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Dietary Sodium's Effect on Urinary Sodium and Dopamine Excretion in Patients with Postural Tachycardia Syndrome

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1.0 Background

Assumption of upright posture leads to major hemodynamic changes, including a decrease in cardiac output and stroke volume and an increase in heart rate. There is substantial pooling of blood in the lower extremities, the buttocks, and in the splanchnic vascular beds that is felt to be responsible for much of the heart rate increase that occurs with standing. Blood pooling lessens the absolute blood volume in the heart and central circulation, leading to activation of low and high-pressure baroreceptors, causing activation of the sympathetic nervous system and withdrawal of the parasympathetic nervous system. In conditions of low baseline blood volume, cardiovascular responses to standing are more dramatic.

Angiotensin II (ANG II) is synthesized from angiotensinogen via the sequential actions of renin and angiotensin-converting enzyme (ACE). Renin catalyzes the rate-limiting step in this pathway. Renin secretion is stimulated by a fall in NaCl concentration at the macula densa, a decrease in arterial pressure, and an increase in sympathetic tone. ANG II stimulates sodium reabsorption in the proximal tubule, causes arteriolar vasoconstriction, and stimulates aldosterone production, thereby decreasing sodium excretion and maintaining or increasing plasma volume.

Dopamine (DA) also plays an important role in the regulation of plasma volume, especially at the level of the kidney. Plasma DA has a renal vasodilatory effect that is associated with increases in urine flow and excretion of sodium, phosphate, calcium and magnesium. In addition, intrarenal DA, synthesized from filtered DOPA, induces natriuresis via inhibition of tubule sodium reabsorption. DA also promotes natriuresis by inhibiting renin secretion and decreasing ANG II-stimulated sodium reabsorption. Renal DA production is stimulated by extracellular fluid volume expansion and a high sodium diet, whereas a low salt diet decreases renal DA synthesis.

Postural tachycardia syndrome (POTS) occurs in approximately 500,000 Americans, predominantly in young women. POTS is a chronic condition of consistent orthostatic tachycardia (> 30 bpm increase) and symptoms that are worse on standing but relieved by lying down. Orthostatic symptoms include palpitations, lightheadedness, chest pains, dyspnea, tremulousness, blurred vision, and mental clouding. When studied in balance on 150 mEq sodium/day, many patients with POTS have a low plasma volume. Furthermore, POTS patients have inappropriately low plasma renin activity and aldosterone for their level of hypovolemia.

Physicians often prescribe a high sodium diet for the treatment of POTS. The rationale behind this approach is that sodium in the diet will help subjects to retain fluid, and thereby raise blood volume and/or blood pressure. We will compare urinary DA and sodium responses to low and high dietary sodium intake in POTS and controls. Assessment of urinary catechols under these circumstances, well developed in our laboratory, permits a legitimate characterization of renal DA production and its metabolites since these collectively represent an index of DA synthesis in the kidney. Urine will be collected for 24hr intervals throughout the study for analysis of

creatinine, electrolytes, and catecholamines, so that we can look at differences during transitional periods as well as during sodium balance. Plasma levels of DOPA and catechols allow us to assess DOPA available to the kidney, as well as the amount of sympathetic stimulation. We will also measure plasma renin activity (PRA) and aldosterone.

We have preliminary evidence that a high salt diet raises plasma volume (as determined by changes in hematocrit) and blunts the tachycardia with standing. This treatment is also felt to decrease symptoms in some patients with POTS. We will now use the DAXOR BVA-100 Blood Volume Analyzer to test whether patients with POTS have a blunted plasma volume expansion in response to a high sodium diet and assess whether raising the amount of sodium consumed in the diet can lessen orthostatic symptoms in POTS. We will look at associations between alterations in urinary sodium and DA and changes in plasma volume.

2.0 Rationale and Specific Aims

Patients with POTS may not adequately expand their plasma volume in response to a high sodium diet. Mechanisms involved in the regulation of plasma volume, such as the renin-angiotensin-aldosterone system and renal DA, may be impaired in POTS and may respond inappropriately to changes in dietary sodium.The purpose of this study is to determine (1) whether changes in dietary sodium level appropriately influence sodium excretion in POTS; (2) whether changes in dietary sodium level appropriately influence DA excretion in POTS; (3) whether a high dietary sodium level appropriately expands plasma volume in POTS; and (4) whether patients with POTS have improvements in their orthostatic tachycardia and symptoms as a result of a high dietary sodium level.

