Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

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Project Title:	Beta-2 adrenergic receptor polymorphic variants: an endotype of salt sensitive hypertension
Version Date:	09/08/2026
Version Name/Number:	v4

For Intervention/Interaction studies, submit a Detailed Protocol that includes the following sections. If information in a particular section is not applicable, omit and include the other relevant information.

1. Background and Significance

1. Importance of the Problem. Over 1/3 of adults in the USA have hypertension (**HTN**), of which 51.3% (over 34 million Americans) have uncontrolled blood pressure (BP)[1], leading to a high prevalence of cardiovascular (CV) complications mediated by elevated BP[1]. Among the major hurdles to be addressed in HTN are: 1) the lack of specificity in treatment programs, and 2) the unclear mechanisms by which genetic and environmental factors (e.g. dietary Na^+ intake) affect BP and CV function. Clearly, the first hurdle is – for the most part – a consequence of the second. A recent document from NIH provides the following guide to address these issues.

"To address interindividual variability [responder/ non-responder phenomena] in […] complex diseases [e.g., obesity, asthma, type 2 diabetes, hypertension, chronic kidney disease, and cardiovascular diseases], it has been instructive to take a lesson from the asthma field, noted for its early recognition of prioritizing elucidation of disease endotypes over phenotypes"[3]. Like asthma, HTN "is now considered an umbrella diagnosis for several diseases with distinct mechanistic pathways [endotypes] & variable clinical presentations [phenotypes]. A phenotype covers the clinically relevant or obvious properties of the disease [for example, HTN could be salt resistant, salt sensitive, orthostatic, pregnancy induced, etc.]. However, phenotypes do not necessarily show the direct relationship to underlying disease etiology and pathophysiology that may be a key for treatments to work; **endotypes** [or endophenotypes which require a genetic component] are meant to do that. Another aspect is that several endotypes can lead to the same phenotype or umbrella diagnosis. Classifying complex diseases [e.g., HTN] using pathogenetic mechanism-based subtypes (endotypes/endophenotypes) may have other advantages in epidemiological, genetic, and treatment-related studies"[3].

Recent reviews have reported that for nearly 50% of the essential (primary) HTN population, all of whom have salt sensitive blood pressure (**SSBP**), a dozen or more endotypes potentially underpin their pathophysiologic mechanisms. To date, the pathophysiologic mechanisms of these endotypes ultimately involve dysfunction in sodium/volume homeostasis and the components of the two major pathways that regulate this process--- the renin-angiotensin-aldosterone (**Aldo)** system (**RAAS**) and the renal vasculature, i.e., renal plasma flow (**RPF**)[10,11].

In most cases, one or the other system is adversely affected, and the alternate system tends to compensate for the altered sodium handling. This project focuses on one of the less understood endotypes: beta-2 adrenergic receptor (**β_2AR**) gene-mediated dysfunction in SSBP.

This endotype is **unique** because preliminary and published data suggest that the mechanism(s) causing the SSBP association with a common **β_2AR risk diplotype** ($Arg^{16}Arg/Gln^{27}Gln$) **may or may not** be the direct consequence of the primary effect of the diplotype---a gain of function for the β_2AR gene. On one hand, increased β_2AR levels could **directly** activate the two major Na^+/volume homeostatic pathways (Aldo and RPF) resulting in Na^+ retention and SSBP. However, it is possible that the cause of the SSBP may be **indirect**: maladaptation of the Na^+/volume homeostatic systems that "believe" that the β_2AR-induced vasodilation is the result of volume depletion and therefore Na^+ retention is required – a maladaptation like that documented in other CV conditions – most notably, in congestive heart failure (**CHF**). Either of the two mechanisms could lead to SSBP, but with quite different therapeutic implications. Thus, **the project herein will assess the two contrasting hypotheses regarding the mechanism(s) underlying the β_2AR endotype-associated SSBP.**

These two different possibilities are reminiscent of the apparent contradictory results for the same diplotype – in **asthma**. The "**risk**" diplotype ($Arg^{16}Arg/Gln^{27}Gln$) is associated with bronchodilation, and as such should act as a protective mechanism (akin to an endogenous beta agonist); however, its prevalence is surprisingly higher in asthma[12]. The opposing diplotype that we call "**non-risk**" ($Gly^{16}Gly/Glu^{27}Glu$) associates with lower asthma risk but it also describes improved responsiveness to exogenous beta agonists[13]. The numerous β_2AR-endotype investigations in the asthma field successfully converged on genotyping patients as a measure to ensure better responsiveness to beta agonist therapy[14]. However, in the CV system the apparent contradictory results have yet to be leveraged. The prevalence of the "**non-risk**" diplotype is higher in heart failure patients, yet it confers better responsiveness to beta-blockers[15]. In contrast, the "**risk**" diplotype ($Arg^{16}Arg/Gln^{27}Gln$) displays decreased vasodilation response to isoproterenol infusion[9,16,17], potentially related to an already overstimulated vasodilation pathway at baseline (see our **Preliminary Results** for RPF). These apparently conflicting results for the β_2AR diplotype remain unresolved in the CV field. While many PubMed publications link β_2AR gene variance to asthma and CV function, none connect it to SSBP, except those from our group – **a knowledge gap we aim to begin addressing in this proposal**.

2. Scientific Premise: Recent scientific advances can now be leveraged to identify, within the complex HTN population, homogeneous subsets that share a common, often novel, root basis. As noted above, understanding the mechanism(s) responsible for the altered physiology in these subsets will provide an opportunity to hone treatment programs to an individual level, with the anticipation of better attainment of BP goals. We recently identified a **novel hypertensive endotype** involving β_2AR gene variation, and its likely effects on receptor function. In two hypertensive cohorts, individuals homozygous for the β_2AR **risk** diplotype $Arg^{16}Arg/Gln^{27}Gln$ display SSBP[4-6]. Importantly, the prevalence of this diplotype is 13-32%, i.e. 8-21 million hypertensive Americans[18]. This functional diplotype inhibits β_2AR downregulation[7-9] and drives increased β_2AR expression (gain of function). Further, to assess mechanisms at a cellular level, we generated a mouse model that carries the risk ($Arg^{16}Arg/Gln^{27}Gln$) β_2AR diplotype (**β_2AR Mut**), which displays SSBP and therefore is translationally relevant.

The following preliminary and published data provide support for our hypotheses:

a. Both mice and human β_2AR risk homozygotes have SSBP.
b. In human β_2AR risk homozygotes, the SSBP is caused by a fall in BP on the restricted salt diet (**RES**), not the traditional rise in BP on the liberal salt diet (**LIB**).

c. In humans, the β_2AR risk vs. non-risk diplotype displays higher RPF on a LIB diet, yet an inappropriate change in RPF response to Angiotensin II (**AngII**) in the face of SSBP (no difference from non-risk homozygotes).
d. In human β_2AR risk vs. non-risk homozygotes, Aldo levels on a LIB diet are inappropriately high.
e. In the mice carrying the β_2AR risk diplotype, males have an appropriate decrease in the renal artery resistive index (**RI**) on the LIB diet – i.e. an increase in RPF – while the females have an inappropriate increase in RI – i.e. a decrease in RPF. We had insufficient data to address this question in the human observational cohort.

These data suggest at least two likely mechanisms for the β_2AR endotype-associated SSBP (**Fig. 1**). The primary defect in the endotype (increased β_2AR levels): **either 1) directly activates** the two major Na^+/volume homeostatic pathways (Aldo and RPF) resulting in Na^+ retention **or 2) indirectly activates** volume mediating sensors inappropriately signaling volume depletion. Thus, in response to the gain of function - vasodilation induced in the in β_2AR risk homozygotes - the two key salt retaining mechanisms Aldo production and renovascular function appear to inappropriately increase Na^+ reabsorption, volume expansion and SSBP. While either process could lead to SSBP, the therapeutic implications for the two mechanisms are quite different.

