Visualizing Vascular Mechanisms of Salt Sensitivity

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1.0 Background

Salt sensitive blood pressure preferentially affects black Americans

Salt sensitive blood pressure (SSBP) is a physiologic phenotype defined by a parallel change in blood pressure in response to dietary salt load. SSBP is fundamentally related to the body's inability to clear salt, leading to elevated blood pressure and a life-long increased risk of cardiovascular disease. SSBP was determined to be an independent risk factor for developing cardiovascular disease in hypertensive patients almost two decades ago[1], and more recently SSBP was found to portend all-cause mortality[2]. It is a prevalent condition that affects up to 50% of normotensive, 75% of hypertensive[3], 74% of black American adults[4], and is estimated to be 6% more prevalent among females than males[5, 6]. Black Americans are preferentially affected by this phenotype, and represent one of the most at-risk population for cardiovascular diseases related to SSBP. Salt sensitivity is increased with the occurrence of metabolic syndrome associated with abdominal obesity[7] and insulin resistance[8]. Current tests for SSBP require dietary salt loading, are time-intensive and, more importantly, provide limited mechanistic information on the incompletely characterized mechanisms of tissue salt storage.

Tissue sodium homeostasis is maintained by renal and lymphatic mechanisms

While renal dysfunction is implicated in SSBP[9], emerging evidence suggests that lymphatic mechanisms additionally regulate tissue salt storage and blood pressure[10] and may contribute significantly to systemic vascular resistance[11, 12]. The lymphatic system is a central component of the body's circulatory system, functioning in the regulation of tissue sodium homeostasis, in the clearance of high-density lipoproteins[13] and metabolism of fatty acids[7]. Lymphatic clearance of tissue salt may represent an extra-renal system[14] capable of regulating blood pressure[15, 16] such that inhibition of this pathway is implicated in salt-sensitive hypertension[17, 18]. Patients with essential hypertension, who were not evaluated for salt sensitivity, display elevated tissue sodium in peripheral tissues[19]. Similar profiles of elevated tissue sodium have been reported in patients with chronic kidney disease[20]. Additionally, impaired lymphatic clearance and renal dysfunction may also contribute to obesity[21, 22], which is an established risk factor for cardiovascular disease. Our overarching hypothesis is that impaired lymphatic and renal vascular function is associated with elevated tissue sodium and fat storage in persons with SSBP.

Magnetic resonance imaging (MRI) tools for measurement of lymphatic and renal function associated with tissue sodium and fat deposition

The critical limitation to interrogating lymphatic and renal clearance of sodium in vivo rests with a lack of noninvasive imaging technologies sensitive to sodium, lymphatic, and renal physiology. To address these limitations, we have recently developed a novel, noninvasive magnetic resonance (MR) lymphangiography technique[23] and applied standardized MRI protocols for measuring tissue sodium and related fat deposition in adults with impaired lymphatic clearance. We found evidence of lymph stasis and tissue salt deposition that

correlated with local subcutaneous adipose tissue volume[24]. Here, we will use noninvasive MRI to test whether similar lymphatic pathways are impaired in persons with SSBP, leading to tissue salt and fat storage, in comparison to the involvement of renal dysfunction in SSBP tissue profiles.

In addition to MRI, several external biophysical measurement tools exist to measure limb volume and tissue water content. These instruments include a bioimpedance spectrometer (LDex U400, Impedimed Limited) for measuring extracellular water content in the limbs, and a tissue dielectric probe (MoistureMeterD Compact, Delfin Technologies, Inc.) for measuring water content of the superficial dermis and adipose layers. These instruments aid in clinical diagnosis of other lymphatic disorders (the LDex is FDA approved to diagnose unilateral lymphedema of the arms or legs), which leads us to hypothesize that they may provide information on salt handling physiology if there is a lymphatic component to the disease. However, few studies have applied these devices in concert with MRI. We will apply these devices to study body composition related to SSBP and determine their potential use in screening of the phenotype.

