxide (NO) metabolism of which several mechanisms have been postulated including: abnormally elevated asymmetric dimethylarginine (ADMA) level- a potent endogenous competitive inhibitor of NO [3], overproduction of free radicals which have been identified in patients with elevated cholesterol and coronary artery disease and reduction in NO synthesis [4-6]. Impaired availability of NO has been implicated in the development of coronary artery disease, and poorly controlled hypertension [7].

1.2 Dietary Sodium Levels

According to the most recent report by institute of medicine (IOM), most Americans consume an average of sodium in excess of 3400 mg. This far exceeds the center for disease (CDC) recommendation[8] of less than 2300 mg or less than 1500 mg for high risk groups with hypertension, diabetes, African Americans, or chronic kidney disease. The American Heart Association has recently recommended an average dietary sodium intake not exceeding 1500 mg across all age groups [9].

Dietary sodium have been implicated in endothelial dysfunction, with increasing evidence linking salt intake to increased risk of cardiovascular disease and elevated blood pressure [10]. Excess salt intake in mammalian study has demonstrated increased mortality by accelerating arteriosclerosis and renal parenchyma damage [11]. Strazullo at al. performed a meta-analysis that suggested a significant increased risk of stroke and total cardiovascular events among those with a high salt diet [12].

1.3. Endothelial dysfunction and oral salt diet

Short-term high salt intake has been shown to produce reductions in NO as reported by Dishy et al. [13]. Elevated ADMA was also implicated in patients with high salt diet by a study reported by Fujiwara et al. [14]. Many of these studies have assessed patients on their native diets, and thus may be confounded my many unknown variables. Most of these studies were also carried out on middle aged or older patients with cofounding risk factors such as hypertension. We propose to study young to middle aged subjects who will undergo acute, but steady state HIGH and LOW sodium diets, and assess the acute changes in measures of endothelial events. Differences in measures of endothelial function between the diet phases might suggest that dietary sodium intake is an important variable that would need to be controlled in future studies of endothelial function.

2.0 REACTIVE HYPEREMIA PULSITLE ARTERIAL TONOMETRY (RH-PAT)

2.1 RH-PAT Device

The principle of PAT, a finger plethysmographic device that allows the isolated detection of pulsatile arterial volume changes, has been described [15, 16]. This device (Itamar Medical Ltd., Caesarea, Israel) consists of two finger-mounted probes, which include a system of inflatable latex air-cushions within a rigid external case. The probe design allows the application of a constant and evenly distributed near-diastolic counter-pressure within the entire probe, which increases sensitivity by unloading arterial wall tension, and prevents venous blood pooling to avoid venoarteriolar reflex vasoconstriction. Pulsatile volume changes of the fingertip are sensed by a pressure transducer and transferred to a personal computer where the signal is band pass-filtered (0.3 to 30 Hz), amplified, displayed, and stored.

2.2 RH-PAT and Other Methods for Assessing Vascular Endothelial Function in Vivo

Reactive hyperemia, which is due to dilation of small resistance vessels, is partly mediated by endothelium-derived NO and, therefore, the magnitude of the hyperemic response serves as an indicator for the status of endothelial function [17, 18]. Moreover, using strain-gauge plethysmography, an excellent correlation between peak forearm blood flow response during RH and that to intra-arterial infusion of the endothelium-dependent vasodilator acetylcholine has been demonstrated, indicating that the magnitude of RH may serve as an index of peripheral endothelial function [19]; RH-PAT permits the noninvasive assessment of peripheral vascular reactivity by measuring digital pulse volume at rest and during RH. Notably, although local, systemic, and environmental factors may modulate digital pulse volume, this parameter also depends on the bioavailability of NO [20]. Moreover, endothelium-derived NO has been shown to be particularly important for the regulation of vascular tone in areas rich in arteriovenous anastomoses, such as the finger tip [21]. The effect of NO synthesis inhibition by N^{ω}-nitro-L-arginine methyl ester (L -NAME) on the PAT response to reactive hyperemia was investigated recently[22]. In that study, 19 healthy volunteers underwent RH-PAT testing, according to the same protocol as was used in the present study, before and after administration of L-NAME into the brachial artery. Importantly, L-NAME administration was associated with a significant 61% reduction ($p < 0.05$) in the RH-PAT index values, indicating that this index depends on endothelium-derived NO and, therefore, represents a marker of peripheral

