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Official Title: Mechanism and Effects of Manipulating Chloride Homeostasis in Acute Heart Failure

Date: 9/28/2022

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HRP-503B – BIOMEDICAL RESEARCH PROTOCOL

Protocol Title: Mechanism and Effects of Manipulating Chloride Homeostasis in Acute Heart Failure

Principal Investigator: Jeffrey M. Testani, MD, MTR, Associate Professor of Medicine: Cardiology

Version Date: Version #13: 09.15..2022

Clinicaltrials.gov Registration #: NCT03446651

Does this study have a Clinical Trials Agreement? No

Will this study have a billable service? No

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital (YNHH) or Yale Medical Group (YMG) to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either YNHH or YMG (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? **Yes**

Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? **Yes**

Will you be using any new equipment or equipment that you have not used in the past for this procedure? **No**

Will a novel approach using existing equipment be applied? **No**

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes YNHH patients, including patients at the HRU, the Principal Investigator (PI) and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. **By submitting this protocol as a PI, you attest that you and any coinvestigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH**

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SECTION I: RESEARCH PLAN

1.0 Statement of Purpose: The overarching goal of this proposal is to develop a comprehensive understanding of the biology and therapeutic potential of sodium-free chloride supplementation. While sodium homeostasis has been the focus of substantial investigation, very little research has been devoted to understanding chloride homeostasis. Thus, this proposal is designed to obtain the full spectrum of information pertaining to chloride, such as novel areas with great interest by the scientific community (i.e. modulation of the WNK-kinase system and the use of exosomes), to more practical/basic questions (i.e. what happens to sodium chloride balance when a patient is challenged with chloride). The proposed multi-site inpatient study has been designed to serve as a real world efficacy study. With extensive biobanking and analysis of samples in the proposed setting, we will be able to deliver a great wealth of information on the biology and therapeutic potential of manipulating chloride homeostasis in heart failure.

We have seen that many heart failure therapies demonstrate measurable benefit in highly controlled environments, but lack effectiveness when studied in decompensated patients receiving standard decongestive therapies. As such, this study seeks to understand the effects of chloride supplementation on volume-overloaded patients concomitantly treated with IV diuretics.

2.0 Probable Duration of Project: Subject enrollment is planned to last 5 years, with a target rate of 25 patients per year. Enrollment of up to 5 patients into the open-label arm will take place over the course of 12 months. Biomarker measurement will occur on a rolling basis staggered from specimen collection by approximately 6 months. Completion of enrollment, statistical analysis, and publication is planned to be complete within 6 years.

3.0 Scientific Background and Study Rationale

Scope of the problem. Nearly 7 million Americans are affected with heart failure (HF), and its prevalence is expected to increase to nearly 10 million by 2030.[1] At age 40, an American's lifetime risk of developing HF is 1 in 5.[1] HF causes tremendous morbidity and mortality, with over 1 million hospitalizations in the U.S. each year and with HF listed on 1 in 9 death certificates respectively.[1] While HF is generally regarded as the inability of the heart to pump sufficient blood, on a population level, congestion is the primary driver of symptoms leading to hospitalization.[2-5]

Our understanding of sodium homeostasis and its management in HF is incomplete: It has been recognized for some time that HF is a sodium avid state, and that retained sodium is the primary driver of volume overload.[6] The simplistic paradigm has followed that salt should be avoided or minimized as much as possible. However, this fails to consider that the normal (appropriate) response of the kidney to a restriction in salt is compensatory neurohormonal activation and increased sodium avidity.[6] While it is known that administering saline to an otherwise healthy person with hypovolemia (e.g., dehydration from diarrheal illness) will correct the neurohormonal activation and sodium avid state, it has been assumed that this physiology would not apply to HF. This is due, in part, to the fact that many HF patients with overt volume overload continue to display sodium avid physiology. Thus, it is commonly believed that the ability of salt to "quench" the sodium avid state in HF is defective and thus salt administration would only lead to worsening volume overload. However, it is important to stress that we have limited understanding of the actual sensor(s) for sodium avidity in HF beyond difficult to measure concepts such as effective arterial under-filling.[7]