3.0 Animal Studies and Previous Human Studies

In an unpublished study (Biaggioni et al., Vanderbilt University) comparing 150 mEq sodium versus 10 mEq sodium in the daily diet, there was a significant effect on the orthostatic change in heart rate in some patients with POTS. The orthostatic tachycardia was diminished in the moderate sodium diet (30±3 bpm) compared with the low sodium diet (53±7 bpm). In another small unpublished study, we fed POTS patients diets consisting of 10 mEq/day sodium (low), 150 mEq/day sodium (normal) and 300 mEq/day sodium (high) for 5 days each. Our preliminary findings in 5 patients indicated greater orthostatic tachycardia in the low salt diet (43±12 bpm) than with either a normal (29±9 bpm) or high salt diet (29±10 bpm).

4.0 Inclusion/Exclusion Criteria

Inclusion:

- Premenopausal patients with POTS and healthy volunteers, 18-50 years old, who are non-smokers and free of medications with the potential to influence blood pressure
- Patients 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

- Only female participants are eligible. Since 80-90% of POTS patients are female, and there can be differences in measures with the menstrual cycle, including a small number of males might introduce a significant amount of noise.
- Able and willing to provide informed consent

Exclusion:

- Smokers
- Overt cause for postural tachycardia, i.e., acute dehydration
- Significant cardiovascular, pulmonary, hepatic, or hematological disease by history or screening results
- Positive pregnancy test or breastfeeding
- Hypertension defined as BP>145/95 off medications when supine or needing antihypertensive medication
- Other factors which in the investigator's opinion would prevent the participant from completing the protocol, including poor compliance during previous studies or an unpredictable schedule
- Unable to give informed consent

5.0 Enrollment/Randomization

The participants with POTS will be recruited from patients referred to the Vanderbilt University Autonomic Dysfunction Center. Healthy volunteers will be recruited from a population of previous participants in autonomic studies, through the ResearchMatch.org database, and through advertising and emails around the Vanderbilt community.

When contacting appropriate study candidates, the investigator will describe the complete protocol. The participants will then be given a written informed consent form that has been approved by the Vanderbilt Institutional Review Board. The subject will be given adequate time to read the consent form, ask questions, and if satisfied by the responses, sign the form. Consent or refusal to participate in this study will not affect medical care. No modifications or waiver of the elements of consent will be necessary in the execution of this study. Consent procedures will take place in the Autonomic Dysfunction Center at Vanderbilt University Medical Center.

Randomization tables will be used to determine whether the 10 mEq sodium/day or 300 mEq sodium/day diet will be consumed first.

6.0 Study Procedures

Participants will be given a screening medical exam, including a pregnancy test, 12 lead ECG in supine and upright positions, and blood for a CBC and a Comprehensive Metabolic Panel. If the pregnancy test is positive, the subject will not continue with the study. Patients will often be identified from a prior assessment at the Vanderbilt Autonomic Dysfunction Center. Screening procedures, other than the pregnancy test, will not be repeated if results are available from within the previous 3 months. Individuals shown to be an appropriate candidate for the study will be told to abstain from medications that could affect blood pressure or blood volume for at least 3 days prior to and during admission for the study. Individuals who take such medication routinely will not be included in the control group. Patients with POTS will be advised to consult

their primary care physician before stopping any medication and to contact the Principal Investigator or an Autonomic nurse if any problems occur prior to admission. Approved participants will be studied on an experimental diet with a fixed amount of sodium consumed per day (confirmed by analysis of 24hr urine sample) and will consume no substances that interfere with catecholamine synthesis, release or assay.