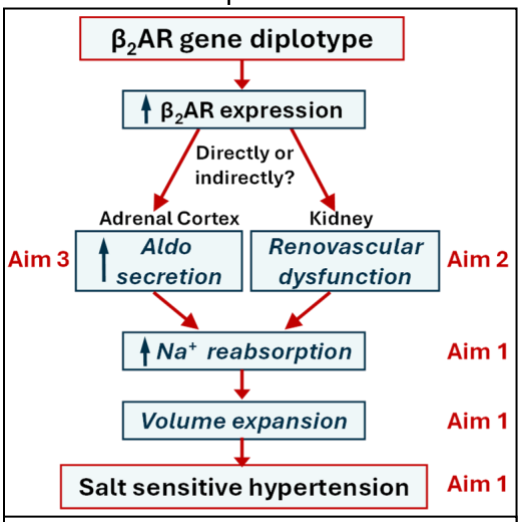


Fig. 1. Potential mechanisms underlying the β_2AR endotype-associated SSBP.

3. Effect on Scientific Knowledge/Clinical Practice This translational research project will elucidate the mechanisms by which a functional β_2AR gene diplotype interacts with Na^+ intake to modify Aldo levels and/or renovascular function and thus affect SSBP. Successful completion of this project may modify scientific knowledge/clinical practice in at least three ways. **First**, a genotype approach would allow early identification of individuals at risk for inappropriate circulating Aldo levels and/or renovascular dysfunction during Na^+ replete conditions, with subsequent salt sensitive HTN and HTN-related CV complications. **Second**, this project may lead to changes in the treatment of HTN from the present non-specific, "trial and error", algorithm-based approach to a more specific, genotype-directed drug paradigm. If our hypotheses prove correct, this mechanistic project will provide the scientific basis for a future clinical trial to determine the efficacy of chronic β_2AR-specific blockade in improving Aldo levels, renovascular function and SSBP in individuals homozygous for the β_2AR diplotype. **Third,** the in-depth mechanistic knowledge to be gained from this project may lead to the identification of novel signaling pathways in the adrenal that are dysfunctional in the β_2AR diplotype carriers, thus generating novel pharmacological targets for specific, individualized therapeutic approaches. Specifically, this may include the development of new drugs that target the adrenal β_2AR signaling mechanisms or β_2AR-AT_1R interactions. This is important since current beta-blockers are mostly β_1AR-specific, which may limit their clinical usefulness in this patient population.

4. Changes in the Field if Proposed Aims are Achieved. Despite substantial observational data that the mechanisms responsible for the prevalence and severity of various diseases differ in women than men, the current algorithmic-based approaches do not reflect these differences clinically or in research studies. Success in documenting the validity of the hypotheses being evaluated herein would encourage physicians and scientists to perform similar three-step approaches. **1**) Acknowledge that chronic illnesses are NOT necessarily diseases but syndromes of a collection of individual diseases. **2)** The underlying specific mechanisms can be determined using a translational approach combining deep phenotyping of a controlled human cohort with focused preclinical studies. **3)** Creating a four-step paradigm to achieve specific, individualized therapy:

- Identify the specific **mechanism** of the individual diseases;
- Identify the precise **treatment/prevention** plan that is mechanistically based;
- Assess if there is a **biological sex** component;
- Identify the specific **person** with the disease (using genotype).

2. Specific Aims and Objectives

Hypertension (**HTN**) treatment is moving towards a more refined individualized approach because the present algorithmic, population-based approach has met with limited success. More than half of hypertensives do not achieve blood pressure (**BP**) goals[1,2]. In recent decades, scientific advances have been used to identify specific, homogeneous subsets of HTN that share a common root basis, now referred to as **endotypes** [3]. Linking genetic factors to phenotypes, endotypes help identify more precise therapeutic mechanisms. One **novel hypertensive endotype** involves the **beta-2 adrenergic receptor** (**β_2AR**), where individuals homozygous for the $Arg^{16}Arg/Gln^{27}Gln$ β_2AR risk diplotype display salt sensitivity of BP (**SSBP**)[4-6]. Importantly, the prevalence of this diplotype of 13-32%, i.e. 8-21 Million hypertensive Americans[1]. This functional diplotype inhibits β_2AR downregulation[7-9] and drives increased β_2AR expression. We generated a mouse model that carries the risk ($Arg^{16}Arg/Gln^{27}Gln$) β_2AR diplotype (**β_2AR Mut**), which displays SSBP and therefore is translationally relevant.

As we have reported in other SSBP endotypes, one or both of the two key sodium (**Na^+**)/volume homeostatic systems may be dysregulated, increasing Na^+ reabsorption, volume expansion and SSBP. **1) Aldosterone (Aldo) production.** The β_2AR risk diplotype associates with increased β_2AR expression in human adrenals and with increased Aldo levels on a liberal Na^+ (**LIB**) diet [4-6] in young, but not old, hypertensives. **2) Renovascular function.** The β_2AR risk diplotype associates with higher renal plasma flow (**RPF**) on a LIB diet in young, but not old, hypertensives. This effect (likely related to increased vascular β_2AR expression) is a potential normal RPF response to SSBP. However, on a restricted Na^+ (**RES**) diet, the β_2AR risk and non-risk homozygotes had a nearly identical percent change in RPF response to angiotensin II (**AngII**)--an inappropriate response to SSBP.

These data suggest two alternate hypotheses for the β_2AR endotype-associated SSBP. The primary defect in the endotype (increased β_2AR levels): **either 1)** directly activates the two major Na^+/volume homeostatic pathways (Aldo and RPF) resulting in Na^+ retention **or 2)** indirectly activates volume mediating sensors inappropriately signaling volume depletion. Either process could lead to SSBP, but with quite different therapeutic implications. We will address these contrasting hypotheses in three translationally structured **Aim**s:

Aim 1: **Hypothesis – *The β_2AR risk diplotype identifies, in humans, a subset of young hypertensives with SSBP, higher β_2AR tissue expression* and *altered urine Na^+ excretion.*** ***Approach***: In β_2AR risk vs. non-risk hypertensives (<51 years), we will assess prospectively: 1) SSBP, 2) levels of β_2AR expression (*ex vivo* in PBMCs and superficial inferior epigastric arteries (**SIEA**)) and 3) the renal response to an acute salt load. ***Results**:* Risk homozygotes will show higher β_2AR levels and increased SSBP, with lower BP on a RES (but not on LIB diet). Delayed Na^+ excretion in the β_2AR risk vs non-risk group would suggest a RPF restriction, while an accelerated excretion would suggest increased Aldo. Differences in responses by sex are not anticipated.

Aim 2: **Hypothesis – *In humans and mice, the β_2AR risk diplotype identifies a SSBP subset mechanistically characterized by dysfunctional RPF responses to changes in Na^+ intake.*** **Approach:** In β_2AR risk vs. non-risk hypertensives (<51 years) and β_2AR Mut vs. wild type (WT) mice on LIB and RES diets, we will assess: **1)** *in vivo* RPF and BP basally (humans and mice) and in response to AngII and angiotensin-converting enzyme inhibition (**ACEi**) (humans); **2)** *ex*

vivo β_2AR and AngII levels in human SIEA and mouse carotid vessels, as well as vascular reactivity studies in the same vessels (β_2AR-specific agonism/blockade, AngII/ACEi). **3)** in β_2AR Mut vs. WT mice, we will contrast BP and RPF responses to *in vivo* treatment with β_2AR-specific blocker ICI 118,551 vs. ACEi Enalapril. ***Results:*** Vascular β_2AR and AngII levels will be higher in risk vs non-risk homozygotes, with reduced RPF responses to AngII, and decreased *ex vivo* vasoconstriction/vasorelaxation responses to AngII and β_2AR-specific stimulation, respectively. Finally, it is uncertain whether the β_2AR-specific blocker or the ACEi will result in a lower BP in β_2AR Mut vs. WT mice.