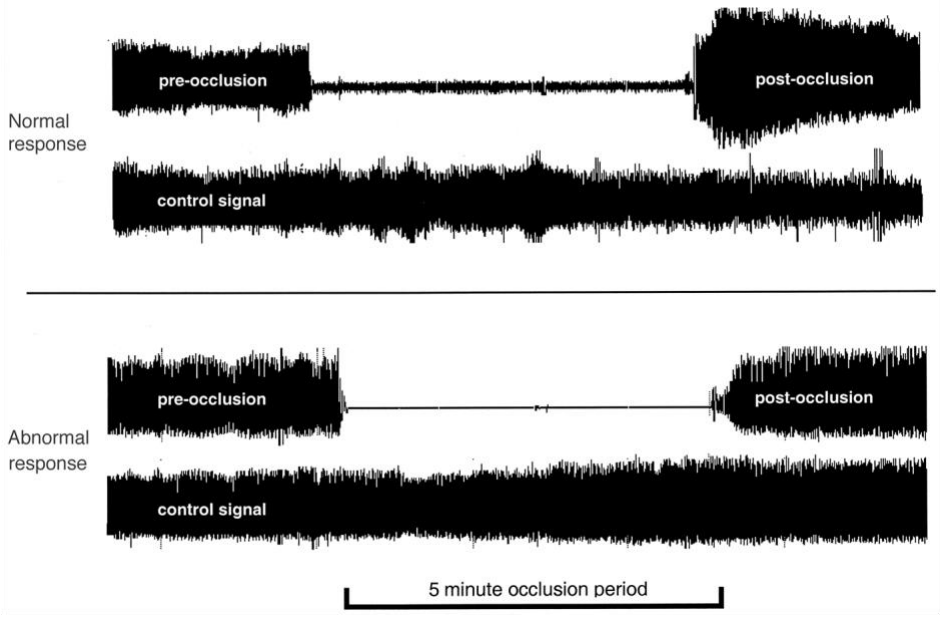

Figure 5: RH-PAT responses before and after brachial artery occlusion.

endothelial function. In accordance with these findings, it was demonstrated that factors known to affect endothelial function, including various cardiovascular risk factors and the presence of CAD, influence RH-PAT index values in a similar manner as flow-mediated dilation (FMD) of the brachial artery, a widely-used method to evaluate peripheral endothelial function [15]. Furthermore, a significant relationship between the PAT response to reactive hyperemia and flow-mediated dilation of the brachial artery has been shown, suggesting that vascular reactivity of the digital vasculature, as measured by the RH-PAT index, is influenced by endothelial function in a magnitude and direction similar to that of the brachial artery [15].

Vasodilatation of a peripheral conduit artery following an increase in luminal blood flow and internal-wall shear is known as flow-mediated dilatation (FMD). In practice it is performed following a period of distant limb ischemia. Strong evidence suggests that FMD is a valid endothelium-dependent and NO-specific index of endothelial function. To measure FMD we will adhere to current guidelines [23, 24].

3.0 EX-VIVO ASSESSMENT OF ENDOTHELIAL FUNCTION

The endothelium can no longer be viewed as the passive inner cover of the vessel wall. It plays a central role in cardiovascular regulation. Endothelial cells produce a variety of vasoactive substances that can act locally or at distant sites. Among these substances nitric oxide (NO) is probably the most heavily studied, and its reduced bioavailability is widely accepted to be responsible for endothelial dysfunction. Reduction of NO bioavailability can result from a defective eNOS expression/activity or from an excessive NO degradation, usually through an interaction with superoxide (O^{-2}) that generates peroxynitrites ($ONOO^{-}$). Endothelial damage is the first step of vascular remodeling. Unfortunately, the endothelium has a limited ability to repair itself and hence prevent or reverse endothelial dysfunction. In animal models, direct information on the anatomical and function integrity of the endothelium can be easily obtained. In humans, endothelial function is usually assessed indirectly as the NO-dependent vasodilatory capacity of a given vascular bed to an endothelial stimulus either mechanical (shear stress, reactive hyperemia) or pharmacological (NO-dependent vasodilators, NOS inhibitors). This indirect approach is however subjected to intra-patient variability, confounding effects, and cannot distinguish between anatomical and functional alterations. Other clinical indicators of endothelial dysfunction are represented by circulating substances such as VCAM-1, ICAM, vWF, ADMA, and thrombomodulin, indicating pathological activation of the endothelium in the setting of a low grade inflammatory state. Relatively recently, more direct *ex vivo* approaches have been developed to study the human endothelium, these include the study of mature circulating endothelial cells that may detach at an increase rate due to ongoing endothelial damage and can be isolated and incubated. Similarly, circulating endothelial progenitor cells, discovered in 1997, can be obtained and cultivated to study mechanisms of vascular repair and regeneration. A third method involves the harvesting of viable endothelial cells via an endothelial biopsy, this allow the study of gene and/or protein expression.