Accumulating evidence suggests more salt can sometimes be beneficial in HF: A growing body of recent evidence challenges the assumption that salt is universally detrimental in HF. Dr. John Burnett's group recently conducted an experiment where they varied the sodium intake from

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0.25g/day to 5.8g/day in ~20kg dogs undergoing HF induction with rapid pacing.[8] Not surprisingly, they found massive suppression of the renin angiotensin aldosterone system (RAAS) with high salt. However, what was remarkable was that filling pressures and body weight were not different between groups and ejection fraction and clinical HF symptoms were significantly better in the dogs on high salt diet.[8] There have also been multiple observational and more than 10 randomized trials of modifying sodium intake in human HF.[9-25] In these studies, there was substantial heterogeneity in the effects of high vs. low salt on biomarkers with some studies showing both improved volume status (natriuretic peptides) and RAAS activation, some showing improved RAAS activation, but at the expense of worsened volume status, and some showing worsening in all parameters. Several studies in acute decompensated HF have shown that high salt diet or administration of sodium chloride intravenously can improve diuresis, renal function, and outcomes.[17, 26-28] In studies reporting hospitalization and mortality, the effect was also heterogeneous, but many studies actually demonstrated substantial benefit from a high salt. These conflicting data on the risk/benefit of salt restriction motivated the American Heart Association/American College of Cardiology and European Society of Cardiology guideline committees to remove the specific recommendation on the quantity of sodium intake for HF patients.[29, 30]

A critical advance would be disassociating the beneficial from harmful effects of salt: From the existing data on salt in HF we can conclude: 1) Increasing salt intake is a double-edged sword with competing benefit and harm. 2) Whether a harmful or beneficial effect will predominate varies greatly across study populations. 3) It is unclear what factors drive this variability. Although further research to determine the optimal salt intake in HF is clearly warranted, disassociating the beneficial sodium avidity "quenching" aspects from the volume retaining aspects of salt would be a more direct path.

It has been known for decades that the kidney "sees" salt through chloride rather than sodium. Sodium is unequivocally the primary driver of extracellular volume, and thus responsible for volume overload in HF.[6] However, renal salt sensing appears primarily driven by the chloride anion rather than sodium cation. It has been known for decades that salt sensitive renal responses such as tubuloglomerular feedback and renin release are determined by chloride rather than sodium. For example, loading rats with various chloride-free salts, ($NaHCO_3$, NaAcetate, $NaNO_3$, $KHCO_3$, CaGluconate) failed to suppress renin secretion. However, when rats were loaded with sodium chloride or various sodium free chloride salts (KCl, cholineCl, $CaCl_2$) significant suppression of renin occurred.[31] Nearly identical observations have been made for tubuloglomerular feedback.[32, 33] Furthermore, with selective depletion of either chloride or sodium by dialysis with maintenance of euvolemia, only chloride depletion causes an increase in plasma renin activity (PRA).[34]

The WNK-Kinases are the molecular sensors for chloride and the "master regulators" of sodium-chloride transport: Recently, a family of serine-threonine kinases (With-No-Lysine (K), WNK) has been demonstrated to play a key role in the regulation of electrolyte homeostasis, the actions of the renin-angiotensin-aldosterone system, and the transporters upon which loop and thiazide diuretics work.[35-38] Notably, chloride appears to bind directly to the catalytic site of at least two of the WNK kinases, inhibiting their auto-phosphorylation and activation.[39-43] In the setting of low chloride, auto-phosphorylated WNK kinases regulate solute transport through phosphorylation of SPAK (STE20/SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress–responsive kinase 1). These kinases then phosphorylate highly conserved residues of major sodium transporters in the kidney such as Na-K-2 chloride co-transporter (NKCC2) and electro-neutral sodium chloride cotransporter (NCC).[40, 41, 44-57] NKCC2 is the target of loop diuretics and NCC the target of thiazide diuretics, transporters that mediate >25% of renal salt reabsorption (>140 grams of sodium a day in health).[49, 58, 59] Thus, small aberrations in their regulation can have substantial influence on sodium

