Dietary Prevention of Heart Failure in Hypertensive Metabolic Syndrome

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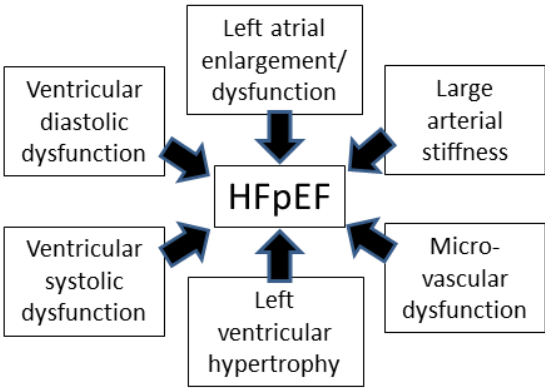
BACKGROUND AND SIGNIFICANCE

Burden of HF in Veterans: In 2014, veterans with heart failure (HF) accounted for nearly 1,200,000 VA outpatient visits and 40,000 primary-diagnosis hospitalizations, and decompensated HF remains the most common cause for hospital admission in the veteran population (Heidenreich 2016). Patients with HF suffer functional decline and poor quality of life, and half die within 5 years after diagnosis.(Owan et al. 2006). Within the VA system, mean annual costs per patient with HF were $30,719 in FY 2010 (Yoon et al. 2016). The overall societal burden of HF is increasing; by 2030, over 8 million Americans will have heart failure (HF), and will account for $70 billion/year in medical costs (Heidenreich et al. 2013). The burden and costs of incident HF will proportionally fall even more heavily upon the VA, since HF risk factors are more frequently present in veterans than in non-veterans even when matched for age (Fryar et al. 2016). These observations highlight the *crucial role of HF prevention within the overall vision of the Veterans Health Administration* to "emphasize prevention and population health."

In the more than 10,000 veterans hospitalized with new-onset HF each year, nearly 80% have systemic hypertension (HTN) (Heidenreich et al. 2010). HTN confers the highest population-attributable risk for incident HF in patients without previous myocardial infarction (Gottdiener et al. 2000), particularly when accompanied by the metabolic syndrome, a constellation of obesity, insulin resistance, and dyslipidemia (Nicklas et al. 2006). In turn, HTN and the metabolic syndrome are associated with cardiovascular abnormalities that promote HF, including left ventricular hypertrophy and diastolic dysfunction, increased large-arterial stiffness, and impaired ventricular systolic reserve (Wang et al. 2015). More than half of veterans have HTN, over 40% are obese, and at least 25% have metabolic syndrome; the prevalence of all of these factors is increasing over time (Fryar et al. 2016), and it remains unclear why only some persons in this large at-risk group develop HF. This critical knowledge gap impairs the development of targeted preventive strategies for HF in veterans.

Heart failure with preserved ejection fraction, a multifactorial entity: In older adults with HTN and metabolic syndrome, HF most commonly develops in the setting of preserved left ventricular ejection fraction (HFpEF), i.e. normal or only mildly reduced chamber contractile function (Kitzman et al. 2001). HFpEF is more common in women than men, but nonetheless already constitutes nearly one-third of HF in veterans (Ather et al. 2012). As the number of female veterans increases and the overall veteran population ages, the incidence of HFpEF will likely increase over time. In recent years, the mechanistic understanding of this condition previously termed 'diastolic' HF has evolved. While ventricular diastolic dysfunction does predict incident HFpEF and increases mortality in existing HFpEF (Persson et al. 2007, Lam et al. 2011), HFpEF is now appreciated as a failure of cardiovascular reserve in multiple domains (Borlaug et al. 2010), *Figure 1.*

Figure 1: Multiple contributors to HFpEF



For example, increased large-arterial stiffness is frequently present in and can help diagnose HFpEF (Weber et al. 2013). Large-arterial stiffness exacerbates the effects of impaired systolic and diastolic functional reserve on cardiac work efficiency and filling pressures (Kawaguchi et al. 2003), and is a key determinant of exercise intolerance in HFpEF (Kitzman et al. 2013). Despite normal ejection fraction, global longitudinal ventricular strain, a more sensitive measure of systolic function than ejection fraction, progressively decreases across age-matched controls, hypertensives with diastolic dysfunction, and HFpEF (Kraigher-Krainer et al. 2014); low longitudinal strain also predicts hospitalization and death in HFpEF (Shah et al. 2015). Left atrial enlargement and dysfunction provide important diagnostic and prognostic information for HFpEF (Obokata et al. 2013, Freed et al. 2016). Recent data demonstrate microvascular oxidative stress and inflammation as well as coronary and peripheral microvascular dysfunction in human HFpEF, which suggests a systemic syndrome

induced by multiple comorbid conditions rather than a solely cardiac insult (Paulus et al. 2013, Franssen et al. 2015, Maréchaux et al. 2016). An effective preventive strategy for HFpEF should incorporate this complexity.

Dietary sodium restriction to prevent hypertensive heart disease: one size fits all? Dietary sodium restriction to prevent HTN-associated cardiovascular disease, longstanding conventional wisdom based on its blood pressure (BP) lowering effects, has recently become highly controversial (Oparil 2014). Despite its broad-based recommendation, the potential exists for harm related to neurohormonal activation during sodium restriction. In prominent publications from large cohort studies, low dietary sodium intake has been associated with both increased and decreased long-term cardiovascular mortality (Mozaffarian et al. 2014, Mente et al. 2016). With regard to incident HF, the findings are similarly mixed, and depend on the population studied and the approach used to assess dietary sodium content (Konerman et al. 2016). The methodological challenges in linking sodium intake to cardiovascular disease in cohort studies, including systematic measurement errors in sodium intake, reverse causality, and residual confounding, are well-described (Cobb et al. 2014). The Institute of Medicine recently concluded that the "paucity of evidence… strongly points to the need for further research to better define relationships between sodium intake and [cardiovascular disease] risk... (Institute of Medicine 2013)"

Just as importantly, this epidemiological debate obscures the possibility of individual differences in response to sodium intake. Controlled studies in humans have identified a salt-sensitive blood pressure (BP) phenotype, i.e., persons in whom BP is substantially higher during high- than low-sodium intake, and conversely those with salt-resistant BP, whose BP responses to changes in sodium intake are negligible. Risk factors for the salt-sensitive phenotype include age, HTN, obesity, and the metabolic syndrome. Independent of baseline BP or previously diagnosed HTN, the salt-sensitive BP pattern triples the risk for cardiovascular morbidity and nearly doubles the long-term mortality hazard (Morimoto et al. 1997, Weinberger et al. 2001). The American Heart Association recently affirmed salt-sensitivity as an independent cardiovascular risk factor with important public health implications, and recommended further study of this phenomenon (Elijovich et al. 2016).

Salt sensitivity: a novel HF risk profile? One of the most extensively studied animal models of HFpEF is the Dahl S (salt-sensitive) rat, an inbred model with hypertensive response to high dietary sodium intake. In the Dahl S rat, BP increases after 1-2 weeks of 8% sodium consumption. By 12 weeks of age, left ventricular hypertrophy, diastolic dysfunction, and increased arterial stiffness are present. By 16-20 weeks of age, HF becomes evident with increased left ventricular end-diastolic pressure and lung weight (Klotz et al. 2006). Salt-sensitive animal models of metabolic syndrome demonstrate similar cardiovascular phenotypes (Matsui et al. 2008, Franssen et al. 2015). The mechanisms are remarkably consistent across a broad variety of salt-sensitive animal models: high sodium intake drives oxidative stress, microvascular inflammation, and cardiovascular injury; low sodium intake largely prevents such damage (Rodriguez-Iturbe et al. 2004).