Endothelial biopsy has been used to quantify eNOS expression (by measuring the protein expression of eNOS), its activity [by measuring Ser-1177 phosphorylated eNOS (P-eNOS)] and also NO-dependent reactive oxygen induced stress (by measuring nitrotyrosine). Ideally samples from a suspected damaged artery (coronary) should be obtained, either during routine cardiac catheterization or after the procedure has been performed, however this is not always possible to

do. Vein sampling has the advantage of its accessibility and it has reported a good correlation (r=0.7) for protein expression of eNOS, P-eNOS and nitrotyrosine from samples obtained form a peripheral artery and the brachial artery.

4.0 HYPOTHESIS & SPECIFIC AIMS

4.1 Hypothesis

In this pilot crossover study, we will test the hypothesis that markers of vascular endothelial dysfunction will be exaggerated acutely with an extreme high-sodium diet compared to an extreme low-sodium diet.

4.2 Specific Aims

1. To assess whether RH-PAT (marker of peripheral small vessel endothelium dependent nitric oxide mediated vasodilatation) will be reduced in subjects on a high sodium diet compared with a very low sodium diet.
2. To assess whether brachial artery flow-mediated vasodilatation (marker of endothelium dependent nitric oxide mediated vasodilatation in a conductance vessel) is reduced on extreme high sodium diet compared to a low sodium diet.
3. To assess whether circulating markers of endothelial function will be reduced in subjects on a high sodium diet compared with a very low sodium diet.
4. To assess whether calf blood flow in response to reactive hyperemia (CBF-RH) correlates with RH-PAT.

5.0 INCLUSION/EXCLUSION CRITERIA

5.1 Inclusion Criteria

- Subjects will be enrolled in the parent study "Dietary Salt in Postural Tachcyardia Syndrome" funded by R01 HL102387
- Postural Tachycardia Syndrome
    - Diagnosed with postural tachycardia syndrome by the Vanderbilt Autonomic Dysfunction Center
        - Increase in heart rate ≥30 beats/min with position change from supine to standing (10 minutes)
        - Chronic symptoms consistent with POTS that are worse when upright and get better with recumbence
- Control Subjects
    - Healthy, non-obese, non-smokers without orthostatic tachycardia
    - Selected to match profiles of POTS patients (gender, age)
    - Not using vasoactive medication
- Age between 18-60 years
- Male and female subjects are eligible.
- Able and willing to provide informed consent

5.2 Exclusion Criteria

- Overt cause for postural tachycardia (such as acute dehydration)
- Inability to give, or withdrawal of, informed consent
- pregnant

- Other factors which in the investigator's opinion would prevent the subject from completing the protocol.

6.0 ENROLLMENT, RANDOMIZATION & BLINDING

6.1 Recruitment

For the parent study, the patients with orthostatic intolerance will be recruited from patients referred to the Vanderbilt University Autonomic Dysfunction Center. Control subjects will be recruited from the Autonomic Research Database, the VICTR Research Participant Database, and advertising within the Vanderbilt Community. For this protocol, subjects enrolled in the parent study will be approached about this sub-study. Subjects will be assured that they are not required to participate in this study even if they choose to participate in the parent study.

6.2 Randomization

There will be no randomization for this specific sub-study. The order of diets (low sodium vs. high sodium) will be performed as a part of the parent study, and not as a part of this sub-study.

6.3 Blinding

None.

7.0 STUDY PROCEDURE

7.1 Screening

All subjects will be previously screened and evaluated as a part of the parent study. No further screening will be performed exclusively for this study. Women of childbearing potential will have had a serum pregnancy test as a part of the parent study. Pregnant women will not be allowed to participate.