2.0 Rationale and Specific Aims

The following hypotheses and specific aims were designed in response to a recent call from the American Heart Association (AHA) for i) standardized biomarkers to enable the clinical diagnosis of SSBP and ii) improved understanding of human tissue salt storage mechanisms[25].

Hypothesis 1: Tissue sodium content is elevated in adults predisposed for SSBP; tissue sodium and adiposity provide biomarkers for SSBP. Aim 1: Black adults without hypertension (20 male; 20 female) with BMI range=18 to 40 kg/m^2 and age range=18-55 years will be assessed for SSBP according to recent guidelines[26], and scanned using MRI to quantify tissue sodium and fat fraction in the lower extremities. Multivariable analyses will be applied to quantify relationships between tissue sodium, fat fraction as a surrogate of BMI, age, and sex with SSBP status. Impact. Unique tissue sodium and fat profiles may provide internal, mechanistic biomarkers of SSBP that could be used as endpoints in clinical trials of SSBP.

Hypothesis 2: Tissue sodium content is elevated in tissues affected by impaired lymphatic flow in adults with SSBP. Aim 2: The same participants will be scanned using noninvasive MR lymphangiography to quantify lymphatic flow velocity in the lower extremities. Non-parametric tests will be applied to evaluate the association between lymphatic flow and tissue sodium content in adults evaluated for SSBP. Impact. Identifying the extent of lymphatic dysfunction in SSBP could motivate novel treatment strategies to improve lymphatic pumping and tissue sodium clearance.

Hypothesis 3: Elevated tissue sodium content is associated with reduced renal perfusion in adults with SSBP. Aim 3: The same participants will be imaged using noninvasive MR spin-labeling techniques to measure kidney perfusion (ml blood/100g

tissue/min). Non-parametric tests will be applied to evaluate the association between kidney perfusion and peripheral tissue sodium content in adults evaluated for SSBP. Impact. Imaging of renal cortical circulation, where sodium-filtering nephrons are located, related to tissue sodium stores in SSBP may have potential to portend salt sensitive hypertension.

The overall goal of this work is to provide noninvasive imaging tools to assess a more complete spectrum of mechanisms underlying SSBP, which will motivate the use of these tools in future clinical trials to evaluate risk of SSBP or therapies to modify tissue sodium storage.

Study procedures will evaluate healthy and obese normotensive black adults using a set of already tested non-invasive MRI techniques developed at Vanderbilt University Institute of Imaging Science (VUIIS) and biophysical instruments available at the VUMC (IRB #103172, #160199). Participants will also be assessed for SSBP using current standard practices for measuring changes in blood pressure after periods of high salt diet and low salt diet through the Clinical Research Center following IRB-approved protocols developed by collaborator Dr. Deepak Gupta (IRB #151943).

3.0 Inclusion/Exclusion Criteria

Inclusion criteria will include identification as black race, age between 18 and 55 years, and BMI between 25 and 40 kg/m^2. Additionally, subjects must be normotensive or pre-hypertensive adults who are willing to adhere to study diets and who are able to provide informed consent.

The following exclusion criteria will apply:

- Prevalent cardiovascular disease or use of medications for cardiovascular disease
- Current or prior history of hypertension or use of blood pressure lowering medications
- Current or prior history of diabetes mellitus or use of anti-diabetic medications
- Prevalent renal disease (eGFR < 60 ml/min/1.73m^2), abnormal serum sodium or potassium
- Current or prior smoker
- Current pregnancy
- Current steroid use
- Contraindications to MRI
- Persons who are deemed clinically unsuitable for an MRI by their treating physician and/or VUIIS MRI technologist
- Severe claustrophobia
- Open wounds on the top of the feet or hands or at locations of interest for measurement of bioimpedance and tissue dielectric.

Also excluded are subjects incapable of giving informed written consent:

- Subjects who are non-English speaking
- Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
- Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
- Prisoners

4.0 Enrollment/Randomization

We will enroll 20 black adults (10 female and 10 male) who meet the inclusion and exclusion criteria. To analyze study hypotheses, and for comparison to normal weight individuals, we will share data from the study cohort of Dr. Deepak Gupta (IRB #151943).