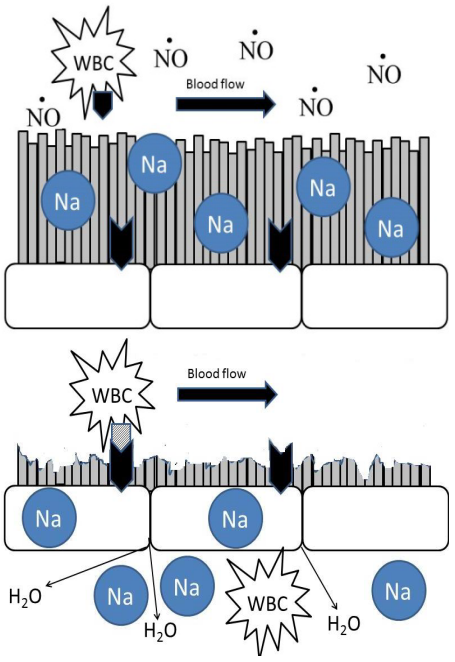
Data from human endomyocardial biopsies strongly imply that oxidative stress and microvascular inflammation promote the development of HFpEF in the setting of HTN and the metabolic syndrome (Franssen et al. 2015). In hypertensive humans, increased systemic oxidative stress is associated with reduced exercise capacity and HFpEF-related cardiovascular changes, including left ventricular hypertrophy, diastolic dysfunction, and arterial endothelial dysfunction (Giner et al. 2005, Dekleva et al. 2007). Dietary sodium restriction in salt-sensitive persons reduces systemic oxidative stress, but increases it in salt-resistant persons, despite causing similar neurohormonal activation in both groups (Laffer et al. 2006, Al-Solaiman et al. 2009). Independent of baseline BP, salt-sensitive hypertensives have higher left ventricular mass and larger left atrial size than salt-resistant hypertensives (Bihorac et al. 2000). Salt-sensitive hypertensives more commonly have peripheral microvascular dysfunction (de Jongh et al. 2007) and microalbuminuria, which worsens during high sodium intake (Bigazzi et al. 1994). Left ventricular diastolic function, large-arterial stiffness, and microvascular endothelial function are abnormal in salt-sensitive subjects and can improve with dietary sodium restriction (Musiari et al. 1999, de Jongh et al. 2007, Todd et al. 2010). These observations suggest that physiological differences in the response to dietary sodium could contribute to HFpEF risk.

Improving phenotyping for salt-sensitivity: At the present time, describing the salt-sensitive phenotype in humans requires BP measurement under conditions of high and low-sodium balance (i.e., conditions where sodium intake equals excretion) following one or more weeks of constant sodium intake (Chen et al. 2009), or

measuring the BP response to rapid dietary sodium loading then depletion with diuretics over a two-day inpatient protocol (Weinberger et al. 2001). Since the BP response to changes in sodium intake is normally distributed in the population, arbitrary thresholds for BP change are used to establish the salt-sensitive phenotype. Salt-sensitivity was originally defined as a change of ≥10% in mean BP between low- and high-sodium conditions; other studies have used thresholds of 3-10 mmHg change in mean or systolic BP. Whatever threshold is used, both sets of measurements are subject to random fluctuations in BP as well as random error in BP assessment that can reduce reproducibility. Automated 24-hour ambulatory BP monitoring can reduce this variance and is recommended by experts in the field (Elijovich et al. 2016). Nonetheless, due to the need to institute and maintain meticulously controlled diets as an outpatient or measure the inpatient BP response to physiological provocation, the salt-sensitive phenotype is challenging to define in clinical practice. As recently stated by the American Heart Association, a biomarker for salt-sensitivity is needed and "should emerge from… protocols that use arbitrary BP cutoffs to define the phenotype." Such a biomarker could then be "validated by [its] association with characteristics of the phenotype (Elijovich et al. 2016)."

An ideal test for salt-sensitivity and accompanying HFpEF risk would be directly linked to vascular dysfunction, oxidative stress, and inflammation, and could identify the at-risk phenotype independent of BP changes and without dietary modification. The endothelial glycocalyx (eGC) is a gel-like proteoglycan-glycoprotein polymer which lines the lumen of blood vessels. The role of the eGC in diverse conditions, including HF with acute pulmonary edema, has recently been reviewed (Tarbell et al. 2016), and the direct relationship between the eGC and BP has previously been demonstrated in humans (Oberleithner 2012). An intact eGC serves several critical functions, including limiting immune cell interaction with vascular adhesion molecules and transducing shear stress into nitric oxide production (Oberleithner et al. 2007, Nijst et al. 2015). The eGC buffers circulating sodium cations due to its high negative electrical charge density (Oberleithner 2014). This buffering slows the entry of sodium (and accompanying water) into the interstitium and endothelial cells, which otherwise would directly contribute to edema and vascular stiffening, respectively (Oberleithner et al. 2007). Moreover, chronic high sodium exposure can damage the eGC (Oberleithner et al. 2011). Accordingly, the eGC could modulate both the mechanisms and consequences (vascular oxidative stress and infiltration of immune cells, hypertension, arterial stiffening, interstitial sodium and fluid retention) of salt sensitivity during acute and prolonged high sodium intake (*Figure 2*).

Figure 2: Intact and damaged endothelial glycocalyx - schematic



Endothelial glycocalyx assessment: *In vivo* assessment of eGC thickness and its relationship with microvascular function may be performed with sublingual sidestream darkfield (SDF) microscopy (Goedhart et al. 2007). In this technique, a specialized camera uses 540 nm green light-emitting diodes to image red blood cells passing through the sublingual microvasculature. Images are collected with a blunt-tipped probe at 4-5 locations over a 1-2 minute period, then analyzed offline. Software identifies the distribution of viable microvascular segments and the percentage of time they are filled with red blood cells (RBC filling %). These data correspond to microvascular density and perfusion, respectively. The difference between the overall perfused vessel diameter and the median red blood cell column width, the perfused boundary region (PBR), is inversely related to the thickness of the eGC in a given vessel segment. In a large population-based cohort, Lee et al. confirmed a strong inverse relationship between RBC filling % and PBR (linear regression r^2=0.48), i.e. that microvascular perfusion was inversely related to eGC thickness (Lee et al. 2014).

While SDF microscopy enables simultaneous evaluation of microvascular function and eGC thickness, this technology is not widely available. However, red blood cells have a very similar glyocalyx layer to the eGC. Following enzymatic damage to the eGC, identical changes occur in the red blood cell glycocalyx following repeated contact with the damaged endothelium (Oberleithner 2013). This suggests that impaired red blood cell glycocalyx integrity would mirror the vascular risk posed by a damaged eGC. The erythrocyte "Salt Blood Test" (SBT) is based on the principles: 1) red blood cell sodium buffering capacity directly relates to the

thickness (and anionic charge) of the glycocalyx, and 2) red blood cell aggregation and sedimentation in the presence of dextran is affected by surface electrical charge. The SBT measures the "erythrocyte salt sensitivity" (ESS) from the sedimentation rate after mixing blood samples with a standardized sodium/dextran solution (Oberleithner et al. 2013). In other words, if the weaker negative charge of a damaged red blood cell glycocalyx is neutralized by adherent sodium cations, dextran-coated erythrocytes will no longer electrostatically repel each other and will sink faster in a capillary tube.

Sodium is only one component of an overall eating pattern: Individual micronutrients, including sodium, are consumed in combination and correlate with one another. Accordingly, research and guidelines have shifted toward dietary patterns rather than focusing solely on single dietary components. The sodium-restricted Dietary Approaches to Stop Hypertension (DASH/SRD) eating plan is recommended by current guidelines to prevent HTN-associated heart disease (Eckel et al. 2014). Compared with the typical American diet, this dietary pattern contains more low-fat dairy, whole grains, fruit, vegetables, legumes, nuts, and seeds, and lower amounts of red meat. In addition to reducing sodium, cholesterol, simple carbohydrate, and saturated fat intake, the DASH/SRD should increase intake of potassium, magnesium, calcium, phytochemicals, and other antioxidants (Karanja et al. 1999). In salt-sensitive animals, increased intake of potassium (Kido et al. 2008), magnesium, calcium (Goodwin et al. 2006), and antioxidants (Bayorh et al. 2006) attenuates cardiovascular dysfunction and the progression to HF even if high sodium intake continues.

In middle-aged and older adults, incident HF and survival after HF diagnosis are inversely proportional to DASH/SRD dietary concordance (Levitan et al. 2009, Levitan et al. 2013). In the Nurses' Health Study and the Health Professionals Follow-up Study, increased DASH/SRD adherence from baseline over a 4-year period was associated with lower incidence of coronary artery disease and stroke over the next 4 years. This study suggests that middle-aged and older adults who newly adopt the DASH/SRD can reduce the development of cardiovascular disease (Sotos-Prieto et al. 2015). Unfortunately, concordance with this eating pattern is declining over time in the U.S.(Mellen et al. 2008), and new approaches are needed.

Implementing dietary modification to reduce cardiovascular risk: Most previous studies of the DASH/SRD have only provided prepared meals, lasted only several weeks, and focused primarily on BP lowering (Sacks et al. 2001). The PREMIER and ENCORE trials examined the effects of longer-term DASH adherence (Appel et al. 2003, Blumenthal et al. 2010); both reduced BP, and some cardiovascular measures improved in ENCORE. Neither intervention promoted sodium restriction, likely to be of increased importance in veterans with HTN and metabolic syndrome (Elijovich et al. 2016). Moreover, both trials were conducted in affluent, early middle-age participants (average age ~ 50 years) and used frequent in-person dietary counseling (13-18 visits), making generalizability uncertain. As the Clinical Science R&D Service approval to submit this application states, "given that dietary interventions are difficult and resource-intensive, [our group is] encouraged to propose an innovative approach that will uniquely address the research questions… the difficulty in translating the results of such interventions into the real world should be addressed."