The history will include (but not be limited to):

- Framingham Risk factors (age, gender, smoking, hypertension, dyslipidemia)
- Metabolic diseases (metabolic syndrome, polycystic ovary syndrome, diabetes)
- Inflammatory diseases (e.g. rheumatoid arthritis).

The physical examination will include (at minimum):

- Height
- Weight
- Waist circumference
- Hip circumference
- Orthostatic vital signs.

7.2 Study Design

The study will involve a crossover design in which each subject will be assessed (as below) while on a very low-sodium (10 mEq/day) diet compared with a very high-sodium diet. These acute dietary interventions will be part of the parent study ("Dietary Salt in Postural Tachcyardia Syndrome" funded by R01 HL102387) for 4-5 days at the time of the study.

Dietary success will be assessed using a 24h urine for sodium and creatinine as a part of the parent study.

7.2.1 Blood work

Blood will be drawn and collected in a **fasting state** for future assay and analysis of the following tests:

- Glucose, Insulin (glucose impairment, insulin resistance)
- Fasting lipid profile
- C-Reactive Protein (hsCRP) (inflammatory state)
- Inflammatory cytokines (inflammatory state)
- F2-isoprostanes (oxidative stress)
- PAI-1 antigen, fibrinogen (prothrombotic state)

7.2.2. Urine Collection

A morning urine sample will be collected, and an aliquot saved for urinary isoprostanes.

7.2.3 Pulsitile Arterial Tonometry (PAT) Protocol

- Performed on Day 6 of parent protocol (5 days of low/high sodium diet).
- All studies will be performed in a fasting state or at least 2 hours following a meal.
- Subjects will be studied in the supine position and both hands on the same level in a comfortable, thermoneutral environment.
- A blood pressure cuff will be placed on one upper arm (study arm; non-dominant), while the contralateral arm will serve as a control (control arm).
- RH-PAT probes will be placed on one finger (finger II, III, or IV) of each hand (same finger on both hands). The fingers on either side of the one with the probe will be separated using soft sponge rings.
- Continuous recording of pulsatile blood volume responses from both hands will be initiated.
- After a 10-min equilibration period, the blood pressure cuff on the study arm will be inflated to 60 mm Hg above systolic pressure for 5 min. The cuff will then be deflated to induce reactive hyperemia, PAT recording will be stopped.

7.2.4 PAT Analysis

The RH-PAT data will be analyzed by a computer in an operator-independent manner. As a measure of the extent of reactive hyperemia, the RH-PAT index was calculated as the ratio of the average amplitude of the PAT signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the PAT signal of a 3.5-min time period before cuff inflation (baseline). RH-PAT index values from the study arm were then normalized to the control arm to compensate for potential systemic changes.

7.2.5 Calf Blood Flow in Reactive Hyperemia (CBF-RH)

Calf blood flow (CBF) will be determined using venous occlusion plethysmography and calibrated mercury strain-gauges (EC-6, D.E. Hokanson Inc., Bellevue, Washington, USA) during reactive hyperemia after a 5 min of ischemia of the distal limb. Strain-gauges will be applied to the widest part of the non-dominant calf (~10 cm below patella). Participants will remain quietly supine for 10 min with legs elevated on foam pads above the right atrium to achieve stable baseline measurements of CBF. The venous occlusion cuff is inflated for 4 seconds at

8 seconds intervals, while monitoring the change in resistance in the system, pressure inside the measuring cuff, and 5-10 determinations are performed The plethysmograph output signal will be transmitted recorded using the WINDAQ data acquisition system (DI220, DATAQ, Acron, OH, 14 Bit, 500Hz) and processed off-line. The slope will be express as milliliters (ml) per minute per 100 ml of forearm tissue (ml/min/100 ml tissue). After 1 minute of ankle cuff inflation to 50 mm Hg above subject's systolic blood pressure to occlude ankle circulation, resting blood flow will be measured as the average of the last 4 of 5 plethysmographic measurements for 1 minute. Peak CBF will be then measured after 5 minutes of ischemia using a cuff inflated to 50 mm Hg above subject's systolic blood pressure, using a cuff located proximal to the patella. Again one minute before thigh cuff release, the ankle cuff will be inflated to 50 mm Hg above subject's systolic blood pressure, and measurements of CBF will be repeated for the next 3 minutes.

