Dietary Salt in Rheumatoid Arthritis
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INFLAMMATORY AND VASCULAR RESPONSE TO DIETARY SALT IN RHEUMATOID ARTHRITIS

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1.0 Background and Rationale

Rheumatoid arthritis (RA) is a common, chronic autoimmune inflammatory disease that affects approximately 1% of the population. Cardiovascular disease, primarily due to premature atherosclerosis, is a major cause of mortality in patients with RA; we and others have found that the prevalence of coronary atherosclerosis is increased in RA.[1] Traditional cardiovascular risk factors such as LDL and HDL cholesterol do not differ markedly in patients with RA and controls; however, for reasons that are not clear, hypertension is more frequent in RA. Hypertension is one of the most important modifiable cardiovascular risk factors. We found that the prevalence of hypertension was 53% in RA and 38% in controls (P=0.03) in a study of 167 patients with RA and a group of 91 controls frequency-matched for age, race and sex. [2] In another very large study involving 28,208 patients with RA and 112,832 control subjects the prevalence of hypertension was 31% in patients with RA compared to 23.4% in controls.[3] The high prevalence of hypertension in patients with RA suggests that factors related to RA or its treatment may play a role in the pathogenesis of hypertension.

The cause of increased hypertension in RA is not known. Factors most commonly proposed as risk factors for hypertension in RA include medications, inflammation, oxidative stress and insulin resistance. However we have found that medications such as non-steroidal anti-inflammatory drugs (NSAIDS), [4] and markers of inflammation and oxidative stress were not associated with hypertension in RA.[5] Dietary sodium intake is a major modifiable determinant of blood pressure and more than 90% of adults in the US consumes more than the upper limit of tolerable sodium intake defined by the Institute of Medicine. [6]

High salt intake adversely affects blood pressure, endothelial function, insulin resistance, and inflammation. The negative impact of high dietary salt intake on blood pressure and thus cardiovascular outcomes such as stroke, coronary artery disease, and heart failure is well documented (http://www.cdc.gov/salt/). In addition to increased blood pressure, high salt intake or the predisposition to increase blood pressure with salt (salt sensitivity) is associated with endothelial dysfunction[7, 8] , insulin resistance[9, 10], vascular stiffness[11], and increased levels of markers of inflammation[12] - all present in RA. Restriction of dietary sodium clearly improves blood pressure and improves endothelial dysfunction in the general population [8, 13, 14], with less clear effects on insulin resistance and markers of inflammation. Despite the increased prevalence of hypertension in RA, the effect of reducing salt intake on blood pressure and other cardiovascular risk markers in RA is not known.

In addition, high salt intake may adversely affect autoimmune inflammatory disease and vascular health. Recently, new findings implicate high sodium intake as being pro-inflammatory and important in the pathogenesis of autoimmune inflammatory disease[15]. High salt intake induced the formation of TH-17 cells (that produce interleukin (IL)-17) and exacerbated inflammation in experimental autoimmune encephalitis - a well-established animal model of autoimmunity[15]. TH-17 cells and their product, IL-17, are critical to the pathogenesis of RA[16] and the maintenance of inflammation in patients who respond incompletely to disease modifying therapy[17]. Moreover, TH-17 cells and IL-17 promote hypertension and vascular dysfunction[18]. Thus, decreasing salt intake in RA could decrease not only cardiovascular risk markers but also IL-17- mediated inflammation. However, the effect of modifying salt intake on inflammation and vascular responses in RA is not known.

Recently we have learnt that the human body stores substantial amounts of Na^+ in the skin and these can be measured in vivo. This discovery was made in astronauts who were confined and closely monitored, thus allowing exact measurement of Na^+ intake and output over months. The major finding was that over time large amounts of Na^+ were retained in the body without changes in water balance. This suggested that the "missing" Na^+ was stored. Subsequent studies in animals, and then in humans, showed that the "missing" Na^+ was stored in tissues, predominantly skin and muscles, and could be measured accurately and non-invasively using ^{23}Na MRI.[20] The relationship between tissue stores of Na^+ and inflammation and blood pressure under conditions of varying Na^+ intake is not known. RA can be considered a model disease in which to define these effects.

2.0 Specific Aim

Accordingly, we propose one Specific Aim: to examine the hypothesis that compared to a high-salt diet, a low-salt diet will reduce blood pressure, vascular stiffness, endothelial dysfunction, insulin resistance, inflammation and tissue Na^+ levels in patients with RA.