All participants will receive dietary education and motivational interviewing-based counseling aimed at increasing dietary concordance to the DASH/SRD eating pattern. Motivational interviewing is a non-confrontational, goal-directed, patient-centered counseling method that focuses on resolving ambivalence toward making behavioral changes. Open-ended questioning and reflective listening are used to help the participant explore their motivations and establish realistic goals specific to their personal values and individual life situation (Miller et al. 2012). Motivational interviewing-based counseling is frequently used within VA, e.g. in a nationally-disseminated and recommended training program for substance abuse clinic providers (Drapkin et al. 2016). These techniques are effective for dietary modification, as evidenced by a recent telephone-delivered motivational interviewing-based intervention that increased consumption of fruits, vegetables, and fiber while reducing fat consumption, all key DASH diet goals. The participants in the 6-month study were disadvantaged rural middle-aged and older adults at risk of or with the metabolic syndrome, making the results particularly relevant to Michigan veterans and the current proposal (Blackford et al. 2016).

Motivational interviewing promotes autonomy and willingness to change behavior, and hence complements other treatment modalities. Half of participants in the proposed study will be randomly assigned to the Women's and Men's Hypertension Experiences and Emerging Lifestyles Intervention (WHEELS-I), a program

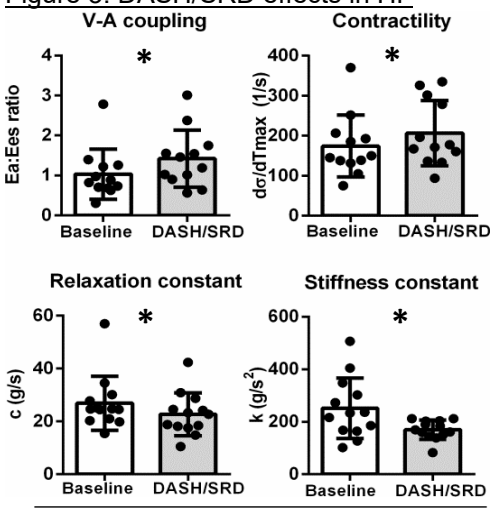
designed specifically to promote DASH/SRD diet adoption via improving cognitive representations. Cognitive representations, enduring beliefs, attitudes, and intentions that are used to structure knowledge and guide behavior, are influenced by genetic, situational, and contextual factors. Easily accessible and accurate cognitive representations enable knowledge integration and continuous learning and increase the ability of patients to cope with stressors and solve problems related to HTN management (Scisney-Matlock 1998). The overall intent of the WHEELS-I program is to use structured messaging to build, reframe, and integrate cognitive representations for the DASH/SRD dietary pattern. As originally conceived, a pen-and paper WHEELS-I intervention In hypertensive middle-aged and older women improved DASH/SRD cognitive representations (knowledge, skills and attitudes) (Scisney-Matlock 1998) and DASH/SRD dietary concordance per the Health Promotion Lifestyle Score at 60 and 90 days (Scisney-Matlock et al. 2006). Subsequently, an email-based WHEELS-I program also improved DASH/SRD adherence at 90 days, with corresponding robust reductions in BP (see *Preliminary data* below).

Overall summary: *This proposal would represent the first study to*: 1) examine cardiovascular changes during short-term consumption and longer-term adoption of DASH/SRD in veterans with HTN and metabolic syndrome, a high-risk group for incident HFpEF; 2) evaluate investigate how cardiovascular functional and structural risk factors for HFpEF in this group differ by salt-sensitivity, the most consistent physiological phenotype in animal models of hypertension-associated HF, 3) and evaluate the performance of a novel biomarker for the salt-sensitive phenotype. These goals will be met while 4) testing the efficacy of cost-sensitive strategies to promote DASH/SRD adherence in veterans with HTN and metabolic syndrome.

C. PRELIMINARY DATA

Cardiovascular effects of DASH/SRD: In our DASH-DHF pilot study, 13 older adults (age 72±10 years) with compensated HF, HTN, and metabolic syndrome consumed a provided DASH/SRD for 21 days. 24-hour BP monitoring, blood and urinary analyses, transthoracic echocardiography, and cfPWV were completed pre- and post-DASH/SRD. Echocardiographic measures of ventricular-arterial coupling (E_{es}:E_a ratio), which measures the efficiency of blood transfer from the heart to the arterial tree, were obtained. We assessed ventricular contractility via pressure-normalized wall stress, which strongly predicts mortality in HFpEF (Zhong et al. 2013). Diastolic function was evaluated with the Parametrized Diastolic Formalism. This kinematic model of ventricular filling analyzes early mitral inflow ("E-wave") patterns to quantify diastolic function via relaxation (*c*) and stiffness-based (*k*) constants. The close relationships between *k* and *c, v*entricular filling pressures, and cardiac mechanics have been invasively validated (Chung et al. 2015).

Figure 3: DASH/SRD effects in HF



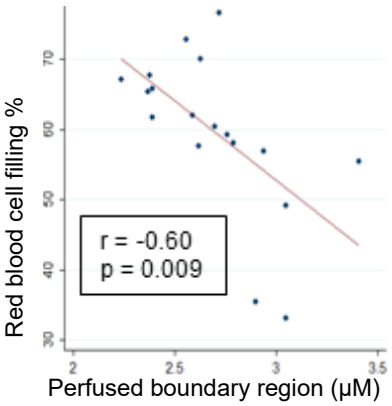
Whiskers: ± SD; *: p < 0.05

Food diaries demonstrated excellent DASH/SRD adherence, corroborated by decreased urinary sodium (3352±1593 to 1478±933 mg/24h; p<0.001) and increased urinary potassium excretion (2284±793 to 2925±1024 mg/24h; p=0.04). Despite treatment with multiple antihypertensives in nearly all subjects and overall well-controlled baseline BP, the DASH/SRD reduced 24-hour mean BP from 89±10 to 84±9 mmHg; p=0.04; cfPWV declined from 12.4±3.0 to 11.0±2.2 m/s; p=0.03. The DASH/SRD significantly improved ventricular stiffness and relaxation, while contractility and ventricular-arterial coupling ratio increased (*Figure 3)* (Hummel et al. 2013). Despite doubling urinary aldosterone excretion, the DASH/SRD reduced urinary F2-isoprostanes (a measure of systemic oxidative stress) by 31%, a similar finding to that in salt-sensitive subjects without HF (Al-Solaiman et al. 2009). This decrease in F2-isoprostanes closely paralleled (r=0.76, p=0.002) achieved reductions in 24-hour urinary sodium excretion on the study diet (Hummel et al. 2012). These data indicate that patients with HTN and HFpEF have a salt-sensitive phenotype, and that the DASH/SRD favorably affects several key predictors of functional capacity and outcomes in HFpEF. Dr. Hummel continues to collect these data (not presented here due to investigator blinding to treatment group) in an NIH/NIA funded study randomizing older patients to home-delivered DASH/SRD vs. usual care following hospital discharge from HF exacerbation.

Left atrial strain and relationship to coronary microvascular function: Dr. Hummel and colleagues are currently investigating the relationship between coronary flow reserve (CFR, via cardiac positron-emission tomography) (Murthy et al. 2014), a measure of coronary microvascular function, and echocardiographic left atrial strain, a sensitive measure of left atrial reservoir function. In 75 subjects (49% male, age 63±11 years, body mass index 31±9 mg/m^2, 83% hypertensive) with normal left ventricular ejection fraction and epicardial coronary perfusion, left atrial strain was 28±8% and CFR was 2.0±0.7 (normal values in healthy subjects 45±11% and >2.0, respectively). The CFR was significantly associated with left atrial strain (β: 3.3% per unit CFR, p=0.01) independent of age, gender, obesity, HTN, chronic kidney disease, diabetes mellitus, and coronary artery disease status, and CFR was significantly lower in patients with diastolic dysfunction.

Measurement of endothelial glycocalyx: In an ongoing study, Dr. Hummel and colleagues are using sublingual SDF microscopy and automated software (Glycocheck, Maastricht, Netherlands) to assess microvascular function and eGC thickness in patients with HF and reduced ejection fraction. In 18 subjects (6 advanced HF inpatients, 8 stable HF outpatients, and 4 healthy controls), we have observed an inverse relationship between RBC filling % and PBR (*Figure 4*), i.e. between microvascular perfusion and eGC thickness (Lee et al. 2014).