7.2.6 Protein expression of harvested endothelial cells

A 20-gauge intravenous catheter will be inserted into a superficial forearm vein. Under sterile conditions, 3 J-shaped vascular guidewires will be sequentially advanced into the vein up to 10 cm. Endothelial cells will be retrieved from the wire tips by washing with cell dissociation buffer (Invitrogen) at 4 °C. Endothelial cells will be recovered by centrifugation and fixed with 3.7% formaldehyde in PBS for 10 minutes, washed twice with PBS, transferred to poly-L-lysine coated slides (Sigma), and air dried at 37°C. The slides will be then stored at -70 °C until analyzed.

Using this procedure it is possible to measure basal eNO production and activity measuring venous endothelial expression of total eNOS and activated eNOS (P-eNOS) by quantitative immunofluorescence. Vascular inflammation and oxidative stress can be determined by measuring venous endothelial expression of nuclear factor-κB (NFκB) and nitrotyrosine.

7.2.6 Evaluation of Forearm-Mediated Dilation

Subjects will be fasting for at least 2 hours prior to the study and will be instructed to avoid exercising the day before the study. All studies will be conducted in a quiet, temperature-controlled room. All subjects will rest in the supine position for at least 30 minutes after all instrumentation procedures are done. The arm will be kept extended and immobilized at heart level. Brachial artery diameter will be measured using a high resolution ultrasonography (iU22 xMatrix Ultrasound System, Philips, Andover MA) using a linear array probe with a 5 to 17 MHz frequency range (L17-5 Broadband Linear Array Transducer, Philips, Andover MA). The brachial artery will be imaged in longitudinal sections, 5-10 cm proximal to placement of an occlusion cuff in the dominant forearm just below the antecubital fossa. The probe will be held with a stereotaxic holder with micrometer movement capabilities.

When the clearest B-mode image through the center of the vessel is obtained with optimal contrast between the anterior and posterior vessel walls and the lumen of the vessel, the stereotaxic clamp will be fixed in place. Baseline diameter will be examined before cuff inflation for at least 1 minute. The rapid occlusion cuff (Hokanson) will be inflated and maintained for 5 minutes at 50 mm Hg above the subject's systolic blood pressure to produce transient ischemia. Measurement of post-deflation diameter will be initiated before cuff release and continue for 5 minutes following deflation.

Flow velocity and brachial artery diameter will be continuously measured using duplex ultrasound. Blood velocity will be assessed using an insonation angle <60°. The B-mode will

update synchronously with the R-wave of the ECG while also having a continuous Doppler spectrum recording throughout.

One experienced sonographer will collect all images. Images will be digitized from the video output of the ultrasound machine using a frame grabber on a personal computer. Image acquisition will be gated with an ECG signal so that images will be captured at end diastole at each cardiac cycle.

Brachial artery diameter will be assessed by a single trained operator using an automated, beat-by-beat edge detection and wall-tracking image processing software (Vascular Tools 5.0, Medical Imaging Applications, USA). Peak diameter will be calculated using automated mathematical algorithms. Data will be presented as absolute (mm) and relative FMD response (%). The area under the curve until peak diameter will also be measured and presented.

7.3 Study Outcome Measures

- RH-PAT index
- Brachial artery diameter changes.
- CBF-RH
- Other blood parameters (outlined in section 7.2.1)

8.0 STATISTICAL CONSIDERATIONS

8.1 Primary Analysis

The primary outcome measure will be the RH-PAT index. The primary analysis will involve a non-parametric, paired, Signed Rank test of RH-PAT between all subjects (POTS patients and control subjects) on the high sodium diet vs. the low sodium diet.

8.2 Secondary Analysis

Secondary analyses will include non-parametric paired comparisons between low sodium and high sodium diets of continuous outcome measures (outlined in section 7.3). Secondary comparisons might also assess differences in the dietary sodium induced changes between POTS patients and control subjects.

8.3 Sample Size Calculation

As this is a pilot study, there are no preliminary data available on the RH-PAT index in different sodium diets. Published data indicate that the RH-PAT index in 39 healthy subjects without endothelial function is 1.78±0.08 (mean±SEM) [25]. In the same study, RH-PAT<1.35 was found to be the best discriminator of coronary endothelial function. We will designate a more conservative ΔRH-PAT=0.25 as a clinically significant difference between high sodium diet vs. low sodium diet for the entire group. We expect the standard deviation around the difference will be 0.5 (based on the above data), for an effect size of 0.5. With the aforementioned assumptions for a paired test of continuous data, and a 0.05 two-sided significance level, a sample size of 44 (combined POTS patients and control subjects) would give 90% power to detect this difference [26]. To account for drop-outs, we propose to enroll up to 50 subjects in total from the parent study. Using the same assumptions, 33 subjects would give 80% power to detect the same difference (in case of enrollment difficulties for this sub-study).