The following recruitment approaches will be used: Vanderbilt CTSA (VICTR) Research Notification Distribution List, the linked email system which reaches Vanderbilt faculty and staff, as well as members of the Middle Tennessee community, and ResearchMatch, a national online registry maintained at Vanderbilt which allows people to self-register and express interest as research participants. Additional patients may be recruited from external sites. IRB approved flyers/advertisements of the study may be distributed in the public local to Vanderbilt and surrounding areas as additional means of subject recruitment for this study. The SUBJECT LOCATOR program is part of a toolset available through VICTR that enables teams to specify inclusion/exclusion criteria for a specific study. The inclusion/exclusion criteria are codified for computable use and combined with data coming through VU Clinical Systems to proactively identify individuals who might qualify for a study. Once a 'match' is made, research study personnel are alerted using confidential messaging or a secure web portal and they may then use the information to further review the subject's information using the Vanderbilt Electronic Medical Record. If the subject is further deemed a candidate for the study, the study personnel will notify the subject's care provider who will then ask the subject if they would be interested in communicating with study personnel.

At the screening visit, history study consent will be obtained, a medical and physical examination administered, and protocol eligibility determined. If a subject meets inclusion criteria, without any exclusion criteria, the subject will be enrolled. Subjects will receive serial low- and high-salt diets in a randomized crossover design. The randomization (using permuted blocks) will occur to one of two dietary sequences: (1) high-salt, washout, then low-salt diet, or (2) low-salt, washout, then high-salt diet. Randomization assignment will occur at screening and before baseline to ensure sufficient time to prepare the subjects' provided meals.

5.0 Study Procedures

<u>Screening Visit</u>

This will occur in the Vanderbilt Clinical Research Center (CRC). Subjects will arrive in a fasting (at least 6 hours with no food or drink) state. After informed consent has been obtained, inclusion/exclusion criteria will be reviewed to confirm that the subject meets study eligibility requirements. Subject's medical history and concomitant medications will be discussed and documented. Subjects will then undergo a physical exam, including measurement of height and weight, and "vital signs" (blood pressure and heart rate). Subjects will have a 5ml (1/3 tbsp) fasting blood draw in order to assess glucose, electrolyte and fluid balance, and kidney function, and will undergo a urine pregnancy test (if the woman is of child-bearing potential). Subjects will speak with study personnel who will review physical activity level and any food restrictions or allergies. You will be asked questions about your ability to undergo an MRI scan, including questions about metal implants. Study personnel will review the low- and high-salt dietary protocols and provide the subject with study diet instructions. If needed, study personnel may then schedule an appointment for the subject to meet with a dietician for further review of dietary needs. If subject agrees to adhere to study diets, then randomization (using permuted blocks) will occur to one of two aforementioned dietary sequences: (1) high-salt, washout, then low-salt diet, or (2) low-salt, washout, then high-salt diet.

The subject will then be given instructions on 24-hour urine collections, which will be obtained at 4 study time points (baseline, end of diet 1, end of washout, and end of diet 2). A baseline study visit will then be scheduled. Additionally, all subjects will be asked to discontinue use of the following over-the-counter-medications (NSAIDS, decongestants, cold medicines) one week prior to study and to remain off these agents until the study is completed.

Baseline Visit

In the 24-hours prior to the baseline visit, the subject will collect urine for quantification of urine sodium excretion and creatinine. For the baseline visit, the subject will arrive to the CRC in a fasting (at least 6 hours with no food or drink) state, return the 24-hour urine collection, and then be asked to lay supine. A physician or study research coordinator will review the subject's medical history to assess for any changes since screening that may potentially exclude the subject from participating. After 1 hour and while the subject is still supine, the CRC nurse will record blood pressure and heart rate (average of 3 measures made 5 minutes apart) in the left arm using an automated cuff. The CRC nurse will also draw venous blood samples (25 ml = 1 3/4 tablespoons) for measurements of a basic metabolic panel and plasma renin. Blood samples (cells, plasma and serum) may also be collected for storage at -80ºC for future investigations. The subject will be given another 24-hour urine collection kit, and diet 1 materials. If the subject was randomized to begin with the low-salt diet, he or she will pick up the prepared low-salt meals from the CRC. If the subject was randomized to begin with the high-salt diet, he or she will receive high-sodium bullion packets.