Figure 4: Microvascular perfusion vs. eGC thickness

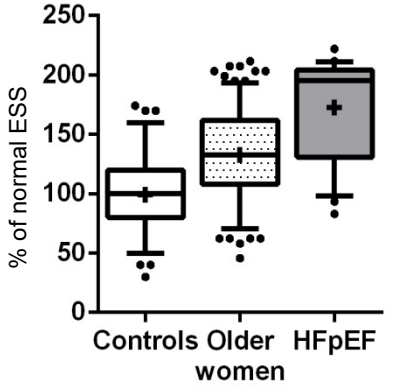


We have also examined the sodium-buffering capacity of the glycocalyx. The mini-SBT (Care Diagnostica, Voerde, Germany) is a point-of-care blood test, and is performed as follows: 1) mix 50 μL of whole blood from an EDTA-potassium (1.8 mg EDTA/ml) tube or minivette into a vial containing 50 μL standardized solution of sodium chloride and dextran (composition proprietary due to pending patent application); 2) gently shake vial to mix; 3) transfer solution to a 75 mm microhematocrit capillary tube and place upright; 4) measure supernatant length following red blood cell sedimentation for 60 minutes. The mini-SBT result is calculated as: *ESS = [supernatant length at 60 min. (mm)/24.1 mm (mean length in healthy controls)] * 100,* and is expressed as a percentage (normal ESS = 100%).The between-subject variance (n=90, ESS 100±34%) is larger than same-day within-patient variance (n=15 tests, ESS 104±6%). Increased variance on within-patient serial testing suggests that ESS is modulated by physiological state (n=109 tests over 5 months, ESS 113±16%).

We have obtained ESS results in hypertensive HFpEF patients (21 samples from 8 patients), and 187 older women without HF. These data are shown with data from 98 healthy controls in *Figure 5*. The ESS in HF was higher than in healthy controls (173±43% vs. 99±32%, p<0.001). In older women without HF, ESS values were intermediate to the other two groups (133±37%, p=0.03 vs. HF and p<0.001 vs. controls) The ESS trended higher in older women with HTN and central obesity (HTN vs. no HTN, ESS 136±38% vs. 125±37%, p=0.052, waist-hip ratio above vs. below median, ESS 144±40% vs. 126±36%, p=0.07).

In addition, the ESS value in older women correlated with C-reactive protein, a measure of inflammation (ρ 0.25, p=0.01), the adipokines leptin (ρ 0.23, p=0.003) and resistin (ρ 0.18, p=0.02), and brachial-ankle pulse wave velocity, a measure of arterial stiffness (ρ 0.16, p=0.03). The relationship between ESS and brachial-ankle pulse wave velocity was independent of other predictors of arterial stiffness, including age, black race, body mass index, and the presence or absence of metabolic syndrome (β 8.7 cm/s per 10% increase in ESS, p=0.049).Taken together, these data indicate that the ESS, a measure of impaired eGC sodium-buffering capacity, is: a) increased in HFpEF, consistent with our central hypothesis that salt-sensitivity contributes to HFpEF, b) increased but retains substantial variability in persons with expected higher prevalence of salt-sensitivity, and c) significantly associated with several parameters that predict incident HF.

Figure 5: ESS values by group

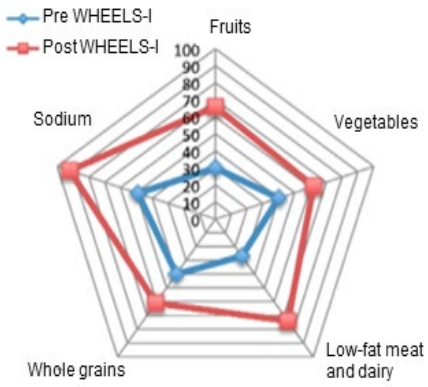


Line: median, +: mean, whiskers 5-95%

Efficacy of motivational interviewing to reduce dietary sodium intake: Dr. Hummel and colleagues recently studied the effects of dietary sodium restriction on 24-hour ambulatory blood pressure and fluid volume as measured by whole-body bioimpedance in 35 patients with chronic kidney disease (age 60±12 years, 62% male, body mass index 33±5 kg/m^2, 95% hypertensive). The 4-week dietary intervention aimed to reduce sodium intake below 85 mmol (1955 mg)/day. The protocol involved four in-person contacts with a research dietitian, who used motivational interviewing techniques to lessen the subject's resistance to change dietary behavior and empower them to make lower sodium food choices (Miller et al. 2012). The intervention significantly reduced (all $p<0.001$) mean 24-hour urinary sodium excretion (by 46%, 170±40 to 92±39 mmol/24h), 24-hour systolic BP (143±22 to 125±19 mmHg) and total extracellular volume (-1.5 liters compared to baseline). These data show that motivational interviewing can reduce short-term sodium intake, promoting beneficial cardiovascular changes in obese hypertensives with chronic kidney disease.

Efficacy of WHEELS-I on DASH/SRD adherence and BP reduction: An email-based WHEELS-I program was recently studied by Dr. Scisney-Matlock in 77 women with HTN (age 62±9 years, 77% African-American), randomized to usual care vs. WHEELS-I (Scisney-Matlock et al. 2015). The WHEELS-I group received email messages linked to web-based surveys to document momentary assessment and performance of DASH/SRD self-care activities. Morning messages (daily for 28 days) were tailored to cognitive representations and requested a brief journaling response, and evening messages (nightly for 56 days) recorded daily DASH/SRD adherence. The study outcomes were DASH/SRD adherence and systolic BP at 30 and 90 days.

Figure 6: DASH/SRD target adherence (% of participants)



Overall participant engagement with WHEELS-I was excellent, with 78% of messages accessed overall and message interaction of 56% sustained at 8 weeks. As shown in *Figure 6*, the adherence to DASH/SRD targets increased substantially in the WHEELS-I group, with the largest improvement from baseline and the best adherence in sodium intake.

Baseline BP was similar between groups. At 30 days, systolic BP decreased in the WHEELS-I group, but not controls (-9±15 mmHg, $p<0.001$ vs. 2±15 mmHg, $p>0.05$); this BP reduction persisted through 90 days (-8±17mmHg, $p=0.004$ vs. 0±13 mmHg, $p>0.05$). The WHEELS-I group participants with baseline BP >140/90 mmHg sustained a large systolic BP reduction (-13±13 mmHg at both 30 and 90 days) (Scisney-Matlock et al. 2015). This BP effect magnitude is similar to that in the original DASH-Sodium trial, where all food was provided to participants (Sacks et al. 2001). These data suggest that the electronically delivered WHEELS-I program has the potential to foster long-term DASH/SRD adoption and accompanying favorable cardiovascular changes in a multi-ethnic, middle-aged to older adult cohort.

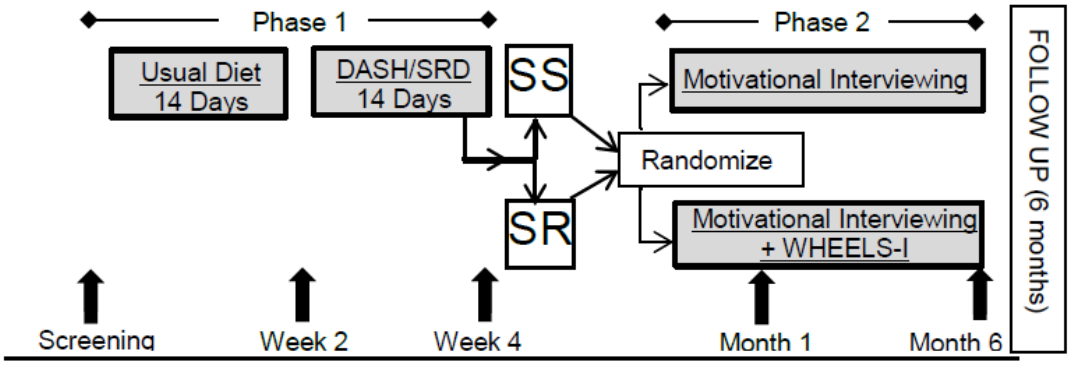
D. APPROACH

***General Design*:** This 7-month dietary intervention study will recruit from the Ann Arbor Veterans Affairs Health System and affiliated Community-Based Outpatient Clinics (CBOCs). We hypothesize that dietary modification with the DASH/SRD will improve HF-associated cardiovascular dysfunction in veterans with HTN and metabolic syndrome, and that differential improvements will occur in those with a salt-sensitive BP phenotype vs. those with salt-resistant BP. The study will take place in two phases (*Figure* 7): *Phase 1* participants will consume their usual diet, followed by a DASH/SRD for 14 days each. Salt-sensitive vs. salt-resistant status will be defined by 24-hour BP measurement at the end of each segment In *Phase 2*, all participants will receive motivational interviewing-based dietary counseling; half will participate in WHEELS-I to promote DASH/SRD adoption over 6 months.