- Pre-study assessments include: H&P, CBC, Comprehensive Metabolic Panel, pregnancy test, 12 lead ECG in supine and upright positions
- 3-day drug withdrawal of any medication with the potential to influence blood pressure
- Day 1: start 150 mEq sodium diets. Diets will contain 60-80 mEq potassium, be free of methylxanthines and low in monoamines and provided by the kitchen of the Clinical Research Center (CRC). The study participants will need to drink 1.5-2 liters of water per day to ensure they are well hydrated. Participants will document that they consumed all food provided by the kitchen and no other food.
    - Start 24 hr urine collection for sodium, potassium, creatinine, and catechols.
    - POTS patients will generally be admitted to the CRC on Day 1, whereas normal volunteers will do initial part of study as outpatients.
- Days 2-5: 10 mEq or 300 mEq sodium diet in a random order starting after 3 meals of 150 mEq Na/day diet. Must consume 1.5-2.0 liters/day of water.
    - Continue daily 24 hr urine collections for sodium, potassium, creatinine, and catechols.
- Day 5: Start 24hr Holter combined ECG monitor and BP monitor
- Day 6: continue study diet and 24hr urine collection for sodium, potassium, creatinine, and catechols;
    - Complete ambulatory BP monitoring;
    - Admit to CRC in afternoon (healthy control subjects only as POTS patients will have already been admitted). Each subject will spend the night in the CRC and remain supine with nothing by mouth overnight
- Day 7:
    - NPO after midnight
    - IV catheter inserted
    - End 24 hr urine
    - Body weight after voiding, before breakfast
    - Posture Study, during which blood pressure and heart rate will be monitored with an automated oscillometric arm cuff (Dinamap) in the supine position, immediately after standing, and at 3, 5, 10, 15, 20, and 30 minutes after standing; blood collection for catecholamines, renin and aldosterone; and symptom rating while supine and standing for up to 30 minutes. The total amount of blood collected will be 30 ml supine and 30 ml in the upright position. Symptom Severity will be evaluated with the Vanderbilt Orthostatic Symptoms Score, a published tool that is sensitive to orthostatic symptoms in patients with POTS
    - Blood collection (2 ml) while supine for BMP (for creatinine and sodium)
    - Plasma Volume Assessment using up to 25 µCi of ^{131}I-Human Serum Albumin (BVA-100 Blood Volume Analyzer, DAXOR Corp.)
        - Blood samples drawn through IV catheter before injection and for ~30 minutes post-injection
        - Total amount of blood drawn will be ~25 ml

        - Test will take ~45 minutes
        - This will be done in the middle of the posture study (after supine assessment but before standing the subject up)
    - Exercise Capacity Test
        - At least 2 hours after eating
        - Will estimate maximal oxygen consumption (VO_2 max)
        - This test will be conducted on a stationary bicycle
        - Effort will be gradually increased while expired air is measured during exhaustive physical work.
        - The test will last approximately 30 minutes and be conducted in the CRC.
        - A mouthpiece with a one way re-breathing valve attached to a breathing tube will be used to collect air samples during the exercise test. Essentially, subjects will breathe room air through a mouthpiece, and then exhale the air into a tube that connects to a machine (metabolic cart). This machine analyzes carbon dioxide and oxygen content, which allows us to calculate the amount of oxygen they are using under resting and exercise conditions.
        - A test will be considered valid and finished when two of the following three conditions are met:
            (1) predicted maximal heart rate is obtained,
            (2) the respiratory exchange ratio reaches 1.15 or higher,
            (3) oxygen consumption plateaus.
        - Verbal encouragement will be given to help subjects achieve a valid exercise test.
        - To assess VO_2 max directly, an individual's expired air is measured during exhaustive physical work.
        - The workload will be gradually increased on the bike by increasing the resistance. As the workload increases, oxygen consumption also increases. Throughout the test period exhaled air will be collected. When subjects can no longer continue, the test will be stopped.
        - Blood pressure will be measured at the end of each resistance-stage. Heart rate data will be recorded continuously.
        - Urine will be collected over the 2 hour period preceding the test and again over the 2 hours following the test.
        - Before and after completing the exercise test, subjects will be required to complete a "warm-up" and "cool-down" session including stretching exercises.
    - Saline Bolus (optional)
        - Subject can receive an optional 2L saline bolus IV to prepare them for discharge to home
    - Study Completed
        - Discharge Subject to home
    - Discharge after testing

The same protocol will be performed with study diets providing 10 mEq sodium/day or 300 mEq sodium/day and 60-80 mEq potassium/day. The order of the diets will be randomly assigned.

To minimize variability due to phase of the menstrual cycle, each participant will undergo testing on the 2 diets during the same phase of the menstrual cycle.

7.0 Risks

Consuming a **sodium- and caffeine-controlled diet** might not be to the subject's taste preferences. If the subject drinks caffeinated beverages regularly, their body may have become used to having caffeine. If they stop caffeine intake suddenly, they might have headaches and fatigue for a few days. They can avoid these symptoms if they cut down gradually on the amount of caffeine in their diet.