If a relatively simple dietary modification has a clinically important effect on inflammation and blood pressure regulation in vivo in patients with RA, this will have far-reaching implications for the treatment of RA and prevention of CV disease in this population.

3.0 Hypothesis

In this study we propose to address the following hypotheses: 1) Reduction in dietary sodium will decrease inflammation in patients with RA. 2) Reduction in dietary sodium will decrease blood pressure in patients with RA. 3) Reduction in dietary sodium will decrease tissue Na^+ in patients with RA.

4.0 General Approach

Our approach will be to gather data on vascular (including blood pressure, augmentation index, vascular stiffness, endothelial function), inflammation (including DAS28, IL-17, IL-6, TNF-α), metabolic (including insulin, glucose, catecholamines), microbiome (stool and saliva), oxidative stress (F2 isoprostanes excretion), and tissue Na^+ measures after, a high-salt and low-salt intake each administered for 8 weeks, in random order, in a crossover study with a 4-week washout period between high-salt and low-salt diets in 22 patients with moderately active RA. It is not possible to blind patients to diet - thus patients and the dietician will know whether a diet is high-salt (HS) or low-salt (LS). However, we will ensure that investigators performing assessments are blind to the dietary intervention.

5.0 Methods

Subjects and recruitment: Patients with RA will be recruited from the rheumatology clinics of the Vanderbilt University Medical Center, by word of mouth from the practices of physicians of the Vanderbilt Rheumatology Practice, from the private practices of community rheumatologists, and by advertising. MyHealth@Vanderbilt and other VUMC-based advertising methods may be utilized. Inclusion criteria will be used to search eStar for

potential participants. Once identified, we will contact the patient's physician to ask for an introduction, with the patient's permission.

a. Inclusion criteria

1. Male and female patients older than 18 years who are willing to participate.
2. Satisfy the ACR criteria for the diagnosis of RA.
3. Have stable disease activity as evidenced by no clinically meaningful change in immunomodulating or corticosteroid therapy in the past 1 month.
4. Have moderate disease activity as reflected by a minimum of 3 swollen and tender joints.

b. Exclusion criteria

1. Pregnancy
2. Receiving dialysis
3. Organ or bone marrow transplant
4. Taking diuretics, uncontrolled hypertension (>160/100 mmHg), or cardiac failure requiring treatment.
5. Severe edema (as judged by the investigator)
6. Diabetes mellitus treated with an insulin pump
7. Major surgery within the previous 3 months
8. Severe co-morbid conditions such as active cancer likely to compromise study participation
9. Unwillingness, or other inability, to cooperate
10. Contraindication to MRI
11. Presence of a condition that could make 24-hour blood pressure monitoring difficult: atrial fibrillation, inability to operate machine, receiving anticoagulants, presence of a condition that in the opinion of the investigator may be exacerbated by blood pressure cuff inflation (e.g., lymphedema).

c. Screening, randomization, and blinding

Patients will be screened for eligibility and those who wish to participate and provide informed consent will enter the study.

d. Design

This will be a random-order, 2-period crossover study with washout.

e. Concomitant therapy

Patients will continue to receive their regular therapy for RA. If a patient's disease flares and requires a significant change in therapy, the subject will be withdrawn from the study, and we will have the patient return for an exit visit.

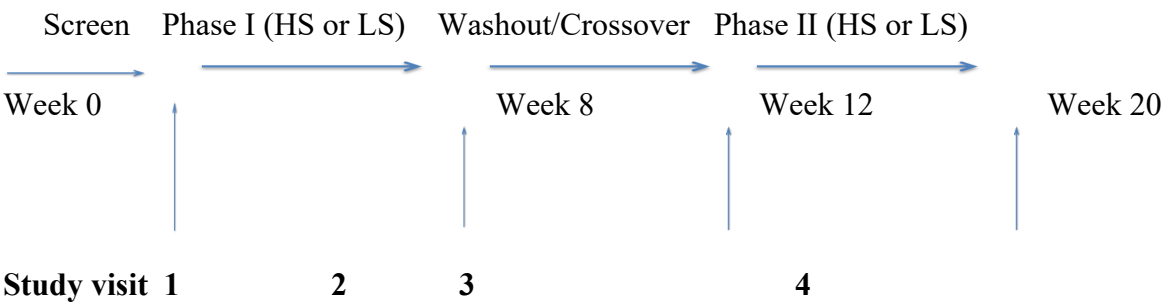
f. Dietary regimen

The order of high-sodium (HS) or low-sodium (LS) diets will be randomized. Patients will be randomly assigned to be on the high-sodium diet (200mmol/24hours x 8weeks) or low-sodium diet (50mmol/24hours x 8 weeks) with crossover separated by 4-week washout period. We will allow a 7-day window on the diet (i.e., to facilitate scheduling the diet can be between 7-9 weeks), and we will allow a 1-week window for the washout