Participants will have a clinical evaluation at 4 time points (denoted by ↑ in *Figure 7* and described below): at weeks 2 and 4 of the *Phase 1* crossover intervention, and at 1 and 6 months of the *Phase 2* DASH/SRD adoption period.

The *Phase 1* primary analysis will evaluate cardiovascular functional changes during usual diet and DASH/SRD diet periods, and test for interaction with salt-sensitivity status. *Phase 2* will assess the performance of WHEELS-I in promoting DASH/SRD adoption, and evaluate cardiac structural changes between salt-sensitive and salt-resistant participants (as they were defined by BP measurement in *Phase 1*). After completion of *Phase 2*, telephone clinical and mailed dietary questionnaire follow-up will occur after 6 months. An optional, in-person visit may also be performed at this time to obtain arterial tonometry data and clinic blood pressure.

Figure 7: Overall study timeline



Abbreviations: DASH/SRD, sodium-restricted Dietary Approaches to Stop Hypertension; SR, salt-resistant; SS, salt-sensitive; WHEELS-I, Womens' and Men's Hypertension Experiences and Emerging Lifestyles Intervention; ⬆= Clinical visit (see text)

Study Subjects: Veterans aged ≥45 years with HTN (here defined as screening systolic BP ≥ 130 and/or diastolic BP ≥ 85 mmHg, or current use of anti-hypertensive drugs) and metabolic syndrome (body mass index ≥30 kg/m^2 and/or waist circumference >94 cm). These data will be confirmed at study screening. Participants must also be willing to participate in the WHEELS-I program by using a smartphone application or email.

Exclusion criteria: On-treatment, resting systolic BP of >160 mmHg (average of 3 values) at screening visit, previous history of HF, left ventricular ejection fraction <50%, moderate or severe valvular heart disease, myocardial infarction or stroke within the prior 6 months, chronic kidney disease with estimated glomerular filtration rate <45 ml/min/ 1.73m^2, unoperated aortic aneurysm for which surgery is indicated, prior hyperkalemia requiring urgent treatment, hemoglobin <9 gm/dL, and investigator-determined factors: severe pulmonary disease (e.g. oxygen-requiring) or hepatic disease (e.g. cirrhosis), severely uncontrolled diabetes (hemoglobin A1c >10%), active cancer other than non-melanoma skin or low-risk prostate cancer, other comorbidity with expected survival <12 months, active alcohol/illicit substance abuse, and/or a history of persistent nonadherence to treatment.

Recruitment strategy: Eligible veterans with HTN and metabolic syndrome documented at their most recent clinical encounter will be identified and allocated randomly into blocks of 10. Beginning with the first block, potential subjects will be mailed a letter inviting them to participate in this study. The letter will be followed-up in one week by a telephone call from the study coordinator to discuss the study, pre-screen for eligibility, and schedule the first visit if interested in participation. In order to enroll 110 participants, we plan in-person screening visits for 130 pre-screened veterans who have expressed preliminary interest in the study, (i.e. we anticipate 20 screen-fails). Given staffing, space, and equipment availability, recruitment will take place over 3.5 years with the final in-person clinical study visits (*Figure 1*) in the second quarter of year 4.

Study procedures: will be performed according to the study procedure timeline (*Figure 8*, which includes both phases of the study). All cardiac and vascular measures will be analyzed blinded to dietary assignment, food diary results, 24-hour BP, and urinary electrolytes; 1/10 of cardiovascular studies will be re-analyzed by a second investigator to establish reproducibility and rigor of results. All study data will be recorded in REDCap, a secure research web application, under a unique study ID number assigned to participants.

- History and exam:
  - Clinic BP and heart rate will be measured three times in the seated position and once in the standing position at each visit using an automated sphygmomanometer.
  - Standard methods will be used to measure height, weight (all visits), waist and hip circumference (screening and end of study).
  - Medical diagnoses, clinical events (e.g. hospitalizations) and medications will be updated at each visit.
  - Questionnaires:
    - Center for Epidemiologic Studies Depression Scale and the 36-item Short Form survey (SF-36) will be obtained, as depression and anxiety can affect quality of life and the efficacy of lifestyle change to reduce cardiovascular risk factors
    - The SF-36 Physical Functioning Scale and Instrumental Activities of Daily Living. These are planned because functional limitations affect nutrient intake; in turn, changing dietary patterns can affect muscle mass.
    - GOURMET Barriers questionnaire, to assess psychosocial factors related to behavior change. Sample attached.
    - Physical Activity Scale for the Elderly (PASE), to assess physical activity level before and after the intervention to ensure outcomes are not attributable to changes in physical activity. Sample attached.

Figure 8: Study procedure timeline

	Screening	Phase 1		Phase 2		
		Week 2	Week 4	Month 1	Month 6	Month 6 F/U
History/exam	X	X	X	X	X	X (exam optional)
24-hour BP/Urine		X	X	X	X	
Blood draws	X	X	X	X	X	
FFQ	X				X	X
3-day food diary		X	X	X	X	
Arterial tonometry		X	X		X	X (optional)
Echocardiography		X	X		X	
SDF microscopy		X	X		X	
Questionnaires		X			X	

Abbreviations: BP, blood pressure, FFQ, Food Frequency Questionnaire, SDF, sidestream darkfield microscopy, F/U, follow up

- 24-hour BP measurement: Participants will wear an Oscar II (Suntech Medical Inc, Raleigh, North Carolina) ambulatory BP monitor on the non-dominant arm for 24 hours; BP will be recorded 2 times per hour while awake and 1 time per hour during sleep. Acceptability of recordings for analysis will be assessed according to published international norms (Kikuya et al. 2007).
- Blood tests: *Screening*: comprehensive metabolic panel, complete blood count, B-type natriuretic peptide (BNP), hemoglobin A1C, lipid panel. *All other visits*: basic metabolic panel, insulin, BNP, F2-isoprostanes, aldosterone, IL-6, hs-CRP, TNF-α. These measures were chosen to evaluate the effects of DASH/SRD on renal function, electrolytes, renin-angiotensin-aldosterone system, insulin resistance, and oxidative stress, as well as inflammation markers previously associated with incident HFpEF (Kalogeropoulos et al. 2010). A repeat lipid panel will also be obtained at the end of the study. Whole-blood samples will be used for the mini-SBT (see *Aim 3* for description). Additional samples will be stored for post-hoc biomarker analysis, planned to include syndecan-1 and heparan sulfate, serological markers of endothelial glycocalyx degradation, and the adipokines leptin, adiponectin, and resistin.
- 24-hour urine: *All collections*: sodium, potassium, creatinine, aldosterone, F2-isoprostanes, and microalbumin. These measures were selected to assess DASH/SRD adherence, the relationship between systemic oxidative stress and renin-angiotensin-aldosterone system activation in salt-sensitive and resistant subjects (Al-Solaiman et al. 2009), and chronic microvascular damage. Additional samples will be

stored for post-hoc analysis of biomarkers. The completeness of 24-hour urinary collections will be assessed via creatinine excretion per the method of Knuimann et al. (Murakami et al. 2008).