Aim 3: **Hypothesis – *In humans and mice, the β_2AR risk diplotype identifies a SSBP subset mechanistically characterized by dysfunctional Aldo secretion. Approach:*** In β_2AR risk vs. non-risk hypertensives (<51 years) and β_2AR Mut vs. WT mice on LIB and RES diets (see **Aim 2**), we will assess: **1)** *in vivo* Aldo and PRA levels basally (humans and mice) and in response to AngII and upright posture (humans); **2)** In mouse Zona Glomerulosa cells, we will assess Aldo responses to secretagogues (including a β_2AR-specific agonist) and measure the β_2AR and Aldo biosynthetic enzymes. **3)** in β_2AR Mut vs. WT mice, we will contrast Aldo responses to the β_2AR-specific blockade vs. ACEi (see **Aim 2**). ***Results:*** In risk vs. non-risk homozygotes, Aldo will be increased at baseline and in response to AngII. In β_2AR Mut vs. WT mice, adrenal β_2AR will be increased, with higher serum Aldo levels and Aldo responses to agonists, with no changes in enzyme levels. it is uncertain whether one of the treatments will result in greater Aldo change in β_2AR Mut vs. WT mice.

Both hypotheses explored in our **Aims** suggest that the β_2AR risk diplotype activates the Na^+/volume homeostatic systems. However, whether this activation is direct or indirect has profound therapeutic implications.

3. General Description of Study Design

A.1. ***Rationale***: Our approach is based on two observational studies in hypertensives, in which individuals homozygous for the $Arg^{16}Arg/Gln^{27}Gln$ β_2AR risk diplotype, display SSBP and increased Aldo levels[4-6]. Further, our preliminary data suggest that these associations are driven by the effect of the diplotype in young, but not old, hypertensives on a LIB diet. *In silico* assessments in two eQTL databases also suggest that the β_2AR diplotype associates with increased β_2AR expression in human tissues. Additional preliminary studies in mice that carry the risk diplotype (β_2AR Mut) show that – as compared to WT, β_2AR Mut mice have SSBP. These data suggest two alternate hypotheses for the β_2AR endotype-associated SSBP. The primary defect in the endotype (increased β_2AR levels) can: **either 1) directly activate** the two major Na^+/volume homeostatic pathways (Aldo and RPF) resulting in Na^+ retention **or 2) indirectly activate** volume mediating sensors inappropriately signaling volume depletion with stimulation of these homeostatic pathways. The purpose of this Basic Experimental Study Involving Humans (**BESH**) is to determine, prospectively, the endotype driven by the β_2AR risk diplotype and specifically document three critical steps by associating the risk β_2AR diplotype with: **1)** increased β_2AR expression and protein levels in vasculature and mononuclear cells; **2)** a specific response to an acute salt load: either accelerated or delayed Na^+ excretion, suggesting either a RPF effect or an Aldo effect, respectively; and **3)** increased SSBP.

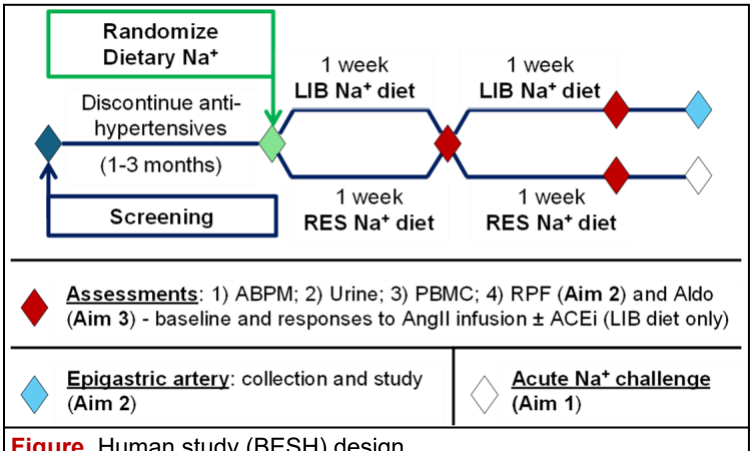


Figure. Human study (BESH) design.

4. Subject Selection

A.2. *Subject Population*: Two groups (n=50/group; see **Statistics**) will be recruited for this BESH study: β_2AR **risk** ($Arg^{16}Arg/Gln^{27}Gln$) and **non-risk** (Gly^{16} $Gly/Glu^{27}Glu$) diplotype homozygotes. The subjects to be enrolled will be newly recruited from either the MGB Biobank that currently contains DNA and clinical data on >165,000 subjects, or the ~45,000 subjects amongst the ~11 million subjects in the MGB research patient data registry (RPDR) that have our HTN phenotypic characteristics. They will NOT be from our HyperPATH cohort. We have used these sources to recruit 60-125 subjects/study into seven different mechanistic genetic studies. We estimate that, based on our previous studies, about 29,000 subjects (~55% women) meeting our inclusion/exclusion criteria will be invited to participate. Approximately 15% will complete a recruitment questionnaire, of which ~4000 will have a cheek swab for genotyping. ~2000 will have one or the other genotypes, with ~250 black subjects. Of these, ~50% will be eligible (~500 men, ~500 women, and 125 black subjects). Thus, we will enroll about 10-15% of these individuals, enriched by black subjects that will comprise 20% of the population rather than the 10% in our original pool. Equal numbers of male and female participants will be included.

A.3. *Eligibility.* Inclusion criteria*:* ages 18-50 years; HTN as defined by the primary physician; on ≤ two anti-hypertensives; normal renal, liver, metabolic, electrolyte, CBC, and thyroid tests. Exclusion criteria*:* Pregnancy; renal, neurologic, and cardiac diseases other than HTN; diabetes; secondary HTN as assessed by history, physical examination, screening blood and urine tests; any drug therapy, except for anti-hypertensives and replacement thyroid medication; and no oral contraceptives/implants/ERT within 2 years of study.

5. Subject Enrollment

Phone Screen

Subjects that respond to the previously described recruitment methods will complete a telephone screen to determine if they meet basic eligibility requirements (see included Telephone Script). Potentially eligible subjects will then be scheduled for a screening visit.

Screening Visit 1
Subjects meeting basic eligibility requirements from the Telephone Screen will be invited to meet for a screening physical exam at the Center for Clinical Investigations (CCI) outpatient facility at Brigham and Women's Hospital (BWH). Research coordinators will obtain informed consent for Screening Visit 1. A licensed physician investigator will be available for backup if consent is obtained by a research coordinator. The consent form for the main study will be made available to participants at or prior to Screening Visit 1. The screening visit will involve obtaining vital signs, reviewing the screening consent form, and performing a cheek swab for rapid turnaround genotyping results of the B2AR allele.

Screening Visit 2
If participants qualify after the first screening visit, they will proceed to the second screening visit. A licensed physician investigator will obtain informed consent for the main study. This screening physical and history exam will take place at the CCI outpatient facility at BWH. Prior to arriving for the screening visit, the potential subject will be mailed and asked to complete a Personal Medical History form and Family Medical History form in addition to the research consent form. The screening visit will involve reviewing the completed forms, conducting a physical exam by a study physician, reviewing the study consent form, completing an EKG, and a blood draw for laboratory studies (serum glucose, sodium, creatinine, potassium, liver tests, TSH, lipid panel, hematocrit, urine HCG pregnancy test, and ECG).