(i.e., washout can be 3-5 weeks). Subjects will choose a rotation of low-sodium meals from a predetermined list from a commercial vendor (Mom's Meals) that will be used to provide 2 meals (lunch and dinner)/day that the vendor will deliver at approximately 7 day intervals. If there is disruption in shipping the meals, the dieticians will direct the participants on which foods to purchase to continue the diet without interruption. Participants will be reimbursed for needed diet purchases upon submission of receipts.

Staff of the Vanderbilt Diet, Body Composition, and Human Metabolism Core (under the supervision of Dr. Silver) determine breakfast and snacks appropriate for the HS and LS diets and provide instructions to subjects. These Na^+ intake levels are in the range of high current intake estimates (~12 g/d) and AHA recommendations (~4 g/day). HS and LS phase menus will be standardized for potassium and calorie composition and will be similar in the HS and LS phases except for the addition of table salt and salty snacks during the HS phase. Treatment order will be randomized with each diet treatment for a period of 8 weeks and a 4-week wash-out period between treatments. To facilitate diet conformity we will provide patients with salt packets and high-salt snacks or drinks, and we will also purchase and provide a supply of a low-sodium breakfast for patients (e.g., a selected breakfast cereal). These items will be curbside pickup for participants on Visit 1 and 3 from the Metabolism Core. Study personnel will communicate with patients at approximately 1-2 week intervals during the HS and LS diets to provide nutrition counseling, resolve problems, optimize diet adherence, and coordinate visits. If patients feel hungry or lose weight on the prepared meals, study dietitians will provide patients with a list of allowable supplementary dietary items that patients can prepare at home and add to the prepared meals and snacks provided. During the washout period (weeks 9-12) subjects will resume their habitual diet.

Schematic of Crossover Design



6.0 Study Procedures

a. Screening

Patients with RA who fulfill inclusion and exclusion criteria will be offered the opportunity to discuss the study with a member of the study team by telephone or video conferencing. If they agree to participate in the study, they will indicate the same by signing a copy of the informed consent document, either electronic or paper. Consented subjects will then be screened for participation in the study.

If participants meet criteria to continue during the virtual screening, they will be scheduled for an in-person screening at the VUMC Clinical Research Center. A kit will be mailed to participants before the in-person screening which will include a face mask to be worn by the participant when they come to VUMC.Patients will speak by telephone with the CORE dietitian for assessment of dietary intake,

selection of meals to be delivered, and diet instructions. Then, enrolled patients will be seen at baseline (study visit 1, week 0 - randomization) and then at visits 2 (week 8), 3 (end of washout week 12), and 4 (week 20) (See Schematic).

b. Baseline Data Collection

Screening will be divided into two parts: a virtual screening and an in-person screening. During the virtual screening, clinical details, including self-reported height, weight, joint count, current and past smoking history, menopausal status, duration of menopause, family history of cardiovascular disease, and current and past estrogen replacement history will be obtained. Cumulative medication history and cardiovascular therapies will be obtained from the patient and the medical record. Participants will complete an online health assessment questionnaire at baseline and at each visit.

Participants will be called no earlier than 72 hours in advance of their visit and no later than 12 hours prior to their visit to answer questions on COVID-19 exposure and symptoms per VUMC CRC protocol.

When participants arrive at VUMC wearing their face masks, they will have their temperature checked and will be asked about COVID-19 symptoms before they can enter the CRC.

During the in-person screening, weight, waist and hip circumference, and blood pressure will be measured. Joints will be examined for swelling and tenderness. Study staff will review directions on collection of 24-hour urine, stool collection, and blood pressure monitoring. Participants will be provided a kit containing containers for urine and stool collection and an at-home blood pressure monitor for participants to measure their blood pressure weekly for the duration of their participation in the study. An insulated bag will be provided to transport materials to VUMC at each study visit. Kit contents will be replaced at each visit. The at-home blood pressure monitor will be returned to study personnel at the end of the study to be cleaned.