- Dietary assessment: Estimated nutrient intakes from the Block Food Frequency Questionnaire (collected at screening and at the end of study) will be analyzed (NutritionQuest, Berkeley, CA) and scored for DASH/SRD concordance (Mellen et al. 2008). Three-day food diaries will be performed during weeks 2 and 4 of *Phase 1* (i.e., the final week of usual diet and home-delivered DASH/SRD) and at Month 1 and Month 6 of *Phase 2*; these will be initially reviewed for completeness by the study dietitian and analyzed with Nutrition Data System for Research (2015 version; Nutrition Coordinating Center, Minneapolis, MN) through the Michigan Nutrition Obesity Center (MNORC);
- Arterial tonometry: Carotid-femoral pulse wave velocity (cfPWV) will be obtained with a Sphygmocor applanation tonometer (AtCor Medical, Itasca, IL). Pulse waveforms are obtained with gentle tonometer pressure above the carotid and femoral arteries, with cfPWV calculated using the distance between the two sites and the wave transit times, measured using the R wave from simultaneous ECG recording. Radial artery pulse waveforms will also be obtained to measure other parameters of potential interest, e.g. central pulse pressure, aortic augmentation index, and aortic characteristic impedance (Townsend et al. 2015).
- Two- and three-dimensional (3D) echocardiography: B-mode 2D, M-mode, and 3D echocardiographic images will be obtained with the Philips EPIQ 7 ultrasound platform (Philips Electronics, Andover, MA) and recorded via study ID number on DVD and placed under study ID number in the Ann Arbor VA echocardiogram database. Standard cardiac structural, valvular, and functional parameters, including standard measures of diastolic function (e.g., E/A and E/e' ratios), will be measured as per current guidelines. Speckle-tracking of 2- and 4-chamber apical views will be used to obtain global longitudinal strain of the left ventricle using the Philips QLAB program. Parametrized Diastolic Formalism analyses will be performed as described (Chung et al. 2015). 3D images will be used to measure left atrial volume, left ventricular mass, and ejection fraction (Gottdiener et al. 2004, Nagueh et al. 2009, Lang et al. 2015).
- Sublingual sidestream darkfield (SDF) microscopy: Images of the sublingual microcirculation will be obtained and analyzed using an integrated platform that includes a SDF microscope probe and automated software (Glycocheck, Maastricht, Netherlands) (Lee et al. 2014). See *Aim 3* for additional description.
- Safety Monitoring: Patients will perform home BP monitoring with their own personal cuff or a provided Omron 7 series automated cuff (Omron Healthcare, Lake Forest, IL). Monitoring will be daily during the run-in period and *Phase 1* of the study, and at least weekly thereafter – if systolic BP is >180 or <100 mmHg on more than one occasion, or if symptoms suggest hyper- or hypovolemia (e.g., worsening/new edema, dizziness), the participant will be scheduled for an in-person safety visit. Targeted history and physical will be performed by a clinician-investigator, with laboratory or other testing obtained based on the clinical presentation. Antihypertensive medications may be adjusted at this visit if needed for participant safety. Safety visits may also be scheduled for worsening glycemic control in diabetic participants. See *Human Subjects Protection* section for additional details.

Study phase 1: Phenotype veterans with HTN and metabolic syndrome as salt-sensitive or salt-resistant; determine effects of a 2-week DASH/SRD intervention vs. control diet on HFpEF functional cardiovascular risk factors in salt sensitive compared to salt-resistant veterans.

Introduction: Animal models, cohort studies, and our preliminary work suggest that the DASH/SRD would favorably affect cardiovascular parameters that predict incident HFpEF in veterans with HTN and metabolic syndrome, and have a stronger effect in salt-sensitive individuals. *Our working hypothesis* is that the DASH/SRD will produce significantly greater improvement than control diet in HFpEF risk factors including large-arterial stiffness (carotid-femoral pulse wave velocity [cfPWV], primary outcome), left ventricular diastolic function, and left ventricular systolic function. *We further hypothesize* that the improvement in these measures will be greater in salt-sensitive veterans. We will test our hypotheses in a 2-sequence, 2-period, 2-treatment comparison of DASH/SRD vs. control diet.

Phase 1 study design: Consented participants will have clinic BP measured at the screening visit and will enter a two-week usual diet period, during which antihypertensive medications, with the exception of calcium channel blockers, will be weaned off under Dr. Hummel's supervision and with guidance from daily home BP monitoring. During this time period, the participant will consume their usual diet. Potential subjects with on-treatment, resting, average systolic BP of > 160 mmHg at the screening visit will be excluded from further

participation, as will those with symptomatic hypertension or systolic BP of > 180 mmHg on more than one occasion during the ad-lib dietary period.

Following the usual diet period and the week 2 visit, participants will consume the DASH/SRD diet for 14 days each. This time period is sufficient to demonstrate changes in BP and arterial stiffness, which peak at 2 weeks after dietary sodium modification (Gates et al. 2004). In addition to in-clinic BP measurement, 24-hour ambulatory BP will be obtained at the end of each dietary sequence. We expect that the DASH/SRD will reduce BP across the cohort, but differentially on an individual basis (Sacks et al. 2001).

The salt-sensitive vs. salt-resistant phenotype will be defined as a change in 24-hour mean BP of ≥8 mmHg between control and DASH/SRD dietary segments (Castiglioni et al. 2011). Based on previous studies in patients with HTN, we expect that 30-50% of participants will be classified as salt-sensitive (Elijovich et al. 2016). The 24-hour ambulatory BP monitoring and 24-hour urinary collections will be used to provide an alternate definition of salt-sensitivity. Specifically, the quantitative Sodium Sensitivity Index will be calculated as the change in 24-hour mean BP divided by the change in 24-hour urinary sodium excretion between DASH/SRD and control diet periods (Castiglioni et al. 2011).

The *Phase 1* primary outcome is the cfPWV compared at the end of usual diet and DASH/SRD. This parameter was chosen because of its association with incident HF in community-dwelling elderly (Tsao et al. 2015) and its high sensitivity and specificity for HF in older adults with unexplained exertional dyspnea (Weber et al. 2013). Key secondary outcomes include ventricular diastolic function (*k*; ventricular stiffness) and systolic function (global longitudinal ventricular strain) and left atrial reservoir function (left atrial strain).

Dietary intervention: At the week 2 visit, a printed guide to the DASH/SRD produced by the U.S. Department of Health and Human Services will be provided, and participants will complete a standardized questionnaire that establishes their cognitive representation of the DASH/SRD (Scisney-Matlock et al. 2006). Baseline nutrient intake will be assessed with the Block Food Frequency Questionnaire and Nutrition Quest software (NutritionQuest, Berkeley, CA). This instrument has previously been used to assess DASH/SRD concordance (Mellen et al. 2008). Following this visit, participants will consume 14 days of DASH/SRD provided meals, designed by Dr. Hummel. These will be prepared, pre-packaged, and home-delivered twice weekly by PurFoods, LLC. The study DASH/SRD sodium content will be 50 mmol (1,150 mg/2,100 kcal), as used in the DASH-Sodium study (Sacks et al. 2001). Both diets will be calorie-adjusted to maintain stable body weight using the National Institutes of Health body weight planner (Hall et al. 2011). Nutrient consumption during *Phase 1* will be assessed by 3-day food diary during the second week of each dietary period; results will be corroborated by 24-hour urinary sodium and potassium.

Sample size and power: The sample size is based on a combination of logistical considerations and power required to detect important differences in the primary endpoint of change in cfPWV in the context of a randomized crossover design. Sample size is based on a 2-sequence, 2-period, 2-treatment crossover design for continuous endpoints (using paired t test for mean differences). We conservatively assume a within-subject correlation of 0.70 for cfPWV measurements, based on our DASH-DHF pilot (0.81) and literature showing same-day correlation >0.90 (Wimmer et al. 2007) using the Sphygmocor device. We also assume approximate 10% dropout rate during Phase 1 of our study. Under these assumptions and using a two-sided type I error rate of 5%, 100 subjects, 50 allocated to the DASH/SRD-control diet sequence and 50 allocated to the control-DASH/SRD diet sequence, provides >80% power to detect an effect size (mean treatment difference/standard deviation) of 0.22 and >90% power to detect an effect size of 0.26. For comparison purposes, the effect size of cfPWV changes with DASH/SRD in our preliminary work in HFpEF has been 0.4-0.6; in dietary sodium restriction studies in healthier populations than we plan to study, the effect size is at least 0.2-0.4 (Seals et al. 2001, Todd et al. 2010). This sample size provides >80% power to detect an effect size of 0.5 in cfPWV change between salt-sensitive vs. salt-resistant subjects. This is a between-groups comparison based on a two-sample t test and two-sided Type I error of 5%.

Analysis plan: We will examine the distributions of outcomes under each treatment (e.g., DASH/SRD and control diet), as well as by salt-sensitive phenotype. Graphical approaches such as boxplots and scatterplots will be used, allowing identification of outliers, linearity, and correlation of measurements within subject and across time. If the distributional assumptions of our models are not met, transformations will be employed.

Obvious outliers unrelated to data entry error will prompt reconfirmation of acquisition methods and quality by another blinded investigator. We expect no carryover effects due to the two week duration of each dietary segment, a sufficient interval to match sodium intake and excretion even in subjects with chronic kidney disease (McMahon et al. 2013), who have more difficulty achieving this balance.