Stopping intermittent medications might worsen symptoms. POTS patients will be supervised by a nurse and physician, and will be monitored on the CRC during most of the duration of their medication withdrawal. If necessary, medications will be restarted. Healthy subjects will not be allowed to participate in this study if they routinely take medication that alters blood pressure or heart rate.

There are minor risks and discomforts associated with **blood sampling**. We will insert a plastic catheter into the vein to allow drawing blood without repeated sticks during the study. This may cause a brief period of pain and possibly a small bruise at the site. Occasionally, a person feels faint when their blood is drawn. There is a small risk of bleeding after removal of the catheter and possibly a bruise at the site, which can be prevented by tight compression on the site. Rarely, an infection develops which can be treated.

Blood pressure cuff: some may find it uncomfortable to hold their arms with an inflated cuff placed around the forearm, or finger, in a relatively fixed position, or have the cuff inflated frequently.

Electrodes: Sticky patches will be put on the chest and limbs to record electrical activity from the heart or for the body impedance measurements. This might be uncomfortable. This can occasionally cause a rash.

Rating symptoms may be inconvenient.

24-hour urine collection. Collecting urine for 24 hours might be an inconvenience. We try to make it more convenient by fitting the toilet with a collection device and/or providing a urinal for their use.

As part of this research study, participants will receive a **small amount of radioactive substance** called Iodine-131. Iodinated I-131 albumin (Volumex), injected intravenously for determination of total plasma volume, is a radiopharmaceutical and, as such, requires that care be taken to ensure minimum radiation exposure consistent with proper patient management. Each 1 ml dose of Volumex contains 25 microcuries in a pre-filled injection kit. This radioactive substance will expose participants to a small amount of radiation (x-rays). The radiation dose that they will receive from each procedure is about the same amount that they would receive over a period of four months from natural background radiation. Natural background radiation comes from naturally occurring radioactive material that is present in everyone's body, from outer space (cosmic radiation), and from naturally occurring radioactive material in soil, rocks,

and building materials. This procedure will be performed a maximum of 2 times at an interval of at least several weeks. **I-131 tagged human albumin** is a human blood product. This product has been screened and heat-treated for at least 10 hours at 60°C. Experts believe that this treatment kills the encapsulated viruses (like HIV and Hepatitis C). Recent studies have shown that the risk of getting a disease from human blood products such as this is extremely small. However, some individuals may not want to receive human blood products for religious or other ethical concerns. Prior to having this test, and for two days afterwards, the subjects will be given SSKI (iodine 5%/potassium iodide 10%, strong iodine solution) as a safety precaution.

Exercise test. The risks involved in this exercise test may include abnormal blood pressure, fainting, irregular heartbeat, and in the most rare instances, heart attack, or even death. Every effort will be made to minimize these risks by continuously monitoring participants throughout the exercise testing. Participants will be encouraged to inform the study investigator if they feel dizzy, ill-feeling, or other symptoms, during or after the test.

We cannot foresee any other risks, but there may be previously unknown or unforeseen risks. By not allowing pregnant females to participate, we will eliminate any risks these procedures might have for a pregnant woman.

8.0 Costs and Compensation to Participants

Costs to Participants

Study related materials will be provided to the participants without charge. The Inpatient stays will be covered by a grant from the Vanderbilt Institute of Clinical and Translational Research (VICTR). Assay and testing costs will be covered by an NIH grant. The participants will be responsible for their own travel expenses to come to Vanderbilt and any extraneous expenses related to their time in Nashville.

Participant Compensation

Participants will be compensated at a rate of $450 for the entire study ($175 for each phase and an extra $100 at completion). For patients with POTS, this may help to defray some of their travel expenses. For the healthy control subjects, this will compensate them for 2 stays on the CRC and 2 separate weeks of diet (which may not be pleasant). If participants are unable to finish the study or withdraw from the study, their compensation will be rated according to how much of the study they completed.

9.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

The P.I. and at least one co-investigator will review data from subjects enrolled in this study on a bi-monthly basis.

Adverse events will be monitored on an ongoing basis by Drs. Satish Raj & Emily Garland (both of whom will be responsible for tracking adverse events in this study). Any adverse event of a serious or greater nature will be reviewed immediately with the P.I.

The adverse event will be described with the following information: description of the event; outcome of the event; duration of the event; relationship to study procedure; requirement, if any, for treatment or intervention; and outcome.