Participant Consent
As per above, all potentially eligible subjects will be mailed a copy of the research consent form to review prior to their screening visit. Every attempt will be made to acquaint each prospective subject with all of the procedures involved in this study during the screening visit. A research study physician will obtain informed consent. We will also ask if the potential subjects would like to be placed on a research database list in order to be notified of future research studies in the Divisions of Endocrinology at BWH. Potential participants who do not speak English be given a written translation, in a language understandable to them, of the entire English version of the consent form approved by the MGB IRB and following the MGB IRB guidance on Obtaining and Documenting Informed Consent.
Laboratory values must fall within the "Acceptable Laboratory Values" range (see attached). Laboratory tests that fall outside the Acceptable Laboratory Values range will be discussed with the research participant and if the subject is deemed ineligible to participate in the study, a letter will be sent to the subject to inform them of his/her exclusion. The letter will also recommend that the subject follow up with their primary care provider. We will also call and/or email the subject to make sure s/he understands why s/he was excluded and to make sure the subject is following up with their health care provider. If the subject does not have a primary care provider, arrangements will be made to locate one for them using the BWH BIMA referral line.

6. STUDY PROCEDURES

- Remuneration as applicable. Indicate if payments to subjects are made upon completion of study visits/certain procedures and how remuneration is pro-rated, particularly for non-completers.

Procedures will be performed using resources from the Center for Clinical Investigation (**CCI**), following techniques established over three decades in our > 1,000 person HyperPATH cohort. ***Run-In Period.*** Anti-hypertensive medications are discontinued following standard procedures for ~one month (**Fig.**). ACE inhibitors (**ACEi**), angiotensin receptor blockers (**ARB**), or Mineralocorticoid Receptor Antagonist (MRAs) will be withdrawn for ~3 months with appropriate amlodipine coverage if needed until 1 month before start of study when all antihypertensives are stopped. Subjects are trained to obtain and record home Blood Pressure (BP)s and e-mail them to the study team daily. Additionally, a 1-1 live contact is made every 3 days throughout the washout and Assessment Protocol. The protocol is stopped, and previous therapy restarted for the onset of cardio- or cerebrovascular symptoms, a rise in diastolic BP >15 mmHg or systolic BP >30 mmHg, or an absolute BP >180/109 mmHg on 2 consecutive days.

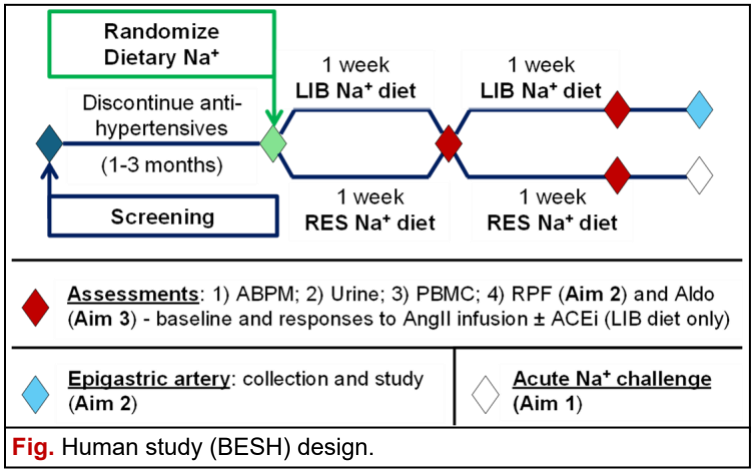


Fig. Human study (BESH) design.

Dietary Na^+ Assessment Protocol. (**Fig.**) **1)** Subjects complete two controlled dietary phases in random order in a cross-over design, usually separated by 0-6 days of a regular diet (or longer in women because of the need to be studied during the follicular menstrual phase): 7 days of a Liberal (LIB) diet (200 mmol Na^+/day) and 7 days of a Restricted (RES) diet (10 mmol Na^+/day). Both diets are isocaloric, with 100 mmol/day K^+, 20 mmol/day calcium, and are caffeine and alcohol-free. **2)** The start of the diet in women is timed so that Day 7 coincides with the follicular phase of the participant's menstrual cycle (day 2-7 of cycle), confirmed by estrogen and progesterone levels. **3)** On the final morning of each diet, subjects begin a 24-hr urine and a 24 hr BP Ambulatory Blood Pressure Monitor (**ABPM**) (**ST-1**) and will be admitted overnight to the in-patient CCI unit. A spot urine will be obtained for Na^+ and creatinine to confirm Na^+ balance. **4)** The subject is kept NPO and supine overnight, and at ~ 7 AM, lines and a BP monitor (Dinamapp) are placed to collect and record BP every 2 minutes. Approximately 30 minutes later, blood is obtained for basic metabolic, lipid panels, inflammatory markers and hormones and for isolation of Plasma Peripheral Blood Mononuclear Cell (PBMC) (**ST-2**). **5)** Then, a *Para-aminohippurate* (*PAH)/ Angiotensin II (AngII) protocol* is performed (**ST-4**). **6)** The subject will then receive lunch with the alternate Na^+ diet, discharged, maintain the alternate diet, and be re-admitted in ~7 days for the same protocol with the opposite Na^+ diet. The time between diets will be longer in menstruating women, as noted above. In the HyperPATH cohort, we have successfully completed the core study in ~1000 subjects. The protocol runs between 6-7AM and 12-1PM. **7)** If they have agreed to participate in an additional study, they are discharged but maintained on the same diet for 2-3 days. Then, they return to the outpatient unit. **a)** If on LIB diet, an ***Superficial Inferior Epigastric Artery (SIEA) isolation procedure*** is

performed (**ST-3**). **b)** If on RES diet, an ***Acute Salt Load protocol*** is performed (**ST-5**). **8)** At study completion, subjects are discharged on pre-study medications.

Special Techniques (ST)

1. 24-hour ambulatory BP: The Brigham and Women's Hospital (BWH) Ambulatory Blood Pressure Core will perform this measurement using Microlife WatchBP.
2. PBMC cell study: 40 ml of whole blood will be obtained supine after overnight fast. The PBMC will be separated by gradient density centrifugation and stored at -80C until studied within one week of isolation.
3. SIEA isolation procedure: SIEA will be isolated by Dr. Dennis Orgill (plastic surgeon). SIEA (located by ultrasound) is easily harvested under local anesthesia. The area is infiltrated with 0.5% lidocaine with epinephrine, 1:200,000. A low transverse incision of ~2 cm is made and a 2-4 cm^3 dissection of fat, with the artery, placed in a sterile cup with saline, delivered to the laboratory, closed and checked in 1 week.
4. Renal Plasma Flow (RPF) /ANGII/captopril study: RPF will be assessed as described by us. Briefly, 60-90 minutes after the completion of the PAH/AngII protocol, the PAH but not AngII infusion is continued. Then, captopril (25 mg) is given; 60 minutes later, a blood sample is obtained for PAH, a repeat AngII infusion is given. PAH clearance is calculated from the plasma concentration and the infusion rate.
5. Acute salt studies: At 0700, subjects ingest distilled water, 5 ml/kg body weight, over 30 min and voids within 30 min. A 0.45% saline infusion is started to give 60mEq of Na^+ over 1 hour. At time 0, blood is drawn for Na^+ and creatinine. Voided urines are collected for 5 hours. Fractional Na^+ excretion is calculated from serum Na^+, creatinine clearance, and Na^+ excretion rate (percentage of filtered load).