During the in-person screening, patients who have not had a complete blood count (CBC), AST, ALT, alkaline phosphatase, and creatinine performed in the previous 3 months will have a blood sample drawn for these tests to exclude serious co-morbidity. Women of childbearing potential will have a urine test performed to exclude pregnancy at each visit.

c. Patients will be asked to weigh themselves once a week and the same time of day and to report that weight to the study staff. Weight will be measured at each visit.**Procedures for Visits (the following procedures will be performed at visits noted)**

1. Disease Activity (all visits)

At each visit we will assess a RA disease activity (DAS 28 score) by joint examination and questionnaire as previously described.[19]

2. Blood and urine collection (all visits)

Sample collection: The day before each visit patients will collect a 24-hour urine specimen. At each visit we will also obtain a urine sample and draw venous blood for laboratory testing. An aliquot of the 24-hour urine sample will be frozen and stored for later assays that will include electrolyte and creatinine levels to assess compliance with the diet. An aliquot of the urine sample collected at the

study visit will be frozen and stored for later assays that will include F2-isoprostane measurements. Venous blood will be drawn for measurement of hsCRP, ESR, glucose, insulin, and inflammatory mediators including IL-17, IL-6, and TNF-α as we have previously described[16,17,18].

Laboratory measurements of mediators: Validated assays are available for each of the inflammatory mediators described. Samples are drawn at the same time of day to minimize circadian variation. We immediately separate, aliquot, and store both plasma and serum at -70ºC. If possible, samples are only thawed once. DNA will be extracted and stored for all patients for potential future studies not proposed here. Serum, urine, plasma, stool, saliva, and blood cells will be stored for analysis of mediators that may affect risk of RA and its complications and response to therapy or the effects of salt.

3. Ambulatory blood pressure (all visits)

To obtain data regarding blood pressure response to varying sodium intake in RA patients, ambulatory blood pressure will be monitored for 24 hours before each study visit. Patients will be taught to fit the device at home at Visit 1, if they have not used the machine previously. If the patient is unable to fit the device then the device will be fitted at the end of the study visit. Patients will also be given an event/activity diary to complete. The 24-hour ambulatory blood pressure results will be used to determine day and nighttime blood pressure response and nocturnal dipping. If the patient is unable to return the blood pressure machine and diary, we will provide a prepaid package to have the device and diary sent back to us.

4. Endothelial function (PAT) and vascular stiffness (AiX and PWV) (all visits)

High-salt intake adversely affects endothelial function and leads to vascular stiffness [7,8,11]. To determine the effects of high-salt intake on endothelial function and vascular stiffness in RA, we will measure augmentation index (AiX) and pulse wave velocity (PWV) at each visit. Subjects will undergo non-invasive pulse-wave analysis using the commercially available SphygmoCor system (AtCor Medical, Sydney, Australia). This system uses a transfer function to calculate central arterial pressure from measurements obtained at the radial artery by a hand-held tonometer (Millar pressure tonometer, PWV Medical, Sydney, Australia). After at least 10 minutes of supine rest, peripheral blood pressure will be measured twice using an automated sphygmomanometer (Dinamap Pro 110, GE Healthcare, WI, USA) and augmentation index, aortic (carotid to femoral) pulse-wave velocity (PWV) and brachial (carotid to radial) PWV will be determined using applanation tonometry. The tonometer will be held at the point of maximal pulsation and pressed lightly against each respective artery (i.e., radial, carotid, and femoral arteries.) Measurements are recorded after at least 12 consecutive beats, and the quality of the waveforms confirmed by the quality control function provided by the software. Since augmentation index is influenced by heart rate, an index normalized for a heart rate of 75 beats per minute is used.

Endothelial function will be measured using peripheral arterial tonometry (PAT), a finger plethysmographic device allowing isolated detection of pulsatile arterial volume changes will also be used to assess endothelial function in relation to the salt diet. 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 counterpressure 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 processed and stored. The change in flow post-occlusion reflects endothelial function.

5. MRI measurement of tissue Na^+ content (visits 2 and 4)

Each subject will have two MRIs. These will be performed at the end of the low-salt and high-salt phases. We will quantify Na+ content in skin and muscle by ^{23}Na MRI using a 3.0 T Phillips Achieva scanner, equipped with a ^{23}Na coil. Scans will be performed in the Vanderbilt University Institute of Imaging Science. This will take about an hour. Urine pregnancy testing will be performed before the MRI in women of childbearing potential. The MRI will be scheduled on the same days as visits 2 and 4 if possible; however, it is possible that for logistic reasons this may not always be possible and the MRI and in that case the MRI and clinical visits will occur on different days.