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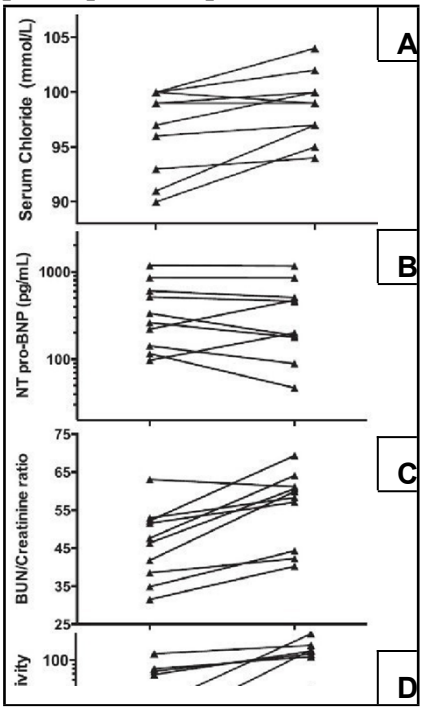
balance.[60, 61] In addition to direct regulation by phosphorylation, the WNK system modulates protein abundance of its targets and constituents by multiple factors such as clathrin-mediated endocytosis, altering intracellular trafficking, and gene expression.[53, 62-83]

Chloride is important in human HF: The constellation of observations and biology described in the paragraphs above led us to hypothesize that chloride may play a significant role in human HF. Over a series of 7 peer-reviewed publications, we have demonstrated several key findings:[84-90] 1) Chloride and sodium levels are much less closely linked than one would assume (r^2=0.19), and many patients have isolated low chloride or isolated low sodium **(Figure 1A)** 2) Low chloride, rather than low sodium, is strongly associated with diuretic responsiveness **(Figure 1B)**. 3) Chloride is linked to renin levels (r=-0.46, p<0.001), but sodium has no incremental association (p=0.49). 4) Chloride depletion appeared to be an important contributory mechanism to hypochloremia in HF patients as the fractional excretion of chloride was higher [1.1% (0.6-3.4) vs. 0.6 (0.3-1.5), p=0.04)] and urine osmolality was lower (301±54 vs. 366±139 osmol/L, p=0.002) in hypo vs. normochloremic patients. 5) Chloride levels were prognostically very important, whereas sodium had no independent association with mortality **(Figure 1C)** 6) The prognostic importance of low chloride appeared to be primarily restricted to patients with depletional, rather than dilutional, hypochloremia (p interaction <0.0001).

Investigation of new compounds that often take decades before they will be available at the bedside, and when available, are generally very expensive. Given that chloride is a macronutrient and available as the counter ion in a number of different FDA approved formulations, amino acids, and dietary supplements, additional research and deployment to patients can occur rapidly and with a fraction of the expense of new drug development.

Efficacy trials of decongestive therapies are conducted in hospitalized HF patient. In this setting, intercepting the patient before usual care is initiated is remarkably challenging as patients arrive via multiple portals of entry 24 hours a day. Furthermore, there is great heterogeneity in therapy with respect to type, intensity, and duration of treatment. These factors introduce substantial heterogeneity into the measurements and makes it difficult to keep participants on protocol.



Transitional Care Clinic (YTCC) is a 5-bed nurse

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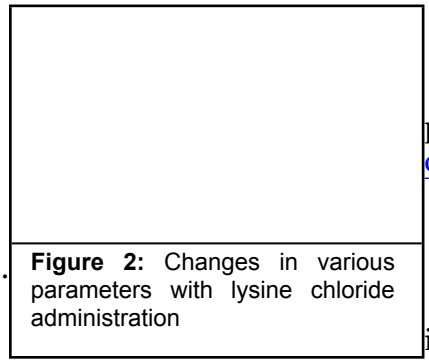