ORDER OF PROCEDURES

- At ~ 7 AM, tubes are place in a vein in each arm and a BP monitor is placed on one arm to collect and record BP every 2 minutes.
- Approximately 30 minutes later, approximately 60 ml (4 tablespoons) of blood is obtained to measure various hormones, lipids markers of inflammation and a basic metabolic panel, Para-aminohippurate (PAH), and to isolate from the blood one of its components (Peripheral Blood Mononuclear Cells (PBMC). 40 ml of whole blood will be obtained supine after overnight fast. The PBMC will be separated by gradient density centrifugation and stored at -80C until studied within one week of isolation.
- In one of your arm veins, a *PAH* bolus (8mg/kg), followed by a continuous infusion of 12 mg/min for 45-60 minutes. PAH infusion rate and serum levels are used to measure renal blood flow. At the end of this infusion approximately 20 ml (1 and 1/4 tablespoons) of blood is obtained to measure various hormones and PAH.
- The PAH infusion rate will be the same throughout the protocol (12 mg/min).
- The PAH infusion continues in one arm and an angiotensin II (ANGII) infusion (3ng/kg/min) is begun in the other arm. These two infusions run together for 30-45 minutes. At the end of this infusion approximately 20 ml (1 and 1/4 tablespoons) of blood is obtained to measure various hormones and PAH.
- The ANGII infusion rate will be the same throughout the protocol (3ng/kg/min).
- For 60-90 minutes after the completion of the PAH/AngII infusion, the PAH but not AngII infusion is continued. At the end of the 60-90 minute approximately 20 ml (1 and 1/4 tablespoons) of blood is obtained to measure various hormones and PAH.
- With the PAH infusion continuing, a captopril (25 mg) tablet is then given; 60 minutes later approximately 20 ml (1 and 1/4 tablespoons) of blood is obtained to measure various hormones and PAH.

- After the blood draw, with the PAH infusion continuing in one arm at 12 mg/min, an ANGII infusion (same dose – 3 ng/kg/min) is begun in the other arm. These two infusions run together for 30-45 minutes, starting approximately 60 min after captopril administration. At the end of this infusion approximately 20 ml (1 and 1/4 tablespoons) of blood is obtained to measure various hormones and PAH.

If the subject completes the entire study, he/she will receive a total of $2,000.00 to compensate for time and transportation expenses.

7. Risks and Discomforts

We identify several potential risks associated with participation in this study.

Alteration of anti-hypertensive medications
Hypertensive subjects will be followed without specific anti-hypertensive therapy for approximately 2 drug-free weeks during the high and low salt diet study periods. Subjects will be in close contact with study investigators to ensure prompt and careful control of blood pressure (described below).

Hypertensive medication washout
We have safely and effectively performed anti-hypertensive medication washouts using the methods below in over 2,000 individuals over the past 25 years using our IRB approved *Standard Operating Procedure for Blood Pressure Research Protocols*:

To ensure that the results we see are due to our study interventions and not due to participant's previous hypertensive treatment, all participants who are on anti-hypertensive medications prior to the study will enter a washout phase that lasts for at least 2 weeks. If the participant is on an angiotensin converting enzyme inhibitor, angiotensin receptor blocker or an MRA, they are washed out for 3 monhts prior to starting the study diets.

During the medication washout phase and throughout the entire protocol, participants are supervised by the study staff. Participants are trained on how to use an automated sphygmomanometer (Omron, Kyoto, Japan) so they can take daily morning blood pressure recordings at home and report them to study staff daily. Every three days, the participant had direct (in person or via Zoom) contact with a study staff member. If the participant's blood pressure is equal or above 170/109 mmHg, they are asked to contact the study staff. If blood pressure is still increased on two separate occasions 24-hours apart throughout the washout phase or study protocol or they develop clinical symptoms, they will be removed from the study and asked to resume taking their prior antihypertensive medication(s).

Risks associated with performing the study procedure
Blood Collection. Blood draws may cause tenderness, hematoma formation, and rarely infection. Alcohol swabs to sterilize the skin and blood collected by skilled nurses or phlebotomists are employed to minimize these risks. Total blood volume obtained will be less than that permitted by the Mass General Brigham IRB for study of disease populations.

Risks associated with PAH infusions
Para-aminohippurate (PAH) infusions may rarely cause flushing, tingling, nausea, vomiting and cramps. Very rarely PAH may cause a hypersensitivity reaction (including angioedema and

urticaria) or vasomotor disturbances. Subjects may have a sensation of warmth or the desire to defecate or urinate during or shortly following initiation of infusion. Discontinuation of the PAH infusion leads to the resolution of these symptoms.

Risks associated with Captopril
Captopril is a specific competitive inhibitor of angiotensin I converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I to angiotensin II. Administration of Captopril results in a reduction of peripheral arterial resistance in hypertensive patients with either no change, or an increase, in cardiac output. There is an
increase in renal blood flow following administration of Captopril and glomerular filtration rate is usually unchanged. Reductions of blood pressure are usually maximal 60 to 90 minutes after oral administration of an individual dose of Captopril. The duration of effect is dose related. The reduction in blood pressure may be progressive, so to achieve maximal therapeutic effects, several weeks of therapy may be required. The blood pressure lowering effects of captopril and thiazide-type diuretics are additive. In contrast, captopril and beta-blockers have a less than additive effect. Blood pressure is lowered to about the same extent in both standing and supine positions. Orthostatic effects and tachycardia are infrequent but may occur in volume-depleted patients. Abrupt withdrawal of Captopril has not been associated with a rapid increase in blood pressure.
Counterindications. Captopril is contraindicated in patients who are hypersensitive to this product or any other angiotensin-converting enzyme inhibitor (e.g., a patient who has experienced angioedema during therapy with any other ACE inhibitor).
Warnings and precautions. Presumably because angiotensin-converting enzyme inhibitors affect the metabolism of eicosanoids and polypeptides, including endogenous bradykinin, patients receiving ACE inhibitors (including Captopril) may be subject to a variety of adverse reactions, some of them serious. Excessive hypotension was rarely seen in hypertensive patients but is a possible consequence of captopril use in salt/volume depleted persons (such as those treated vigorously with diuretics), patients with heart failure or those patients undergoing renal dialysis. Use of drugs that act on the renin-angiotensin system during the second and third trimesters of pregnancy reduces fetal renal function and increases fetal and neonatal morbidity and death. Infrequently, an increase in potassium can occur. More common is a cough.

Risks associated with Angiotensin II infusions
AngII increases BP. The increase in BP can be accompanied by chest discomfort and rarely cardiac damage. The doses we will use, however, generally increase mean arterial pressure by only 6-10 mm Hg in subjects on a high sodium diet. We have extensive experience with these techniques, having performed such infusions in over 2,000 subjects in the past 25 years. Using these doses of AngII and monitoring BP every two minutes during the infusion of pressor agents, we have had only minor adverse events (<0.5%) and only two episodes of chest discomfort, neither of them cardiac in origin. We believe the potential risks of these infusions are minimal. A clinical investigator will be present for each of the infusions.