MRI Exam

The subject may undergo a baseline MRI scan on the same day as the baseline visit or on a separate day, to occur either before beginning study diets, or at least 1 week after the diet 2 visit. The subject will undergo the following noninvasive magnetic resonance imaging (MRI) scan at the Vanderbilt University Institute of Imaging Science (VUIIS), following an established protocol (no longer than 1.5 hours total) to quantify baseline tissue sodium content, whole-body fat composition, lymphatic function, and kidney vascular function. No MRI methods used require the administration of any exogenous contrast agents, and therefore will be completely non-invasive. In total, noninvasive MRI protocols will be applied within a 90-minute scan session.

Tissue sodium content can be visualized and quantified non-invasively in humans with MRI.[27-31] Sodium content in leg will be imaged with a detector tuned to sodium resonance (Rapid, Germany) and a 3.0T MRI scanner (Ingenia Elition, Philips Medical Systems, Best, The Netherlands). Four tubes containing aqueous solutions with 10, 20, 30, and 40 mmol/L NaCl will serve as calibration standards by relating intensity to a concentration in a linear trend analysis. In parallel, we will quantify tissue water content using conventional MRI methods, including a fat-saturated inversion recovery sequence with spin density contrast.[27-29] The total acquisition time for this protocol is 15 minutes.

Body composition including fat/water fraction will be measured using a whole-body multi-echo Dixon MRI (AMRA® Body Composition Protocol[32], AMRA Medical AB, Linkoping, Sweden). Volunteers will be positioned supine, head-first in the MRI scanner. The total acquisition time for this protocol is 6 minutes.

A 16-channel torso coil will be placed over the lower extremities of the volunteer. Noninvasive MR lymphangiography will be performed to measure lymphatic vascular function in the legs[33]. The total acquisition time for this protocol is 11 minutes.

With the torso coil placed over the abdomen, the kidneys will be imaged with MR relaxometry[34, 35] and an arterial-spin-labeling sequence applied bilaterally over the kidneys and renal artery to measure cortical perfusion. The total acquisition time required for kidney imaging is 10 minutes.

Biophysical Exam

During the baseline visit and at the time of MRI, body composition will also be measured using bed-side, biophysical instruments: a bioimpedance spectroscopy unit (L-Dex U400) and/or a tissue dielectric probe (MoistureMeterD) in the arms and legs, and locations of interest on the body. This will require approximately 15 minutes while resting supine, and results are intended to determine whether accessible, bed-side devices can provide similar information as MRI.

Low - and High -Salt Diets

All low-salt meals will be provided to participant by the study. Subjects will pick up the prepared meals, snacks, and sodium free water at the CRC at times and intervals most convenient to the participant. The low-salt diet will include a maximum of 600mg (26 mEq) of sodium per day. Subjects are encouraged to consume all food as provided and expected to have no substitutions or additions. The calorie needs for weight maintenance will be assessed at screening. The low salt meal plan contains 2500-2800 calories. This calorie level is sufficient for the majority of the research population. Additional low-salt snacks will be added as need should the subject require additional calories. The high-salt diet supplies will consist of the subject's "usual" diet, supplemented with 2 bouillon broth packets each day, which will be provided by the study personnel. Each broth packet contains 48 mEq sodium for a total of approximately 100 mEq additional sodium intake per day. This method conveniently raises total salt intake to >200mEq/day (> 4,600 mg sodium/day) in over 95% of healthy volunteers obviating the need for additional prepared meals and visits.

Subjects, while on the low- and high-salt phases of the diet, will receive calcium supplementation. Calcium will be supplied by 2 antacid tablets taken by mouth; each will provide 750 mg of calcium.