Figure 2: Changes in various parameters with lysine chloride administration

The Yale practitioner run/physician supervised outpatient diuretic treatment center where protocolized care is delivered to decompensated HF patients.(https://www.ynhh.org/services/heart-failure/transitional-care-center.aspx). Here HF patients with volume overload are referred as an alternative to hospitalization. Patients present in the morning to a single location and are treated with protocol driven intravenous (IV) loop diuretics, monitoring, follow-up, etc. The diuretic treatment protocol involves aggressive stepwise escalation of IV diuretics every few hours until urine output is adequate, with addition of metolazone if loop monotherapy fails. These protocols were based upon the NHLBI Heart Failure Network CARRESS and DOSE trial findings/algorithms.[94, 95] As such, "usual care" in the YTCC is state-of- the-art aggressive diuretic therapy. The YTCC allows us to eliminate many of obstacles to the study of decompensated HF and dramatically reduce resources required to complete a study of decongestive therapy. As a result of these advantages, we have studied over 200 patients in the YTCC since its creation.

Preliminary data with relevance to all aims: A pilot study of sodium free chloride administration was performed in 10 stable HF outpatients.[2, 52] The primary purpose of the pilot was to determine feasibility, to ensure volume status wouldn't worsen with the chloride load (this was unlikely, but unknown), and to see if any biologic effects plausibly related to chloride dependent processes could be detected. Details of inclusion/exclusion have been previously published[2]. Briefly, enrollment required a diagnosis of HF, a loop diuretic dose of ≥80 mg/day furosemide equivalents, clinical stability and optimal volume status per an advanced HF physician. The median diuretic dose in those enrolled was 160 mg/day and the median NT-proBNP was 298 pg/ml, indicating a euvolemic population on high dose diuretics. There was no specific inclusion related to serum chloride levels, but the median level was 98 mmol/L and all patients had a chloride ≤100 mmo/L. Seven grams of lysine chloride was administered three times a day, providing 115 mmol/day of sodium free chloride daily. 115 mmol/day chloride supplementation was chosen as this should approximately double daily chloride intake. Patients were instructed to consume a 2-gram sodium diet for the week prior to and during the intervention period. All diuretics and HF medications were maintained constant over the duration of the study.

Compliance with lysine chloride appeared to be good as serum chloride improved in 8 of 10 patients, (p=0.01, **Figure 2A**), however compliance with the 2-gram sodium diet in these outpatients was poor as the pre-intervention median 24-hour sodium excretion was over 5 grams. Given the uncontrolled diet rich in sodium chloride we anticipated null findings. However, we were quite surprised to find striking signals supporting a meaningful change in volume status in these euvolemic patients. Hemoconcentration (a surrogate for improvement in blood volume) occurred in 8 of 10 patients (p=0.036), which is remarkable as this occurs in less than 50% of hospitalized HF patients receiving multiple days of IV diuretics.[98, 99] An improvement in body weight occurred in the majority of patients with 3 patients losing more than 5 pounds. We also saw an improvement in natriuretic peptide levels in 8 of 10 patients, with a relative improvement of 25% in those that did improve (**Figure 2B**, p=0.01). Further support for a reduction in blood volume in these euvolemic patients came from the fact that the blood urea nitrogen to creatinine ratio (p=0.007, **Figure 2C**) and PRA (**Figure 2D**, p=0.005) also increased. Despite the change in volume status in these euvolemic HF patients, kidney function remained stable with no change in creatinine, cystatin C, serum potassium, or the markers of kidney injury neutrophil gelatinaseassociated lipocalin, kidney injury molecule-1, and interleukin-18, p=NS for all. As

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described in detail in the preliminary data section of Aim 3, we observed a remarkable 72% ± 33% reduction in the level of NCC (p=0.0005). This is contrary to the expectation that a reduction in volume and increase in PRA would be associated with significant upregulation of sodium transporters. Thus finding the strong signal of reduction in NCC in this setting is in support of the hypothesis that sodium free chloride supplementation is in fact suppressing WNK-kinase mediated sodium avidity.