An intention-to-treat approach will be used to analyze endpoints, including all randomized participants. Multiple imputation will be used for missing data, which assumes that outcome data are missing at random (i.e., where other variables from the study can account for differences in the primary outcome for observed and missing subjects) (Little et al. 2012). A linear mixed effects model (Verbeke et al. 2000, Diggle et al. 2013) will be used to assess the effect of intervention on the change in cfPWV, with fixed-effect terms for sequence, study period, dietary intervention, and adjusting for baseline cfPWV and other potential confounders, e.g. age, race, body mass index, diabetes status, and renal function. A random-effect term will be included to account for the correlated measurements for each subject. Tests to compare diet will be based on Wald and chi-squared tests based on maximum likelihood or restricted maximum likelihood methods. Alternative covariance structures (other than compound symmetry) will be explored and model fit will be examined using the Akaike Information Criteria (Holford 2004). This approach will also be used for the key secondary endpoints (ventricular diastolic stiffness *k* and global longitudinal ventricular strain) and other cardiovascular parameters (e.g. E/e' ratio). For binary secondary outcomes (e.g. serious adverse events) a similar mixed model approach will be employed (Ezzet et al. 1992). To test whether improvements in the primary and key secondary endpoints are greater in salt-sensitive than in salt-resistant subjects, interaction will be assessed via the linear mixed model described above, with a fixed-effect term for salt-sensitivity phenotype.

Potential problems/alternative approaches: Despite our previous experience in collecting 24-hour BP and urinary data, it is possible that some attempts could be incomplete. If this occurs, the dietary intervention would be continued for an additional 24 hours to repeat the measurement. If despite these efforts, one or both of the 24-hour BP or urine collection are unacceptable in an individual, we would assign the salt-sensitive phenotype if clinic-measured mean BP is >5 mmHg higher following the control diet vs. the DASH/SRD segment; this threshold has been previously used to define salt-sensitivity in metabolic syndrome (Chen et al. 2009). This could affect the distribution of salt-sensitivity slightly, but we expect that few patients will have incomplete data and there will be no bias in missing data by underlying phenotype. The detectable effect size would increase based on the degree of imbalance between the number of subjects defined as salt-sensitive vs salt-resistant. In the event that this imbalance is as high as 70%:30%, the detectable effect size with >80% power would be 0.62 with a two-sided Type I error of 5%. If the distribution of salt-sensitivity is markedly different than expected, we would perform a secondary analysis using the median value or other appropriate cutpoint of the quantitative Sodium Sensitivity Index (Castiglioni et al. 2011).

We do not expect substantial difficulty in obtaining cardiovascular measures; Dr. Hummel is evaluating all proposed echocardiographic parameters in four ongoing studies, including two NIH-funded studies using DASH/SRD as the intervention. A major focus of Dr. Hummel's NIH/NHLBI K23 award has been non-invasive vascular assessment. In the event that one or more of the cardiovascular measures cannot be obtained, multiple imputation methods will be used. In the course of studies conducted by Dr. Hummel's team, >120 subjects have completed dietary interventions, involving provided food and/or dietary counseling, lasting three weeks to four months. Adherence to provided meals has been excellent overall, but it is likely that some non-adherence will occur. The primary analysis will be intent-to-treat; if any subjects are severely nonadherent (via 24-hour urinary sodium or food diary review) we would do secondary analyses excluding these participants.

Study Phase 2: Assess the efficacy of motivational interviewing plus the WHEELS-I program vs. motivational interviewing alone in promoting adoption of the DASH/SRD. Determine the effects of DASH/SRD adoption over a 6-month period on structural cardiovascular HFpEF risk factors in salt sensitive vs. salt-resistant veterans.

Introduction: Structural correlates of HFpEF include left ventricular hypertrophy, which strongly predicts HF onset in older adults, left atrial enlargement, which reflects chronically elevated ventricular filling pressures, and stiff central arteries. No previous studies in participants with HTN and metabolic syndrome have examined the relationship between the salt-sensitive phenotype and these HFpEF risk factors after long-term dietary

modification. Current guidelines recommend the DASH/SRD to prevent cardiovascular disease in hypertensives with metabolic syndrome, but adherence to this dietary pattern is declining nationwide. *Our working hypotheses* are that: 1) When compared to motivational interviewing alone, the addition of the Women's and Men's Hypertension Experiences and Emerging Lifestyles Intervention (WHEELS-I) program, an electronically-delivered tailored messaging intervention, will improve adoption of the DASH/SRD over a six month period, and 2) sustained adoption of the DASH/SRD over 6 months will produce greater improvement in structural cardiovascular HFpEF risk factors in salt-sensitive than salt-resistant subjects, with measures including left ventricular mass index (LVMI, primary outcome), left atrial volume index (LAVI), cfPWV, and BP.

Phase 2 study design: We will test our working hypotheses by providing motivational interviewing-based counseling to all participants and randomizing half to the WHEELS-I program, with both interventions described further below. The primary outcome of *Aim 2* of this proposal, to be tested in *Phase* 2 of the protocol, is the intra- and inter-group mean change in DASH/SRD concordance score (Mellen et al. 2008) from the baseline value obtained at the beginning of the study. This score provides 1 point for fully meeting each of 9 DASH/SRD targets and 0.5 for meeting an intermediate level of each target (e.g. fiber 14.8 g and 9.5g/1000 kcal, respectively). The secondary dietary outcome is the group change in mean 24-hour urinary sodium excretion compared to baseline values. Other outcomes of interest are urinary potassium, microalbuminuria, and the nutrient composition from three day food diaries.

We will also test if DASH/SRD adoption differentially improves HFpEF-associated structural cardiovascular parameters in salt-sensitive vs. salt-resistant subjects (as they were defined by BP response in *Phase 1*). The primary cardiovascular outcome of *Phase 2* is the change from baseline (Week 2 visit) to 6 months in left ventricular mass index (LVMI, indexed to height$^{2.7}$). This measure was chosen because LVMI is a strong risk factor for incident HF (Gottdiener et al. 2000), can decrease after 6 months of dietitian-counseled sodium restriction (Jula et al. 1994, Liebson et al. 1995), and is relevant to the pathophysiology of HFpEF (Melenovsky et al. 2007). Key secondary outcomes include cfPWV (see *Aim 1*), left atrial volume, indexed for body surface area (LAVI) (Melenovsky et al. 2015), and 24-hour mean BP.

Phase 2 data collection: History and exam, questionnaires, blood tests, 24-hour BP and urine collections will be performed at months 1 and 6 of *Phase 2* (see *Study procedures* above). Dietary concordance with DASH/SRD, the measure of exposure in *Phase 2*, will be assessed via Block Food Frequency Questionnaire and 24-hour urine sodium excretion at month 6, and 3-day food diaries immediately prior to the Month 1 and Month 6 visits. Arterial tonometry, echocardiography, SDF microscopy, and the mini-SBT will be performed at Month 6 of *Phase 2* (see also *Figures 7 and 8*). Following *Phase 2,* participants will have follow-up after 6 months via telephone interview to assess cardiovascular events and vital status; subjects will be mailed a Food Frequency Questionnaire to evaluate persistence of dietary patterns after study participation; and there may be an optional in-person visit determined by study staff, based on need, for an additional arterial tonometry procedure and blood pressure check.

Dietary intervention: Phase 2 of the protocol will begin within 2 weeks of the end of *Phase 1*, and typically will begin immediately (i.e., as part of the week 4 visit). During this period, participants will be placed back on their prior antihypertensive therapy, if any, under Dr. Hummel's guidance. Prior to the first *Phase 2* visit, subjects will be randomized to control (motivational interviewing only) or the addition of the WHEELS-I program. At the first visit of *Phase 2*, participants will update their cognitive representation of the DASH/SRD, and have an in-person dietitian session during which motivational interviewing techniques will be employed to explore and support willingness to change dietary habits. If the participant desires, the motivational interviewing portion of the visit may be conducted by telephone. Motivational interviewing-based counseling will continue via telephone during week 2, month1 visit, and at months 2 and 4.

The WHEELS-I program consists of three daily activities: in the morning, reading messages that reinforce the key DASH/SRD diet educational components (e.g., types of foods, number of servings, rationale for recommendations) related to a specific dietary goal, then brief journaling of emotions and thoughts related to this goal; in the evening, record of DASH/SRD adherence during that day. An example: goal, eat 4-5 servings of fruits and vegetables per day; messages, try fresh fruits in season, make a fruit salad of "light" or "natural juice" canned fruits, keep dried fruits in your desk or car for snacks; journaling, "I am learning to appreciate the

sweetness of fruit." The daily activities are designed to take less than five minutes. Participants assigned to the WHEELS-I program may choose whether to receive messages via email, in identical fashion to the study shown in the *Preliminary Data* above, or via a newly created smartphone mobile application. Both methods will link to the same web-based information and surveys, which will collect data and store it under subject number in the Amazon.gov cloud storage system, where data will be accessible only by study personnel.