Adverse events will be graded according to the following scale:
0 = No adverse event or within normal limits
1 = Mild adverse event (transient and mild in nature, and no treatment is necessary)
2 = Moderate adverse event (some intervention and treatment are necessary, but participant completely recovers
3 = Severe adverse event (an event that results in hospitalization, disability, or death or is life-threatening)

The investigator will state his opinion on whether there is a reasonable possibility that the event or experience is elated to a procedure performed as part of this study. Serious adverse events will be reported in writing to the Vanderbilt IRB within 72 hours of occurrence. All study adverse events will be summarized once a year, as part of the annual review report to the IRB.

10.0 Study Withdrawal/Discontinuation

The investigators or Vanderbilt may stop participants from taking part in this study at any time if it is in their best interest, if they do not follow the study rules, or if the study is stopped.

Participants are free to withdraw from this study at any time. We will cease to collect study information at the time of withdrawal of consent. Withdrawal of consent or refusal to participate will not prejudice their health care.

11.0 Statistical Considerations

Statistical Analysis Plan

Primary outcomes will be 24 hr urinary DA and sodium excretion. The primary analyses will involve a comparison of values for urine excretion as a result of a high-sodium or low-sodium diet and between patient and control groups. Secondary endpoints will include the change in plasma volume and measures of orthostatic tachycardia and orthostatic symptoms (VOSS). Differences will be assessed using a Student's t-test and Mann-Whitney U test for group comparisons and a paired t test and Wilcoxon signed rank test for diet comparisons. It is possible that the speed of achievement of sodium balance, as well as cumulative sodium balance, may provide additional information about sodium handling. A mixed-effects model will be developed to describe the time, diet and group effects.

Data will be entered into a Microsoft Excel spreadsheet or a CTSA generated, HIPAA compliant Research Data Capture (REDCap) web-based database 52. SPSS for Windows (version 17.0), *Stata 11.0*, and R (www.r-project.org) will be used for data analysis. The sample size calculations were performed using the software package PS Power and Sample Size Calculations version 3.0.1 53. A co-investigator on this grant, William Dupont PhD, Professor of Biostatistics at Vanderbilt University, will be primarily responsible for the statistical analyses of this study.

Sample Size

Based on a standard deviation of 35 mEq for urinary sodium excretion in a group of 87 normal volunteers on our moderate-sodium diet, a sample size of 17 subjects in each group will have 80% power to detect a difference of 35 mEq with a 0.05 two-sided significance level. A difference of 0.56 μmoles urinary DA will be detectable based on a standard deviation of 0.56 μmoles from our laboratory. Twenty healthy volunteers and 20 POTS patients will be enrolled to allow for data loss and subject dropout.

12.0 Privacy/Confidentiality Issues

All the investigators have completed Vanderbilt training in compliance with the HIPAA regulations. Every effort will be made to protect and respect patient confidentiality and privacy within the limits of HIPAA. Research data will be entered into a password-protected database. The Principal Investigator's Assurance Statement has been submitted with the proposal.

13.0 Follow-up and Record Retention

The study will last 3-4 weeks for each participant, and it will likely take 2-2 1/2 years to enroll the required number of participants. The study results will be retained in our research records for at least six years after the study is completed. At that time any research information in the medical record will be kept indefinitely. Any research information not already in the medical record may be kept indefinitely.

Appendix A Study Procedure Calendar

Day 1	Start 150 mEq sodium with breakfast	Start 24hr urine at 0700hr (sodium, potassium, creatinine, cats)
Day 2	Start 10 mEq or 300 mEq sodium with breakfast	Continue 24hr urines (sodium, potassium, creatinine, cats) 0700-0700hr
Day 3	Continue study diet	Continue 24hr urines (sodium, potassium, creatinine, cats) 0700-0700hr
Day 4	Continue study diet	Continue 24hr urines (sodium, potassium, creatinine, cats) 0700-0700hr
Day 5	Continue study diet	24hr BP
Day 6	Continue study diet	Start 24hr urine(sodium, potassium, creatinine, cats); Admit to CRC, NPO after midnight
Day 7	Continue study diet	End 24hr urine(sodium, potassium, creatinine, cats); Study day with posture study, plasma volume measurement, symptom score, exercise test; Discharge
Repeat on alternate diet during same phase of menstrual cycle		