Rationale for blood volume as an endpoint throughout this proposal: Change in blood volume was selected as the primary endpoint to measure the effect of lysine chloride on volume for several reasons: 1) The majority of metrics of volume status are either imprecise (e.g., physical examination, change in weight or fluid loss), invasive (e.g., right heart catheterization), or of unclear clinical relevance (e.g., bioimpedance or ultrasound derived parameters). 2) A normal blood volume or a change in blood volume in the setting of decongestive therapy is prognostically very important.[98-104] Notably, a change in body weight is not reproducibly linked to improved outcomes.[105] Furthermore, significant change in total body water with tolvaptan did not translate into improved outcomes.[92] Thus change in intravascular volume appears to be an important parameter. 3) An FDA approved semi-automated test with very high precision is available to measure blood volume (http://www.daxor.com/wp-content/uploads/2014/10/bva100brochure.pdf). 4) Although the cost of measuring blood volume is substantially greater to that of a surrogate marker of intravascular volume (i.e., NT-proBNP), the increased variability in surrogate markers is dramatically greater than that of blood volume measurement. Notably, the average coefficient of variation in NT-proBNP levels obtained one week apart in stable HF, is remarkably large (>20% in some studies).[106] Directly measured blood volume appears to have a very narrow variance in stable HF. In n=26 stable outpatient HF patients with blood volume measured twice for research purposes 1 year apart, the coefficient of variation was only 7.6% (personal communication Dr. Wayne Miller, Mayo Clinic). Since the cost of the blood volume test will represent only a fraction of the cost of the overall per patient costs of the proposed prospective randomized trials, the smaller sample size possible the low variance blood volume endpoint will actually substantially reduce total costs.

4.0 Study Design: We propose a multi-site, randomized, double blind, placebo controlled study of 200 patients with decompensated heart failure receiving treatment with IV diuretics for volume overload. Participants will be enrolled at Yale New Haven Hospital and St. Francis hospital. This study was designed to be more pragmatic than the outpatient study (HIC2000022016) proposed in a separate protocol, with a parallel group randomization to 7 days of therapy with either 115 mmol/day of lysine chloride or placebo. 115 mmol/day of lysine chloride was chosen as this represents a similar cumulative chloride load to the higher dose 5-day inpatient study (separate HIC application). The primary endpoint will be the change in blood volume from baseline to 7 days (the range of days may vary between 5-8 days to minimize missing data).

Due to the current pandemic of COVID-19 (SARS-coV2) and to remain in place for the future, the number of days between the V1 and V2 may be shortened (less than 5 days). This will allow for the completion of the study while the patient remains admitted to the hospital. The shortened duration will be utilized if the patient will be discharged before the 5-8-day window and allowing for complete data collection in the safest way possible. Thus, potentially eliminating the need to bring the patient back as an outpatient to complete the V2 in times of a pandemic or reduced visit capacity.

We propose an additional study arm to be completed in up to 5 additional patients. This arm would be an open-label study in patients with decompensated heart failure receiving treatment with IV diuretics for volume overload. The proposed arm would consist of 7

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days (5-8 days to minimize missing data) of therapy with 115 mmol/day of lysine chloride. Patients enrolled into this arm will undergo identical study procedures to the randomized arm, with the exception of receiving only lysine chloride.

i.1 Study Protocol

A: Study Visit 1

1. Patient Arrival & Enrollment

a. **Enrollment:** Patients that meet inclusion/exclusion criteria will be approached during their admission at YNHH (York St 5-1, 5-2, 5-3, 5-4, 5-7, 58 or St. Raphael's Campus Verdi 5 East and Verdi 3 East). If they agree to participate, the patient will sign an Informed Consent form prior to any study procedures taking place.

b. **Vital Signs, Weight and Height:** Baseline vital signs, bladder scan, weight and height are measured at the start of the study visit. Vital signs and weight measurements are repeated at the end of the study visit.

c. **Pregnancy Testing:** A point-of-care pregnancy test is performed for women under the age of 65 who have not had a hysterectomy.