For the first 30 days of *Phase 2*, WHEELS-I participants will receive a structured series of messages that cycle through the DASH/SRD goal areas, e.g. increasing consumption of fruits and vegetables, whole grains, nuts and legumes, and low-fat dairy and reducing intake of sodium, red meat, and sweets. At the Month 1 dietitian visit, all participants will again update their DASH/SRD cognitive representation. Those assigned to WHEELS-I will then decide how frequently to receive morning messages for the next 30 days (evening messages will continue for the next 60 days). Options will include 1) continue daily morning messages, 2) morning messages three times a week, 3) morning messages once a week.

These ongoing messages will be tailored toward challenges identified through motivational interviewing sessions and updating of the DASH/SRD cognitive representation. Message tailoring is a process that includes 1) assessment of individual characteristics relevant to a particular behavior, 2) algorithms that use assessment data to generate intervention messages that address the needs of each user and 3) individual feedback that delivers these messages in a clear and vivid format. Messages will be tailored using the Michigan Tailoring System, an open-source platform developed by the Center for Health Communication Research.

Throughout the first 90 days, if there are less than 3 logins weekly for evening messages noted after 2 consecutive weeks, a message will be sent to inquire how the participant is doing and elicit any problems. At 90, 120, and 150 days, all subjects will be provided the option to continue messages for an additional 30 days. Training for dietitians to facilitate WHEELS-I will be performed by Dr. Scisney-Matlock, the program's creator. As recommended by the NIH Behavior Change Consortium (Bellg et al. 2004), training will be standardized, delivered by a single trainer, and reinforced (every 6 months). Skill acquisition will be confirmed at baseline and retention by facilitating dietitians observed regularly (e.g., every 10 subjects entering *Phase 2*) by Dr. Scisney-Matlock. Receipt of treatment by participants will be assessed via the proportion of messages accessed and responded to. At the end of 16 weeks, all app users will be sent a secure WHEELS-I feedback survey through REDCap, via email. All responses will be automatically uploaded and stored in the secure REDCap application.

The mobile WHEELS application will be downloadable for either iOS or Android from the Apple or Google Play stores, respectively. The participant will receive a unique code to unlock the WHEELS-I application for download. The application will include an overview of DASH/SRD principles, daily notifications and reminders to both influence and measure adherence to the DASH/SRD as above, and positive encouragement for meeting goals. The system will provide a web-based management dashboard for the research team that allows for data collection from the mobile app or email replies, extracts and analysis by the researchers, and adherence to VA security requirements through the use of Amazon.gov cloud hosting.

Sample size and power: The sample size for *Phase 2* is set by the goals of *Aim 1* and influenced by drop-out during the 6 months of the interventions to promote DASH/SRD adoption. We assume that an additional 10% of subjects will drop out during *Phase 2* and that the dropout rate will be equal in salt-sensitive and salt-resistant participants, resulting in 90 subjects with evaluable data. The primary outcome of *Aim 2* of this proposal is the DASH/SRD concordance score from the Block Food Frequency Questionnaire in participants assigned to the WHEELS-I group vs. motivational interviewing alone. Based on national survey data and our preliminary work, we expect the mean baseline (Week 2 visit) dietary score to be 2.5±1.5. A sample size of 90 provides >80% power to detect an intra-group change of 0.6 between baseline and the end of study, based on paired t-testing two-sided Type I error of 5%. This sample size provides >80% power to detect a differential between group change in dietary score of 0.9, based on a two-sample t test and two-sided Type I error of 5%.

With regard to cardiovascular parameters, a sample size of 90 provides >80% power to detect an effect size of 0.6 in the primary endpoint of change from baseline to month 6 in LVMI between salt-sensitive vs. salt-resistant subjects (assuming a 50:50 split of salt sensitive vs. salt-resistant participants), with a two-sided Type I error of

5%. This between-groups comparison is based on a two-sample t test. Conservatively assuming that the standard deviation of the change in LVMI between baseline and 6 months ranges between 4 to 7 $g/m^{2.7}$ (Blumenthal et al. 2010), this effect size translates to mean group differences of 2.4 to 4.2 $g/m^{2.7}$. This would represent a 5-10% decrease in LVMI from baseline in salt-sensitive subjects (vs. no change in salt-resistant), similar to that in previous studies of dietary sodium restriction (Jula et al. 1994, Liebson et al. 1995).

Analysis plan: Paired and 2-sample t-testing will be used to analyze changes in DASH/SRD concordance score as above, as well as changes in 24-hour urinary sodium excretion. The methods to analyze cardiovascular changes in *Aim 2* will be identical to those used in the comparison of salt sensitivity phenotypes in *Aim 1* (see above). Important covariates in the models to compare changes in cardiovascular parameters by salt-sensitive/resistant phenotype will be the degree of DASH/SRD concordance and changes in 24-hour BP.

Potential problems/alternative approaches: While motivational interviewing and the WHEELS-I program have been effective in our preliminary studies, we expect individual variance in DASH/SRD adoption. The primary analysis will be an intent-to-treat framework; secondary analyses will test interaction with the level of DASH/SRD adoption.(Mellen et al. 2008) We expect that participants who successfully adopt DASH/SRD will substantially change their eating habits. The WHEELS-I program does not target weight loss, but since sodium intake correlates with caloric intake (Guenther et al. 2013) it is possible that some veterans may lose weight during *Phase 2*. We do not *a priori* expect differential weight loss in salt-sensitive and salt–resistant subjects. However, since cfPWV and LVMI can both be reduced through weight loss, we will perform analyses adjusted for *Phase 2* weight changes (Blumenthal et al. 2010). If the proportions of salt-sensitive and salt-resistant subjects are not equal (say 60%:40%), we would retain >80% power to detect effect size of 0.61.

Specific Aim 3 (spans Study Phases 1 and 2): Determine effects of DASH/SRD feeding and longer-term adoption on microvascular function, and assess the relationship between endothelial glycocalyx integrity/sodium buffering and cardiovascular response to DASH/SRD.

Introduction: Small-vessel oxidative stress and inflammation are critical mechanisms of cardiovascular injury in salt-sensitive animals, and recent data strongly implicate microvascular damage in HF pathophysiology. The endothelial glycocalyx (eGC) is a thin network lining the lumen of blood vessels, and if damaged may mediate the mechanisms and vascular consequences of salt-sensitivity. *Our working hypothesis* is that the DASH/SRD will improve microvascular function in comparison to control diet, and differentially so in salt-sensitive subjects. We will test this hypothesis using sublingual sidestream dark-field (SDF) microscopy. *We further hypothesize* that poor eGC integrity will identify subjects who have greater cardiovascular improvement with DASH/SRD. This hypothesis will be tested using SDF microscopy and a novel “mini-Salt Blood Test” (see *Background and Significance* above for description of these procedures).

Aim 3 analysis plan: To determine the effects of the DASH/SRD intervention and adoption on microvascular parameters and the differential impact of salt-sensitivity, we will use the approaches described in *Aims 1 and 2* for microvascular density and RBC filling % (measures of microvascular structure and function). To assess the relationship between eGC and salt-sensitivity, we will characterize the relationship between mean baseline PBR and mean baseline ESS (measures of eGC thickness and sodium-buffering capacity) and the salt-sensitive phenotype (as defined in *Aim 1*). We will also assess the relationship of these parameters with cardiovascular functional and structural measures described in *Aims 1 and 2*. For example, we will test whether mean baseline ESS differs by salt-sensitivity phenotype using a two-sample t-test or simple linear regression (to include adjustment for important covariates). Model performance measures will be used to assess the discrimination of PBR and ESS (vs. salt-sensitivity) for changes in cardiovascular function and structure [e.g., area under the receiver-operating-characteristic curve, net reclassification, and integrated discrimination improvement (Pencina et al. 2008)]. Descriptive analyses of baseline and changes in microvascular and cardiovascular function and structure will use graphical and summary statistics.

Potential problems/alternative approaches: Technical factors could prevent SDF microscopy acquisition in a few participants. If this occurs, subjects will still have all other cardiovascular measures as well as glycocalyx assessment with the mini-SBT. We have successfully obtained the mini-SBT in over 200 participants. In the rare event of a technical failure, we would simply repeat the test. Despite supportive data as above, it is

possible that the mini-SBT results will not predict BP changes during DASH/SRD. Given the mechanistic links between eGC function and sodium-induced vascular damage (*Figure 2*), it is also possible that the mini-SBT is more effective than BP changes in identifying subjects with beneficial cardiovascular effects during DASH/SRD.