Diet 1 visit

The same protocol as the baseline visit (described above) will be followed, with the exception that the subject will not undergo MRI. Other study procedures, including return of the 24-hour urine collection, blood pressure, and heart rate measures, obtaining venous blood draw in the supine position will be identical to the baseline visit. The biophysical exam may be performed while subjects are laying supine. After this Diet 1 visit, the subject will enter a minimum seven-day washout period. This will consist of the subject adhering to their "usual" diet. They will be given their 24-hour urine collection kit, to be completed before the "washout" visit.

Washout visit

The same protocol as the baseline visit will be followed, except that subjects will not undergo MRI. After this washout visit, the subject will crossover to the opposite salt diet from week 1. They will also be given their 24-hour urine collection kit.

Diet 2 visit

Subjects will undergo the same procedures as in the Diet 1 visit (see above), except a 24-hour urine collection kit will not be given.

Biomarkers

Blood and urine samples will be coded for subject confidentiality. Measurements of interest will be performed at Vanderbilt University. For possible future investigations, excess blood and urine samples may be frozen and stored in the Vanderbilt University Core lab for Cardiovascular Translational and Clinical Research.

6.0 Risks

Venous Blood draw: This is a routine procedure that is considered standard of care in clinical medicine. At each visit, subjects will undergo a single venous blood draw. All blood draws will be performed by trained personnel using universal precautions to protect both the subject and personnel. The risks to subjects are minimal, but may include pain, allergic reaction, infection or bleeding at the needle stick site. These usually resolve without any specific medical therapy over the course of minutes to days.

MRI: Magnetic Resonance Imaging is routinely performed in clinical medicine. All imaging scans performed in this study are non-invasive (i.e., they do not require any injection of exogenous contrast agents or ionizing radiation) and therefore risks to subjects are low. Power monitoring and gradient switching speeds at 3.0T are applied according to the general FDA guidelines for MRI and controlled by the scanner company software. The effects of magnetic fields in an MRI scanner have been extensively studied and there are no known significant risks with an MRI exam. Subjects may, however, be bothered by feelings of confinement or claustrophobia, and by the noise made by the magnet during the procedure. Subjects will be asked to wear earplugs or earphones while in the magnet. Subjects may not participate in this study if they have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices for which MRI is contraindicated. Questioning prior to enrollment will determine if subjects have had brain surgery for a cerebral aneurysm, or have implanted medical or metallic devices, shrapnel, or other metal, such as metal in the eye. If subjects move rapidly within or around a magnet with a field of 3.0T they may feel dizzy. We therefore advise them to move slowly and, once they are positioned on the table, we will move the person into the magnet slowly. Emergency personnel and equipment will be on hand during the MRI for safety. If at any time subjects feel excessive feelings of confinement or claustrophobia, the scan may be terminated by the subject. The total time on which the participant is in the MRI scanner is estimated to be no longer than 90 minutes.

Biophysical Exam: The external measurements taken during the visit are applied using standard procedures and are fast to administer using non-invasive tools that do not bring on any discomfort to subjects, are FDA-approved or determined safe for human use, and are taken within a couple of minutes while subjects are lying down. Subjects who have open

wounds at the site of measurement, are pregnant at the time of exam, or have an implanted device with a battery, such as a pacemaker, will not be allowed to receive the biophysical exam.

Tolerability and safety of salt diets: While there is theoretic risk of eliciting hypertensive or hypotensive responses with high- and low-salt diets, the research team has decades of experience giving low- and high-salt diets and have demonstrated the salt diets are well tolerated and safe. Indeed, the salt content of the "high-salt" diet is 25-55% higher than the "average' salt diet for people living in middle Tennessee. The low-salt diet is also well tolerated, with low rates (<10%) of withdrawal, which we have accounted for in our sample size and power calculations. We have not observed hypovolemia or hypotension on the low-salt diet, even in lean healthy individuals. All subjects will be monitored for adverse effects and during the study visits a physician will be available to monitor the safety of the subject through the protocol, and between study visits, a physician will be available 24 hours a day by phone and/or pager to subjects.