d. **IV Line Placement & Patient Acclimation**

1. **IV Line Placement:** A peripheral IV line is placed as part of the Standard of Care for receiving IV diuretics during admission at YNHH. If a suitable IV for the blood volume procedure is not available, one will be placed.
2. **If** a **KVO is required to keep the IV line patent, Dextrose 5% fluid will be used.**

e. **Acclimation:** Patient rests in a semi-recumbent position for approximately 60 minutes prior to undergoing blood volume determination and randomization.

f. **Screening:** After the peripheral IV line is placed, blood will be drawn for determination of venous pH via iSTAT and/or YNHH laboratory. If the patient's venous pH is less than 7.25, they will be withdrawn from the study.

g. **Patients will receive one of the following tracers depending on availability:**

1. Iothalamate Administration
    1 IV iothalamate: 1 cc of Iothalamate meglumine is administered intravenously in patients without a contrast allergy

2 Iohexol Administration
    2 IV Iohexol: 1 cc of Iohexol will be administered intravenously in patients without a contrast allergy

i. **Volumex Administration and Daxor Blood Volume Sample Collection**

i.1 Baseline Blood Collection: Blood is collected prior to the Volumex injection to establish a baseline that reflects background radiation.

i.2 Volumex Injection: Up to 25 microcuries of the Volumex tracer (radiolabeled albumin) is injected as an intravenous bolus (IV-push).

i.3 Blood Volume Serial Blood Collection A series of 5 postVolumex blood samples is collected after tracer injection, allowing for complete mixing in the bloodstream.

i.4 Blood Volume Sample Timing: Samples are ideally spaced ~6 minutes apart, and are collected approximately 12, 18, 24, 30 and 36 minutes after Volumex administration. Dextrose 5% fluid will be used to flush the IV line with administration of Volumex and in between blood draws for the blood volume.

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ii. **Standard YTCC or Inpatient IV Diuretic Therapy Protocol ii.1** The IV diuretic therapy prescribed by their treating clinician if inpatient.

iii. **Randomization & 1st Dose of Study Drug (Lysine Chloride or Placebo)** 1 After receiving the Standard IV Diuretic Therapy Protocol, the patient will then receive the first dose of study drug (lysine chloride or placebo). If patient is enrolled into the open-label arm, they will not be randomized but will receive the first dose of lysine chloride.
2 At this time, if a patient typically uses metformin, it will be discontinued for the duration they are on study medication (5-8 days).

- Only if holding metformin is clinically appropriate (per study PI and patient provider).
- Patients that have their metformin held while inpatient will be under the care of their treating physicians who will monitor their glucometer values and treat accordingly.
- Patients that have their metformin and are discharged from the hospital while still taking the study medication will be asked to check their glucose twice daily or per their usual schedule (whichever is greater) for the duration of the time metformin is held.
- If the participant does not own a glucometer, they will be provided a home blood glucose monitoring kit.
- Any consistent glucometer readings greater than 200 mg/dL while outpatient and still on study medication, the patient will be instructed to contact their study physician.

iv. **Blood Samples Collected iv.1 The total amount of blood samples collected at each visit (1 and visit 2) is 100cc.** This includes 36cc for the blood volume and an additional 64 cc collected during the study visits (1 and 2).

v. **Timed-Void Spot Urine Samples and Bladder Scans**

v.1 Timed spot urine samples will be collected periodically during the visit

v.2 Post-Void Residual Volume Bladder Scans: A post-void residual (PVR) volume is measured with a bladder scanner after each timed-void urine sample is collected.

vi. **Deuterium Oxide (D20) Administration**

Oral D20: 10.0 grams of liquid D20 is administered orally.

11.Imodium AD or Imodium(Loperamide)

a. Once on Lysine Chloride/ Placebo if the participant experiences any abdominal upset as unformed stools (loose stools, diarrhea) from the Lysine Chloride/ Placebo. They will have the option of taking over the counter Imodium AD or Imodium(loperamide)"as needed" to relieve those symptoms.