6. Microbiome specimen collection (all visits)

Stool and oral microbiota samples will be obtained from participants at each study visit. Microbiota specimens will be used to examine the relationship between microbiota and disease characteristics as well as responses to salt and will be stored for future studies.

Stool collection: Stool from a single bowel movement will be collected by the subject before each study visit. Subjects will be provided with instructions and a materials kit for collecting stool prior to the study visit. Subjects will bring collected stool to the study visit. Stool will be collected within 24 hours before the study visit and will be kept at room temperature.

Oral microbiota collection: Subjects will be asked to not brush or floss teeth within 12 hours of the scheduled assessment. Subjects will be asked to spit into a sterile container. Approximately 5 ml of saliva will be collected and frozen at each study visit.

Table 1.A Procedures and Labs - Study Summary

Procedure/Lab	Screen, virtual**	Screen, in person	Visit 1 Baseline #1	Visit 2 HS/LS	Visit 3 Baseline #2	Visit 4 HS/LS
History	+		+	+	+	+
Exam		+	+	+	+	+

Joint Count	Self report	+	+	+	+	+
Disease activity			+	+	+	+
Aug Index			+	+	+	+
PWV			+	+	+	+
PAT			+	+	+	+
24-hr Blood pressure			+	+	+	+
Na+ MRI				+		+
CBC		+*				
ALT, AST, creatinine		+*				
Insulin			+	+	+	+
Glucose			+	+	+	+
hsCRP			+	+	+	+
ESR			+	+	+	+
Plasma			+	+	+	+
Serum			+	+	+	+
24-hr Urine			+	+	+	+
DNA/RNA			+			
Stool			+	+	+	+
Saliva			+	+	+	+
Pregnancy testing, urine		+	+	+	+	+

*Labs only drawn at in-person screening if not available from previous 12 weeks.
Augmentation index (Aug Index), Pulse wave velocity (PWV), Peripheral arterial tonometry (PAT), Complete blood count (CBC), Alanine transaminase (ALT), Aspartate transaminase (AST), High-sensitivity C-reactive protein (hsCRP), Erythrocyte sedimentation rate (ESR)

Table 1.B Blood volume (ml) to be collected (including total)

Tubes	Lab	Screen virtual	Screen in person	Visit 1 Baseline #1	Visit 2 Salt/Placebo	Washout/ Crossover	Visit 3 Baseline #2	Visit 4 Salt/Placebo
4ml lavender	CBC and/or ESR		*4	4	4		4	4
3.5ml lt green	ALT, AST, creatinine		*3.5					
10 ml lt green	CRP, Glucose			10	10		10	10
10ml lavender	Plasma (+ insulin)			20	20		20	20
10ml lavender	DNA/cells			10	10		10	10
10ml red	Serum			20	20		20	20
2.5 ml Paxgene	RNA			5	5		5	5
	Total Volume blood per day		*7.5	69 ml	69 ml		69 ml	69 ml
	Total Volume blood overall	283.5 ml						

7.0 Side effects

At every visit study personnel will enquire about adverse effects in a standardized manner, though there is no intervention other than diet. In the event of a positive response, the adverse effect will be assessed by the investigator and the likelihood of a causal relationship with HS/LS diets (definitely, probably, uncertain, probably not, definitely not) and regarding its severity (mild, moderate, severe). Patients who develop serious adverse events causally related to participation in the study will be discontinued from the study.

a. **Side effects of salt supplements and study procedures**: Salt is a naturally occurring compound found in most food products. No side effects have been reported from salt intake, however salt could cause nausea. The amounts being administered for the study will be within safe levels determined from previous similar studies and the general population intake. A high-salt diet may increase blood pressure in some people. We will provide patients with a home blood pressure monitor so that they can take their blood pressure at home once a week and ask them to call us call us if it is greater than 160/100 mm Hg. Venipuncture may cause bleeding or bruising. The automatic inflation of the arm cuff for ambulatory blood pressure monitoring could disturb sleep. MRI: There are no known major