Private health information: This information will be collected during the course of the study. However, only key study personnel will have access to this information, which will be stored in a HIPAA compliant, password protected REDCap database. No protected health information will be shared with employers, insurers, or non-research personnel.

7.0 Reporting of Adverse Events or Unanticipated Problems Involving Risk to Participants or Others

Adverse events (AEs) will be reported according to IRB policies and procedures. Reporting will depend on adverse event severity:

Grading of Severity

0. No AE or within normal limits.
1. Mild AE.
2. Moderate AE.
3. Severe AE resulting in inpatient hospitalization, or a persistent or significant disability/incapacity.
4. Life-threatening or disabling AE.
5. Fatal AE.

AE's of grade three or higher will be reported immediately to the IRB using the Report of Adverse Events per IRB policies and procedures. Every six months the PI will summarize any AE's to the IRB. In conjunction with the IRB, the PI will determine if modifications to the protocol are warranted.

Serious and unexpected adverse events will be reported to our IRB within ten working days. All our adverse events will be reported as required at the time of continuing review. Protocol deviations and violations will be reported to our IRB within ten working days.

8.0 Study Withdrawal/Discontinuation

Subjects may withdraw from the study at any time and should notify study personnel if they wish to withdraw from the study. Subjects may request their biological samples to be destroyed at any time. However, any data or biological samples that have already been used for research cannot be destroyed. Subjects may be discontinued from the study at the discretion of the investigators' (possible reasons listed below). Subjects will receive financial compensation for the visits that they complete.

Possible reasons for withdrawal/discontinuation from study include, but are not limited to:

- Noncompliance with treatment or procedures
- Decision by participant/participant withdraws consent
- Lost to follow-up
- Starting a medication that would exclude the subject (see exclusion criteria)
- Development of a significant medical condition specified in the exclusion criteria
- In female subjects, becoming pregnant during study
- Significant adverse event deemed by investigator to preclude continued participation

9.0 Statistical Considerations

Subjects who are overweight or obese (n=20) will be recruited through this IRB. To analyze study hypotheses, and for comparison to normal weight individuals, we will share data from the study cohort of Dr. Deepak Gupta (IRB #151943). This will include identical data acquired from at least n=20 subjects who are normal weight (18 to <25 kg/m^2) and who meet identical inclusion/exclusion criteria.

Statistical analysis procedures for Aim 1: Statistical analyses will be performed by key study personnel and members of the Center for Quantitative Sciences at the VUMC. The primary statistical objective of Aim 1 is to test the hypothesis that adults who are salt sensitive have higher baseline tissue sodium relative to adults who are not salt sensitive. To test this, we will divide participants into those who are salt sensitive (SS, ≥5 mmHg change in mean arterial pressure ∆MAP following high salt diet compared to low salt diet) or not salt sensitive (NSS, <5 mmHg ∆MAP), according to a review of methods and recommendations from Kurtz et al.[26]. The metric ∆MAP represents a continuous variable of SSBP. A Mann Whitney U test will be applied to determine whether tissue sodium content is significantly different in SS and NSS groups. Based on a prior study of tissue sodium storage in adults who are hypertensive (n=10)

or normotensive (n=12)[19] we anticipate a similar difference in tissue sodium of 6±4 (mean ± standard deviation, mmol/L) between SS and NSS adults. Our study is designed to provide at least 80% power to detect differences in tissue sodium with a two-sided significance of $p<0.05$.

Our secondary statistical objective of Aim 1 is to test the hypothesis that tissue sodium content and potential risk factors for SSBP, including biological sex, age, and tissue fat/water fraction as a sensitive surrogate of BMI, are related to ΔMAP as a continuous variable. We will apply multivariable regression analysis using tissue sodium, fat/water fraction, biological sex, and age as independent variables, and ΔMAP as the dependent variable among all participants. With a sample size of 40 , we should be able to evaluate the four predictors of ΔMAP (each with one degree of freedom) without over-fitting the model.