9.0 RISKS & INCONVENIENCES

1. Having blood drawn may hurt, may cause bruising and may cause lightheadedness or rarely fainting.
2. The finger probes may be tight and uncomfortable.
3. The blood pressure cuffs may be uncomfortable.

10.0 DATA & SAFETY PLAN

10.1 Adverse Event (or Unanticipated Problem) Reporting

Any adverse events of a serious nature will be reviewed immediately with the principal investigator. Serious adverse events will be reported in writing to the Vanderbilt IRB per IRB regulations. All non-serious adverse events will be summarized once a year, during the annual review to the IRB. C. Victor Nwazue MB ChB will be responsible for tracking adverse events in this study.

The adverse event will be described with the following information: description of the event, outcome of the event, how long it lasted, relationship to study medications, whether the event required treatment or intervention, and the outcome.

The definition of events is as follows:

Mild – transient and mild in nature, with no treatment necessary.
Moderate – some intervention and treatment necessary, but subject completely recovers.
Severe – an event that results in hospitalization, disability, death or is life threatening.

The investigator will state his opinion as to whether there is a reasonable possibility that the event or experience is related to the drug.

10.2 Data and Safety Monitoring

There will be no external Data and Safety monitoring committee for this study.

11.0 STUDY WITHDRAWAL or DISONTINUATION

11.1 Principal Investigator Initiated Withdrawal

The principal investigator reserves the right to withdraw the subject from the study after they have provided informed consent, but before study completion. This could occur for one of many reasons, which include, but are not limited to: non-compliance with the protocol, a concern for subject safety, the availability of new knowledge that might affect continued participation in the study, or study termination.

11.2. Study Subject-Initiated Withdrawal

Subjects are free to withdraw from this study at any time. Withdrawal of consent or refusal to participate will not prejudice their health care.

12.0 COMPENSATION

Subjects will be compensated $25 for their time and inconvenience.

13.0 PRIVACY & CONFIDENTIALITY ISSUES

Protected Health Information will be used in this study. The investigators will comply with the patient privacy guidelines of Vanderbilt University Medical Center and the rules outlined by the Health Insurance Portability and Accountability Act (HIPAA).

The research team is comprised of experienced research nurses and research assistants who are aware of the importance of confidentiality of health information. Research records will be stored in a locked office. Digital records will be stored on password-protected computers/servers. Digital data files will be coded so that the subject name is not in the filename or other such identifiers.

Every effort will be made to publish and present the data from this study. At no time will any subject be identified in any such publication.

14.0 FOLLOW-UP AND RECORD RETENTION

14.1 Follow-Up

There will be no further study follow-up after the physiological breathing study is completed.

14.2 Record Retention

Study records will be kept for at least 7 years following the publication or presentation of the data collected as part of this study.

15.0 Study Support

Study costs will be funded from R01 HL102387.

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Vanderbilt University Institutional Review Board
Informed Consent Document for Research

Principal Investigator: Emily Garland, PhD **Revision Date: 5/28/14**
Study Title: A Pilot Study of the Effect of Dietary Sodium Intake on Assessments of Vascular Endothelium
Institution/Hospital: Vanderbilt University

This informed consent applies to healthy persons.

Name of participant: _____ Age: _____

The following is given to you to tell you about this research study. Please read this form with care and ask any questions you may have about this study. Your questions will be answered. Also, you will be given a copy of this consent form.

You do not have to be in this research study. You can stop being in this study at any time. If we learn something new that may affect the risks or benefits of this study, you will be told so that you can decide whether or not you still want to be in this study.

1. What is the purpose of this study?

You are being asked to take part in this research study because you are healthy and are taking part in another study (Dietary Salt in Postural Tachycardia Syndrome). We want to measure chemicals in your blood while you are on a very high salt or a very low salt diet. About 44 people will take part in this study.