risks with an MRI scan; but, it is possible that harmful effects could be found in the future. Even though the tunnel is open, it may bother some patients (claustrophobia), and they hear the noise made by the magnet during the scan. We provide earplugs to reduce the noise. Metal pieces in the body could move during the scan and damage nearby tissues or organs. Patients will not be able to have this scan if they have a non-MRI safe device implanted such as an aneurysm clip in the brain, a heart pacemaker or defibrillator or a cochlear implant. They also may not be able to have this scan if they have iron-based tattoos or pieces of metal (bullet, BB, shrapnel) close to or in an important organ (such as the eye). We will exclude pregnancy by performing a urine test prior to the MRI. Certain metal objects like watches, credit cards, hairpins, writing pens, etc. may be damaged by the machine or may be pulled away from the body during the scan. For these reasons, patients are asked to remove these objects before going into the room for the scan.

b. Reporting of adverse events and noncompliance: Serious adverse events (SAEs) will be reported to the Vanderbilt Institutional Review Board within 10 days of the PI's notification of the event.

An SAE is an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening. This definition implies that the subjects, in the view of the investigators, are at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death.
3. Requires inpatient hospitalization or prolongs existing hospitalization. (Hospitalization for surgeries planned before study entry will not be considered SAEs)
4. Results in persistent or significant disability and /or incapacity.
5. Results in a congenital anomaly or birth defect. This criterion applies if a congenital anomaly/birth defect is diagnosed in a child of a subject who participated in this study and received study drug.
6. Other important medical events. Judgment should determine whether an adverse event should be classified as serious in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or require intervention to prevent one of the outcomes listed above. Examples of such events are allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in inpatient hospitalization.

Non-serious adverse events and instances of non-compliance with the protocol will be submitted at the time of annual review. The progress of the study will be monitored in an ongoing fashion by the PI at weekly lab meetings. There are no plans for a DSMB

8.0 Adherence

Study personnel will be in contact with patients every 1-2 weeks and will assess compliance by dietary history taking.

9.0 Anticipated Results

No previous studies of this nature have been done in RA. We anticipate that low dietary sodium may reduce blood pressure, vascular stiffness, endothelial dysfunction, insulin resistance, tissue Na^+ levels, and inflammation in patients with RA.

10.0 Statistical Methods

a. Sample Size

We will plan to enroll 50 RA patients assuming that many will drop out. The sample size estimation of n=18 was based on two outcomes of major interest: the comparison of the change from baseline for 1) DAS 28 and 2) Skin Na^+ concentrations using a paired t-test comparing post-treatment values after receiving HS or LS diet. However, in the actual data analysis, we will compare the change from baseline to post-treatment value between the two treatment phases (i.e., linear mixed effect models) which will improve power, thus the sample size analyses presented is the most conservative. Sample size was estimated using PS-Power for a paired t-test with a 2-sided test at a significance level of 5%. Assuming that less than 20% of subjects will dropout or have incomplete data is a reasonable assumption based on our previous studies. If the number of dropouts exceeds our estimate we will replace them. For patients who are withdrawn or lost to follow-up without data for week 8 for either arm, an intention-to-treat approach will be applied. For inflammation (DAS28 score) a sample size of 18 patients will provide 95% power to detect a difference between HS and LS arms of 1.0 unit – a clinically meaningful reduction. For Skin Na^+ measurements the standard deviation (SD) is approximately 5.0 mmol/L and a difference between groups of this magnitude represents a clinically important difference. A sample size of 18 patients will provide ~98% power to detect a difference in skin Na^+ of 5 mmol/L, respectively. For secondary outcomes measures (e.g., inflammation (e.g., CRP, IL-6, T cell populations) and vascular function (e.g., 24 h BP) we have >95% power to detect a difference between groups equivalent to 1 SD – a difference likely to clinically relevant.

11.0 Early Withdrawals and Dropouts

Intention-to-treat analysis, which focuses on all randomized patients with at least one follow-up evaluation, will be used as this has been the standard approach used by many researchers and regulatory agencies. For either, or both HS and LS treatment phases of this crossover trial, when a patient drops out without having any follow-up data assessed, data points for the patient will be excluded from the analysis. Due to the nature of the pilot study with a relatively small population, we expect that virtually all patients enrolled in the study will return for a follow-up visit, thus the number of dropouts is likely to be far less than ~20% which we built into our samples size by consenting 22 patients with goal of having at least 18 subjects' data for both treatment phases. Dropouts will be defined as patients who are randomized but never return (i.e., there is no follow-up visits).