Risks associated with study diets
Subjects in this study will be asked to consume a liberal salt diet and a low salt diet. Increasing dietary sodium diet can elevate BP in some individuals. However, the liberal salt diet used in this study (calculated diet to 200 mEq/day) is similar to the "normal" salt diet (150-250 mEq/day) in the United States. We have performed over 2 dozen similar studies in ~2,000 hypertensive and normotensive individuals without adverse events. The liberal and low salt diets will be constructed by CCI Nutrition and Metabolic Core Kitchen and provided to enrollees after they have been interviewed by a research Registered Dietician to calculated caloric needs. Participants will be

instructed on how to check their own blood pressure at home with a study-provided automatic BP device (Omron, Kyoto, Japan). As per our IRB approved *Standard Operating Procedure for Blood Pressure Research Protocols*, they will check blood pressure daily and report results to study staff every 2-3 days. The study diet will be stopped if systolic BP is >169/109 mmHg on 2 occasions. As described above, subjects will consume a low salt diet prior to drug assignment and again at the end of study. Participants will be warned about signs and symptoms related to hypotension/orthostasis and instructed to refrain from strenuous physical activity while on the low salt diet. They will also be encouraged to drink water freely to maintain hydration and minimize orthostasis.

Unexpected laboratory results
A risk from participating in this study is the inadvertent discovery of abnormal laboratory tests that lead to anxiety, distress and added cost to the participant. This could include laboratory tests obtained at screening.

Risks associated with 24hour Ambulatory blood pressure
Participants will undergo at least 2 and up to 3, 24hr Ambulatory BP studies (ABP). ABP requires placing a BP cuff on the arm which is attached to a pump device. It records BP at set intervals during the day with lower frequencies at night. Discomfort from wearing the device includes those associated with arm constriction and disrupted sleep.

Risks of genotyping
All participants will undergo genotyping to determine genotype status at the at-risk loci for striatin gene. This procedure involves a cheek swab sample obtained under direct observation. The sample is identified with a participant code only. Samples are sent via express overnight mail service to Transnetyx for rapid genotype determination at only these loci. Results are reported back to the study team in under 48hrs. Samples are then discarded.
We developed this service with Transnetyx and have used it in several genotype-based recruitment protocols.

Vascular Biopsy
Details of how to isolate of the superficial inferior epigastric artery (**SIEA**) are included in the **Strategy**, **Approach,** and **Special Techniques** sections of the application. The area will be numbed with an anesthetic (Lidocaine). The risks associated with the Lidocaine injection include burning, itching, pain, redness, or swelling at the injection site. The size of the vessel removed will be that of a drinking straw and about 1.5 inches long. Some risks associated with the biopsy procedure include mild discomfort/pain, swelling, bleeding, and scarring at the biopsy site. Bruising and a temporary small depression in the skin and/or bumps under the skin where the vessel has been removed will likely occur. The discomfort lasts for 12-36 hours. We have performed 30 of these procedures without any other adverse events. Subjects will return 1 week after the biopsy to inspect the surgical site and contact study staff if there are any concerns. Removal of this vessel likely occurs more than 1000 times daily in the US as it occurs with abdominoplasty, reconstructive breast surgery, and hernia repair.

Obtaining vessels from a fat biopsy has major limitations. 1) From the buttock, it is uncomfortable, with an increased risk of infection and poor healing. 2) The vessels are difficult to locate. 3) The micro-dissection results in damage to the vessel that renders them useless for functional studies. Obtaining SIEA from the abdomen eliminates these limitations (Dr. Dennis Orgill [a plastic surgeon, see attached letter]). SIEA is easily located in the lower abdomen. It is routinely divided during inguinal hernia repairs and C-Sections. When lower abdominal skin is removed following

pregnancies or massive weight loss, procedures such as a tummy tuck or panniculectomy, both SIEA arteries are completely removed. The anatomical location is reproducible, and with the aid of an ultrasound device - these vessels are easily harvested under local anesthesia, taking about 20 min. The invasiveness of the procedure is like removing a sebaceous cyst. Thus, the procedure is benign, and the vessel loss is inconsequential. Because of the location, the scar heals nicely and is hidden and small. Of the nine previous subjects studied, only one had to be seen post-op, and his wound healed without additional treatment, documenting the minimal risk of the procedure. The area is prepped, draped in the usual sterile fashion, and infiltrated with 0.5% lidocaine with epinephrine, 1:200,000. A low transverse incision of ~2 cm is made over the Doppler mark, a 2-4 cm^3 dissection of fat, including the artery, is taken, placed in a sterile cup with saline, delivered immediately to the laboratory, closed with dissolvable sutures and checked in 1wk.

8. Benefits

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS
Participants are informed that no specific individual benefit is likely to come from participation in this project. However, if this project uncovers a genetic association between factors that could modify the treatment of vascular disease, there may be substantial benefit to our general knowledge of blood pressure regulation and treatment. They may receive individual knowledge benefit with regard to the influence of dietary salt on their blood pressure when participating in the high and low salt diet phases. We feel that these combined potential benefits outweigh the risks.

IMPORTANCE OF THE KNOWLEDGE TO BE GAINED
Successful completion of this project will substantially increase our understanding of mechanisms underlying specific forms of hypertension. Furthermore, the results will provide insights into the genetic basis for the varying susceptibility to vascular complications.

9. Statistical Analysis

Statistical Considerations
Power calculation[59]**:** Our power calculation is based on the difference between the Risk and the Non risk SSBP defined as the systolic BP on the liberal Na^+ diet minus on the restricted Na^+ diet at the end of study in the risk versus non-risk homozygotes. Using our published data for several SSBP studies[11,52,58,60-62] and **Preliminary Data** (P=0.01, means: 14.5 vs 8.3 mmHg, homozygous risk vs. non-risk diplotypes, respectively, with SD 12.5) our sigma varied from ~8-13. The mean differences varied from ~5-8 mmHg. Based on these data, with an α of 0.05 and 50 completers in each of the two diplotype groups, we could detect better than a 45-90% difference in genotype-based SSBP (**Table**).

SSBP (mm Hg)		SD within group for the changes (mm Hg)	Power
Non-Risk n=50	Risk n=50		
8	15	12	0.82
8	15	11	0.88
8	15	10	0.93
8	15	9	0.97
9	15	10	0.84
9	15	9	0.91
9	14	9	0.78

Table. Scenarios providing sufficient power and a 5% 2-sided alpha.

Assuming a completion rate of 90-95%, we will enroll 55 individuals/diplotype group (~27-28 each for men and

women). To conservatively adjust the sample size to include an assessment of covariates, we have used the following approach: If your primary exposure variable is x and you run a regression of x on 1 or more other covariates labeled z_1, z_2,... z_k, then the sample size adjusted for covariate effects is n_adjusted = n / (1 - R^2) where R^2 is the R^2 from running a regression of x on z_1, z_2,... z_k. So, if R^2 = 0.10, then n_adjusted = n/0.9, which is an increase of about 11%---~ 50 subjects/diplotype group.

Statistical plan: Standard statistical factors will be determined for each variable noted in the **Human In vivo Study,** usually using two-way ANOVA followed by the Tukey or Newman-Keuls post hoc test for multiple comparisons.
Our **primary endpoint** is the differences in systolic SSBP between the diplotype groups.
Secondary endpoints are **1)** the differences in β_2AR expression levels in PBMCs and SIEAs by diplotype **2)** the Na+ excretion response to acute Na+ load by diplotype.
Exploratory endpoints are: **1)** systolic and diastolic BPs on each diet by diplotype group; **2)** on each diet and differences between the two diets for IL-6, MCP-1, and 24hr urine cystatin C, albumin, and KIM-1 – by diplotype; **3)** β_2AR protein levels by diplotype, and diet in PBMCs and SIEAs.