Statistical analysis procedures for Aim 2: The primary statistical objective of Aim 2 is to test the hypothesis that tissue sodium content has an inverse relationship with lymphatic flow velocity, which depends on a person's salt sensitivity. To test this hypothesis, we will apply multivariable regression analysis using tissue sodium as the dependent variable, and lymphatic flow velocity, ΔMAP, biological sex, and age as independent variables among all participants.

Statistical analysis procedures for Aim 3: The primary statistical objective of Aim 3 is to test the hypothesis that elevated tissue sodium content is associated with reduced renal perfusion, which depends on a person's salt sensitivity. We will apply similar statistical analyses as Aim 2 to evaluate the relationships between renal cortical perfusion and peripheral tissue sodium content in adults evaluated for salt sensitivity.

10.0 Privacy/Confidentiality Issues

Strict confidentiality will be maintained to the fullest extent by the research team, including keeping all data in a secure, locked cabinet with limited access and/or password protected databases, i.e. REDCap. All specimens will be coded after they are obtained and the code key kept in a locked cabinet. Blood samples will be coded anonymously to remain confidential and identifiers will be kept in a separate, secure, locked location. Samples may be shared with third parties outside of Vanderbilt for future testing but will remain anonymous to the recipient. If samples are accidentally lost, subjects will not be re-contacted to provide a substitute sample. The investigators will not be obligated to keep contact information to re-contact the subject. Subjects may contact the principal investigator at any time to request that samples be destroyed.

The data files generated by the MRI scanner and L-Dex software are coded using a project name and unique number generated sequentially. Only the investigators will have the key to the subject codes. Image analysis will use the coded files. The safety surveys described above include questions about sensitive health issues. This information will remain with the signed

consent form in a secured area near the scanner. These records will remain in the investigators' possession for ten years following the termination of the study and will then be destroyed.

11.0 Abbreviations

^{23}NA: sodium
AHA: American Heart Association
BMI: body-mass index
MAP, ∆MAP: mean arterial pressure, or change in mean arterial pressure following high salt diet compared to low salt diet
CRC: Clinical Research Center
eGFR: estimated Glomerular Filtration Rate
FDA: food and drug administration
MRI: magnetic resonance imaging
RAAS: renin-angiotensin-aldosterone system
REDCap: Research Electronic Data Capture
SSBP: salt sensitive blood pressure
VICTR: Vanderbilt Institute for Clinical and Translational Research
VUIIS: Vanderbilt University Institute of Imaging Science

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13.0 Appendix :

Protocol Overview and Schedule of Events

Procedures and data acquired	Screening Visit	Baseline Visit	Diet 1 Visit	Washout Visit	Diet 2 Visit	MRI visit
Participant information and informed consent obtained	X					
Inclusion/exclusion criteria met	X					
Medical history	X					
Urine pregnancy test (females)	X					
Physical examination	X					
Current medications	X	X	X	X	X	
Vital signs, height, and weight	X	X	X	X	X	X
Heart rate and blood pressure (3 measurements, 5 minutes apart)		X	X	X	X	X
Biophysical examination		X	X	X	X	X
Dietary protocol followed (for 7 days prior to visit)			X		X	
Clinical laboratory sample[a] collected	X[b]	X[b]	X[b]	X[b]	X[b]	
24-hour urine collected		X[c]	X[c]	X[c]	X[c]	
MRI scan						X[d]
Adverse events assessed	X	X	X	X	X	
Compensation offered		X	X	X	X	

a. Blood samples (cells, plasma and serum) will be collected for storage at -80ºC for possible future investigations

b. Blood collection on study visit days will be fasting venous draw

c. Subjects will bring their collection jug to the clinic where it will be processed by study staff for analysis. (Subjects will be provided with urine collection and storage containers and instructed to start their urine collection on the day prior to their clinic visit, 24 hours before they arrive to the clinic).

d. The baseline MRI scan may occur on the same day as the baseline visit or on a separate day, to occur either before beginning study diets, or at least 1 week after the diet 2 visit. Subjects will be compensated for the MRI visit separately.