Possible confounding could occur when a patient is included in the analysis of phase 1 and excluded from phase II due to dropout. We will re-assess baseline balance between patients who are excluded in phase I and in phase II on demographic or clinical characteristics of patients to examine potential confounding by dropouts. For patients who are withdrawn or lost to follow-up after being randomized, the last visit after baseline screen will be used to replace missing values (last observation carried forward, LOCF method). ANCOVA with LOCF method will be used to analyze the data as a more conservative approach. Exploratory analyses will also be performed by examining the findings in the subset of subjects who are adherent to the diet (± 20% of target Na intake).

If the participant at any time requests to withdraw from the study, he or she may do so. We will continue to store and use samples and information obtained before withdrawal unless the participant notifies the principal investigator in writing with a date of notice. In the event of withdrawal from the study, no additional collection of information from the date of withdrawal will be performed. We will enquire from participants who withdraw whether they may be contacted for future studies.

12.0 Statistical Analysis

Baseline characteristics will be summarized for patients who are randomized to the two treatments (HS vs. LS) at baseline. A crossover design eliminates confounding due to uneven distribution of baseline variables because a patient serves as his/her own control, thus a complete balance in baseline data is obtained in the absence of dropouts.
The outcomes of interest are the comparison of the change in blood pressure, vascular stiffness (Aix, PWV), endothelial function (PAT), insulin resistance (HOMA), skin Na^+ concentrations, and inflammation (DAS28 score) between baseline and 1) after 8 weeks of HS diet, 2) after 8 weeks of LS diet. We will use Analysis of Covariance (ANCOVA) which is a form of multiple linear regression model including the blood pressure at the end of each treatment (HS and LS) or the last observation for patients with early withdrawal as an outcome variable, group variable (HS or LS) as a main independent variable, and baseline blood pressure as a covariate. By including the baseline value as a covariate, the regression coefficient for the group (HS or LS) becomes the between-group (HS vs LS) difference in the change in blood pressure response. This method is often superior to the method that used the change in blood pressure directly as the outcome variable (such as student's t-test for the change) because it adjusts for baseline differences as a covariate which reduces measurement errors more effectively.
A similar approach will be used for the vascular stiffness (Aix, PWV), endothelial function (PAT), insulin resistance (HOMA), and inflammation (DAS28 score) and skin Na^+ concentrations.

If unequal distribution of some of the variables is detected by the univariate analyses of baseline characteristics between groups, those variable will be included in a multiple linear-regression model in addition to a set of a prior chosen covariates based on clinical relevance such as age, sex, and race, with knowledge of not overfitting the model

13.0 Data Base Management and Quality Assurance

A data-collection form will be designed in conjunction with a biostatistician so as to minimize missing and erroneous values. Data will be entered into a REDCap database. Interactive edit checks are performed during data entry to ensure that all data values have reasonable ranges and are mutually consistent. These procedures permit the detection and correction of errors when they occur by providing prompt feedback to study staff and thus facilitate a very high level of accuracy.

Before analysis, we will assess all raw data for accuracy and completeness while remaining blinded to study-diet assignment. A unique identification case number will be used to protect the confidentiality of the study participants. REDCap is designed so that identifying data can be prevented from being downloaded into analysis file, thus analysis files will not include personal identifiers. Data is automatically backed up daily on the CRC server. The biostatistician will check for discordances in the data set and perform statistical analyses.

14.0 Protection Against Risk

Risks will be minimized by performing studies in the Vanderbilt University Institute of Imaging Science and the Vanderbilt Nutrition and Diet Assessment Core. All protocols must undergo approval by the Vanderbilt IRB. A history and physical examination will be performed to ensure that subjects fulfill the entry criteria as defined. We will monitor blood pressure during the study. There are no alternative procedures that would allow us to obtain the information outlined in this proposal. Confidentiality and ethical considerations will be addressed as follows. All identifying documents, data, and specimens collected as a result of this study will be retained by the investigator. Access to this material will be available only to the research investigator and his/her staff. If results of this study are to be published, only code numbers will be used for identification purposes. Participants will not be identified by name.

15.0 Privacy/Confidentiality Issues

Participant medical information will be stored in REDCap database. Protected information such as names, social security numbers, and medical record numbers of the study participants will be designated as non-exportable, protected fields. Access to this information will be granted only to members of the study team.

16.0 Follow-Up and Record Retention

Participant medical record information will be stored in the REDCap database for an indefinite period of time.

17.0 Reference List

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