10. Monitoring and Quality Assurance

The clinical studies in this project are not phase III clinical trials by NIH definition. The studies are Basic Experimental Studies Involving Humans (**BESH**). These studies willbe monitored by faculty and research study staff and the Cardiovascular Endocrine Research Group (**CERG**), which acts as a data/safety monitoring board for projects performed by CERG physicians and scientists. The Board meets monthly, usually on the 3rd Tuesday of each month from 1:15-2:30. The chair of the Board for this project is Dr. Ellen Seely, Professor of Medicine, Harvard Medical School. The other physicians on the Board forthis project are Drs. Anand Vaidya, Associate Professor of Medicine, Harvard Medical School, and Gail Adler, Professor of Medicine, Harvard Medical School. There are also two non-physician members of the Board. For each project, none of the board members are involved in the study being reviewed. All members of the Board have completed human-subject protection education and passed a quiz on the responsible conduct of human studies prior to participating in the Board. The data are examined on every subject, as they become available, for accuracy and safety. The Board also reviews all protocols, protocol deviations, and adverse events in their monthly meeting. Since these studies are not phase II or III clinical trials, the Board will not look at the efficacy of the study. Consequently, there will be no interim analysis of the data for efficacy,and there will be no need to adjust the alpha value for significance for multiple analyses of the data except as pre-analyses conditions, as stated in the statistical section of the protocol. The minutes of the monthly Board meeting are signed by the members attending, and both a hard copy and electronic version are kept. Finally, the study will be registered in *Clinicaltrials.gov* if activated.

The IRB reviews protocol compliance at the minimum yearly and sooner if any serious adverse events occur. Any adverse events will be reported to the IRB. The MGB IRB requires reporting within 5 working days of unanticipated problems including adverse events that are related or possibly related and place participants or others at a greater risk of harm than was previously known or recognized. The IRB can suspend or terminate the study if necessary or require modifications to the research plan. Any suspension of the project by the IRB for a serious adverse event will be communicated to the NIH.

All data collected has patient identifiers removed. All samples (blood, urine, etc.) and questionnaires are de-identified. A unique code is used to identify all the samples. A master list linking the participants' names to their codes is kept locked in the Co-I's research area. Only Dr. Williams (**Co-I**) and one research staff member (Ms. Foote) have access to these data. For studies that use a double-blinded study approach, the key to breaking the blinding will be kept by a research group member who is not directly involved in any aspect of the study, such as recruitment. The database where all the data is entered will have no identifiers that would allow someone to identify a particular subject. The database will comply with both IRB and HIPPA regulations.

11. Data and Research Material Sharing

A) Sending Data/Materials to Research Collaborators outside Mass General Brigham

The data collected will comply with the NIH requirements for timely release and data sharing. Data will be shared in the form of publications and presentations in scientific forums. However, the primary dissemination of the clinical study data will be through clinicaltrials.gov. We will register this clinical study on clinicaltrials.gov upon funding of our application. As the NIH has noted, the investigators reserve the right to keep the data restricted in order to perform the initial analyses for this grant proposal and will continue to use the data for further, but not prolonged, exclusive use. Of note, the sharing of these data will be limited by at least the following issues, some unique to this proposal and some not unique. Some of the data obtained in this study is defined to be sensitive in nature, which may restrict its ability to be shared. Data may only be shared with the approval of our IRB and is limited by HIPPA and any other regulations that may be promulgated during the course of this proposal. Data submitted to clinicaltrials.gov meet these qualifications.

DATA TYPE

Types and amount of scientific data expected to be generated in the project: summarize the types and estimated amount of scientific data expected to be generated in the project.

The proposed project will produce data from mouse studies. We will develop a colony of mice that display a mutant form of the beta-2 adrenergic receptor, expressing the $Arg^{16}Arg/Gln^{27}Gln$ diplotype. The mouse strain used for this research project was generated by using the CRISPR/Cas9 method: the point mutations G46A and G79C (leading to functional, $Gly^{16}Arg$ and $Glu^{27}Gln$ amino acid changes) were introduced in C57BL/6J embryos, followed by breeding for positive founders and subsequently germline transmitted F1 mice. The presence of the diplotype was initially verified by sequencing; subsequent genotyping is performed by using proprietary TaqMan SNP Genotyping Assays (Applied Biosystems, Inc.). From mice, our project will produce data from the following methods: 1) endothelial dysfunction analyses in carotid vessels to assess vascular reactivity ex vivo (using pressure myograph analyses); 2) circulating levels of measures of the renin-angiotensin-aldosterone pathway; 3) inflammatory marker assessment in circulation; 4) ex vivo measures of hormones production and aldosterone biosynthetic pathway molecules from isolated zona glomerulosa cells 5) In a model of aldosterone/mineralocorticoid receptor-mediated cardiovascular and renal damage we will assess the *in vivo* and *ex vivo* effects of the diplotype and biological sex on mesenteric vascular reactivity, and cardiac and renal damage; 6) we will assess gene expression by RT-PCR, as well as protein levels using Western blot analyses and ELISA; 7) blood pressure

measurements by telemetry. An open-source-friendly file format will be used to store raw data and for all subsequently processed datasets used for statistical analyses (CSV, an open-source file format).

Also, the proposed project will produce data from human studies. We will collect data from women and men participating in a seven-day liberal salt and restricted salt study from approximately 100 subjects. These hypertensive study participants will have been genotyped for the presence of the risk and non-risk diplotypes for the beta-2 adrenergic receptor. We will collect the following data on both diets in each study participant: 1) aldosterone and PRA responses, 2) beta-2 adrenergic receptor levels, 3) inflammatory markers, and 4) vascular factors.

We expect to generate the following data file types and formats during this project: images (TIFF and JPEG), and tabular (CSV). All raw data files from will be analyzed to generate CSV files (containing RT-PCR data, western densitometry, hormonal levels, microscopy data, and ELISAs), and to enable statistical analysis. The total size of the data collected is projected to be approximately 1,200 GB.

Scientific data that will be preserved and shared, and the rationale for doing so: describe which scientific data from the project will be preserved and shared and provide the rationale for this decision.

Based on ethical, legal, and technical considerations, only the following data produced during the project will be preserved and shared: for human data the following will include blood pressure, renal blood flow, data from blood and urine specimens provided. Appropriate measures such as each subject will have a unique identifier unrelated to any demographic data the link between the person and the unique identifier will be available only to PI, and informed consent forms will reflect those plans. For the animal studies, data from RT-PCR, western blots, histology, blood pressure, renal blood flow, and data from blood and urine specimens will be available.

Metadata, other relevant data, and associated documentation: briefly list the metadata, other relevant data, and any associated documentation that will be made accessible to facilitate interpretation of the scientific data.

To facilitate the use of the data, a README file (TXT) will be generated and deposited into a repository along with all shared datasets. The README file will include links to published data, a description of methods/equipment used, source of antibodies, RT-PCR primers sets, and quality control assessments. A data dictionary will be created to define and describe all variables in the dataset.

Related tools, software and/or code

State whether specialized tools, software, and/or code are needed to access or manipulate shared scientific data, and if so, provide the name(s) of the needed tool(s) and software and specify how they can be accessed.

The raw data generated from the RT-PCR gene expression analyses will be stored in CSV file format, an open-source-friendly file format. These will be available no later than when a publication is accepted for publication or at the completion of our project.

Standards

State what common data standards will be applied to the scientific data and associated metadata to enable interoperability of datasets and resources, and provide the name(s) of the data standards that will be applied and describe how these data standards will be applied to the scientific data generated by the research proposed in this project.

In accordance with FAIR Principles for data, we will use open file formats (e.g., JPEG, TIFF, CSV, TXT, and PDF). Information needed to make use of our data [*e.g., the meaning of variable names, codes, information about missing data, and other metadata*] along with references to the sources of those standardized names and metadata items will be included.