2. What will happen and how long will you be in the study?

If you agree to be in this study, we will use information you have given us for other studies, and we will look at your medical record. We will also use information you have given us for the Dietary Salt in Postural Tachycardia Syndrome study (called the parent study.)

On Day 6 of each phase of the parent study (there are two phases), we will study you either before you have eaten in the morning or at least 2 hours after you have eaten. We will take blood from your arm with a needle (about 1 tablespoon in each time.)

We will then ask you to lie down. We will place a blood pressure cuff on one arm and small probes on one finger on each of your hands. We will place soft sponge rings on either side of the probed fingers to keep them separated. The probes will measure your blood pressure. We will measure the diameter of the artery in your arm using ultrasound. Ultrasound involves putting a probe on your skin to get measurements. It is not invasive and does not cause pain.

After 10 minutes, we will inflate the blood pressure cuff 60 points above the highest number on your normal blood pressure. The cuff will stay inflated for 5 minutes, and again measure your artery using ultrasound. We will then let the air out of the cuff.

During this time, we will also place two cuffs on your left leg - one just above the calf and one just above the knee. A flexible cuff will be placed between these two cuffs and connected to a meter which will measure the amount of blood flow in the calf when the two cuffs above and below are inflated. You will then be allowed to lie quietly for 9 minutes. When you have been at rest for 9 minutes, your blood pressure and calf blood flow will be measured for one minute. For this, we will inflate the blood pressure cuff on your lower leg to a pressure high enough to stop the blood flow. After 1 minute the cuff will be deflated, and your blood pressure and forearm blood flow will be recorded. We will then inflate the cuff placed on your upper leg to stop the blood flow of your leg for 5 minutes (like a tourniquet). At the end of this 5 minutes the cuff will be released, and we will repeat the measurements of blood pressure and calf blood flow for the next 3 minutes.

We will insert a small tube (catheter) into a vein in your forearm. We will then slide a tiny wire into your vein to collect cells from the walls of your vein. We will do this three times. Then we will remove the small tube.

This study will then be complete. The study will last about 2 hours on each study day.

3. Costs to you if you take part in this study:

If you agree to take part in this research study, you and/or your insurance will not have to pay for the tests and treatments that are being done only for research.

For this study it includes the research only procedures noted in section 2 above.

However, you are still responsible for paying for the usual care you would normally receive for the treatment of your illness. This includes treatments and tests you would need even if you were not in this study. These costs will be billed to you and/or your insurance.

4. Side effects and risks that you can expect if you take part in this study:

Inconveniences: It may be inconvenient to come to the CRC.

Blood drawing and catheter in vein: Drawing blood with a needle in a vein may be painful and may cause bruising, bleeding, or rarely, infection. Some people may feel faint when having a needle put in their arm.

Blood pressure cuff: Some may find it uncomfortable to hold their arms or legs with an inflated cuff placed around the forearm, finger, or thigh, in a mostly still position, or have the cuff inflated frequently. The finger probes may be tight and uncomfortable.

5. Risks that are not known:

None.

6. Payment in case you are injured because of this research study:

If it is determined by Vanderbilt and the Investigator that an injury occurred as a direct result of the tests or treatments that are done for research, then you and/or your insurance will not have to pay for the cost of immediate medical care provided **at Vanderbilt** to treat the injury.

There are no plans for Vanderbilt to pay for the costs of any additional care. There are no plans for Vanderbilt to give you money for the injury.

7. Good effects that might result from this study:

a) The benefits to science and humankind that might result from this study. we may learn more about how the autonomic nervous system may affect people with POTS. This may lead to new treatments for this condition.
b) The benefits you might get from being in this study. None.

8. Other treatments you could get if you decide not to be in this study:

This is not a treatment study. You can choose not to be in this study, and nothing about your health care will change.

Principal Investigator: Emily Garland, PhD **Revision Date: 5/28/14**
Study Title: A Pilot Study of the Effect of Dietary Sodium Intake on Assessments of Vascular Endothelium
Institution/Hospital: Vanderbilt University

9. Payments for your time spent taking part in this study or expenses:

You will be paid $25 for completing this study. You may choose to receive a check for this amount or a gift card for the same amount from Target, Walmart, or Amazon.

10. Reasons why the study doctor may take you out of this study:

You will be withdrawn from the study if the study doctors decide it is best for you. If the study doctors withdraw you from the study, you will be told the reason.

11. What will happen if you decide to stop being in this study?

If you decide to stop being part of the study, you should tell your study doctor.

12. Who to call for any questions or in case you are injured:

If you should have any questions about this research study or if you feel you have been hurt by being a part of this study, please feel free to contact Dr. Emily Garland at 615-936-1748 or the research nurse at 615-343-6862.D

For additional information about giving consent or your rights as a person in this study, to discuss problems, concerns, and questions, or to offer input, please feel free to call the Vanderbilt University Institutional Review Board Office at (615) 322-2918 or toll free at (866) 224-8273.

13. Confidentiality:

All efforts, within reason, will be made to keep your personal information in your research record confidential but total confidentiality cannot be guaranteed. The study results will be kept in your research record for at least seven years after the study is over for as long as we need the information for the study. All the information on paper will be kept locked in a secure location. Any information kept in a computer will be through the Vanderbilt CRC data system, which has many safeguards. Only members of Dr. Garland's research team will be able to see any of the information that would identify you. Any research data entered into your medical record will be kept as long as it is needed.

14. Authorization to Use/Disclose Protected Health Information:

All efforts, within reason, will be made to keep your protected health information (PHI) private. PHI is your health information that is, or has been gathered or kept by Vanderbilt as a result of your healthcare. This includes data gathered for research studies that can be traced back to you. Using or sharing ("disclosure") such data must follow federal privacy rules. By signing the consent for this study, you are agreeing ("authorization") to the uses and likely sharing of your PHI. If you decide to be in this research study, you are also agreeing to let the study team use and share your PHI as described below.

As part of the study, Dr. Garland and her study team may share the results of your study and/or non-study linked blood pressure, heart rate, and breathing tests, as well as parts of your medical record, to the groups named below. These groups may include people from the Federal Government Office for Human Research Protections, the Vanderbilt University Institutional Review Board, and the National Institutes of Health. Federal privacy rules may not apply to these groups; they have their own rules and codes to assure that all efforts, within reason, will be made to keep your PHI private.

Principal Investigator: Emily Garland, PhD **Revision Date: 5/28/14**
Study Title: A Pilot Study of the Effect of Dietary Sodium Intake on Assessments of Vascular Endothelium
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The study results will be kept in your research record for at least six years after the study is finished. At that time, the research data that has not been put in your medical record will be kept indefinitely. Any research data that has been put into your medical record will be kept for an unknown length of time.

Unless told otherwise, your consent to use or share your PHI does not expire. If you change your mind, we ask that you contact Dr. Garland in writing and let her know that you withdraw your consent. Her mailing address is

Dr. Emily Garland
AA3228 Medical Center North
1161 21st Avenue South
Vanderbilt University
Nashville, TN 37232-2195

At that time, we will stop getting any more data about you. But, the health data we stored before you withdrew your consent may still be used for reporting and research quality.

If you decide not to take part in this research study, it will not affect your treatment, payment or enrollment in any health plans or affect your ability to get benefits. You will get a copy of this form after it is signed.

STATEMENT BY PERSON AGREEING TO BE IN THIS STUDY

I have read this consent form and the research study has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to take part in this study.

_____ _____
Date Signature of patient/volunteer

Consent obtained by:

_____ _____
Date Signature

_____
Printed Name and Title

Vanderbilt University Institutional Review Board
Informed Consent Document for Research

Principal Investigator: Emily Garland, PhD **Revision Date: 5/28/14**
Study Title: A Pilot Study of the Effect of Dietary Sodium Intake on Assessments of Vascular Endothelium
Institution/Hospital: Vanderbilt University

This informed consent applies to persons with postural tachycardia syndrome (POTS).

Name of participant: _____ Age: _____

The following is given to you to tell you about this research study. Please read this form with care and ask any questions you may have about this study. Your questions will be answered. Also, you will be given a copy of this consent form.

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1. What is the purpose of this study?

You are being asked to take part in this research study because you have postural tachycardia syndrome (POTS) and are taking part in another study (Dietary Salt in Postural Tachycardia Syndrome). We want to measure chemicals in your blood while you are on a very high salt or a very low salt diet. About 44 people will take part in this study.

2. What will happen and how long will you be in the study?

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None.

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Date	Signature of patient/volunteer

Consent obtained by:

Date	Signature
	Printed Name and Title