Data preservation, access, and associated timelines
Repository where scientific data and metadata will be archived
All datasets to be shared will be deposited in Harvard Dataverse (https://dataverse.harvard.edu/)

How scientific data will be findable and identifiable: describe how the scientific data will be findable and identifiable.
The Harvard Dataverse provides searchable study-level metadata for dataset discovery. Harvard Dataverse assigns DOIs as persistent identifiers and has a robust preservation plan to ensure long-term access. Data will be discoverable online through a standard web search of the study-level metadata as well as the persistent pointer from the DOI to the dataset. We will use unique identifiers such as ORCID iDs for people to improve data findability across all dissemination outputs.

When and how long the scientific data will be made available: describe when the scientific data will be made available to other users (i.e., no later than time of an associated publication or end of the performance period, whichever comes first) and for how long data will be available.
All scientific data generated from this project will be made available as soon as possible, and no later than the time of publication or the end of the funding period, whichever comes first. The duration of preservation and sharing of the data will be a minimum of 10 years after the funding period.

Access, distribution, or reuse considerations
Factors affecting subsequent access, distribution, or reuse of scientific data: nih expects that in drafting plans, researchers maximize the appropriate sharing of scientific data.
There are no anticipated factors or limitations that will affect the access, distribution or reuse of the scientific data generated by the proposal.

Whether access to scientific data will be controlled: state whether access to the scientific data will be controlled (i.e., made available by a data repository only after approval).
Controlled access will not be used. The data that is shared will be shared by unrestricted download.

Protections for privacy, rights, and confidentiality of human research participants: describe how the privacy, rights, and confidentiality of human research participants will be protected.
To protect study participant privacy and confidentiality, shared data will use a unique identifier unrelated to any demographic data that will be available only to PI. Informed consent forms will reflect these plans. To ensure participant consent for data sharing, IRB paperwork and informed consent documents will include language describing plans for data management and sharing of data, describing the motivation for sharing, and explaining that personal identifying information will be removed.

Oversight of data management and sharing
Describe how compliance with this plan will be monitored and managed, frequency of oversight, and by whom at your institution (e.g., titles, roles).
Lead PI – Luminita Pojoga (ORCID: 0000-0002-9448-6333), will be responsible for the day-to-day oversight of the laboratory/team data management activities and data sharing. Broader issues of DMS Plan compliance oversight and reporting will be handled by the PI and Co-I team as part of general stewardship, reporting, and compliance processes.

B) Receiving Data/Materials from Research Collaborators outside Mass General Brigham

NA

12. Privacy and Confidentiality

- ☐ Study procedures will be conducted in a private setting.
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected.
- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)
- ☒ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol.
- ☐ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies.
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research.
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens.
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research.
- ☐ Additional privacy and/or confidentiality protections

Subjects will have information collected that may be of a sensitive nature (e.g. DNA). All results will be recorded using a study-specific numerical code to be assigned to the individual participant. Samples (urine, blood or DNA) will be given the same code. A master list linking the participants' names to their codes is kept locked in the Co-I's research area. Only Dr. Williams (Co-I) and one member of the research staff (Ms. Kristen Foote) have access to this data. The database where all the data are entered will have no identifiers that would allow someone to identify a particular subject. The database will comply with both IRB and HIPPA regulations. No photographs or recorded material will be obtained from these subjects. The subject will be informed that the anonymized completed results from the study will be submitted to Clinicaltrials.gov.

The Mass General Brigham IRB reviews protocol compliance at the minimum yearly and sooner if any serious adverse events occur. The Safety and Educational Oversight Committee reviews the Adverse Event log at the annual IRB Continuing Renewal. All physicians and research staff have completed a human-subject protection education coursed and passed tests on the responsible conduct of human studies prior to participating on the project. Unanticipated problems including adverse events will be reported to the IRB via an Other Event per MGB IRB reporting guidelines.The IRB has the ability to suspend or terminate the study if necessary or require

modifications to the research plan if needed. Any suspension of the project by the IRB due to a serious adverse event will be communicated to the NIH.

13. References

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APPENDIX A

Data Monitoring Committee / Data and Safety Monitoring Board Appendix

- *To be completed for studies monitored by Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) if a full DMC/DSMB charter is not available at the time of initial IRB review.*
- *DMC/DSMB Charter and/or Roster can be submitted to the IRB later via Amendment, though these are not required* if this Appendix has been completed and approved by the IRB.

A Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The following characteristics describe the DMC/DSMB convened for this study (Check all that apply):

☒ The DMC/DSMB is independent from the study team and study sponsor.

☒ A process has been implemented to ensure absence of conflicts of interest by DMC/DSMB members.

☒ The DMC/DSMB has the authority to intervene on study progress in the event of safety concerns, e.g., to suspend or terminate a study if new safety concerns have been identified or need to be investigated.

☒ Describe number and types of (i.e., qualifications of) members:
3 physicians, 2 non-physicians

☒ Describe planned frequency of meetings:
Once a month

☒ DMC/DSMB reports with no findings (i.e., "continue without modifications") will be submitted to the IRB at the time of Continuing Review.

☒ DMC/DSMB reports with findings/modifications required will be submitted promptly (within 5 business days/7 calendar days of becoming aware) to the IRB as an Other Event.

The clinical studies in this project are not phase III clinical trials by NIH definition. The studies are Basic Experimental Studies Involving Humans (**BESH**). These studies will be monitored by faculty and research study staff and the Cardiovascular Endocrine Research Group (**CERG**), which acts as a data/safety monitoring board for projects performed by CERG physicians and scientists. The Board meets monthly, usually on the 3rd Tuesday of each month from 1:15-2:30. The chair of the Board for this project is Dr. Ellen Seely, Professor of Medicine, Harvard Medical School. The other physicians on the Board for this project are Drs. Anand Vaidya, Associate Professor of Medicine, Harvard Medical School, and Gail Adler, Professor of Medicine, Harvard Medical School. There are also two non-physician members of the Board. For each project, none of the board members are involved in the study being reviewed. All members of the Board have completed human-subject protection education and passed a quiz on the responsible conduct of human studies prior to participating in the Board. The data are examined on every subject, as they become available, for accuracy and safety. The Board also reviews all protocols, protocol deviations, and adverse events in their monthly meeting. Since these studies are not phase II or III clinical trials, the Board will not look at the efficacy of the study. Consequently, there will be no interim analysis of the data for efficacy, and there will be no need to adjust the alpha value for significance for multiple analyses of the data except as pre-analyses conditions, as stated in the statistical section of the protocol. The minutes of the monthly Board meeting are signed by the members attending, and both a hard copy and electronic version are kept. Finally, the study will be registered in *Clinicaltrials.gov* if activated.

The IRB reviews protocol compliance at the minimum yearly and sooner if any serious adverse events occur. Any adverse events will be reported to the IRB. Unanticipated problems including adverse events will be reported to the IRB within MGB IRB guidelines. The IRB can suspend or terminate the study if necessary or require modifications to the research plan. Any suspension of the project by the IRB for a serious adverse event will be communicated to the NIH.

All data collected has patient identifiers removed. All samples (blood, urine, etc.) and questionnaires are de-identified. A unique code is used to identify all the samples. A master list linking the participants' names to their codes is kept locked in the Co-I's research area. Only Dr.

Williams (**Co-I**) and one research staff member (Ms. Foote) have access to these data. For studies that use a double-blinded study approach, the key to breaking the blinding will be kept by a research group member who is not directly involved in any aspect of the study, such as recruitment. The database where all the data is entered will have no identifiers that would allow someone to identify a particular subject. The database will comply with both IRB and HIPPA regulations.