- The participant will be instructed to inform their treating physician when inpatient and the study team (if discharged) of any abdominal upset, the Imodium will be built into the epic order set(PRN order) for the care team to administer. If the patient is discharged, the PI or sub- I will be notified, and it is deemed appropriate; The study team will provide the participant with the Imodium.
- The participant will be assessed daily for additional or worsening unformed stools, if appropriate the patient will receive additional doses of Imodium according to the manufacturer's instructions in conjunction with approval from the treating physician(inpatient) or study PI or sub- I if (discharged).

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- Imodium over the counter: initial: Take 2 tablets/ capsules (4 mg) once. May take 1 additional tablet/ capsules after another episode of unformed stool. Up to a maximum of (8) 2 mg tablets or capsules or 16mg daily.
- The study team will provide the over the counter Imodium (once the patient is discharged) and the study team will record the number of doses taken per day

Patient Discharge vi.1 Vital Signs: Vital signs are obtained at the end of the study visit prior to discharge, to ensure patient safety.

vi.2 Home Urine Collection: patients will be given 24-hour urine collection containers to continue collecting urine at home.

B: Daily Monitoring and Dose Adjustment If a patient has a serum bicarbonate level less than 22mmol/L at screening, a venous pH and basic blood chemistries will be obtained via iSTAT and/or YNHH laboratory each day during the study period. 1cc of blood will be drawn each day to perform the test. If the patient has been discharged from YNHH and these tests are indicated, additional safety visits will take place (up to 5). For all patients, the results of any safety tests will be reviewed by the study physician and study nephrologist (Dr. Jeffrey Turner). If necessary, the study drug dose will be adjusted to once daily or no doses. The standard dosage is twice a day.

C: Continued IV Diuretic Therapy: The patient will remain admitted until the treating practitioner feels the patient no longer requires IV therapy, at which point they will be transitioned to oral diuretics.

D: Transition to Oral Diuretics: Upon transition to oral diuretics, the patients will be seen every other day (except for weekends) for laboratory monitoring of electrolytes and renal function. 10cc of blood will be drawn at each of these safety visits (which are separate from the additional safety visits that will be conducted based on serum bicarbonate), and a urine sample will also be collected.

E: Study Visit 2: After 7 days of randomized therapy (5-8 to minimize missing data), the patient will return or if still admitted have repeat blood volume assessment and bio-samples collection. The number of days of randomized therapy may be less than 5 days in order to allow for the completion of the visit 2 while the patient remains hospitalized, this would be utilized only during times of a pandemic or when there is an reduced visit capacity.

Patients enrolled into the open-label arm will return after 7 days of therapy with lysine chloride (5-8 to minimize missing data).

F: Follow Up Visit/ Phone Call: One week after the completion of Visit 2, subjects will be given the option to either come in for a follow up visit or complete a follow up phone call. The purpose of this follow up visit is to make sure they are not experiencing any side effects or adverse events after taking the study medication/placebo as well as obtain a current weight, vital signs, a 10cc blood sample and a urine sample. If the subject is not able to come in for the follow up visit, a follow up phone can be done in its place. During the phone call, the subject will be asked their current weight, how they are feeling and will be assessed for any adverse events.

G: Study Locations: This is a multi-site study. Depending on space availability and other logistics, the patient may be asked to complete any daytime study procedures (either study visits or follow up visits) at either the YNHH Hospital Research Unit

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(HRU), at the research group's outpatient clinic space at 135 College Street in New Haven, CT, or at St. Francis Hospital. The St. Francis site will seek IRB approval from their local IRB.

6.1 Genetic Testing & Maintaining Subjects' Privacy

6.2 Types of future research to be conducted using the materials: Subjects' genetic material will be collected for genome sequencing. One of the goals of this study is to determine if variations in human genome influence HF risk, progression and response to treatment. The accrued genetic material from subjects will permit potential future Genome Wide Association Studies (GWAS) regarding the interaction between genetic material and HF disease progression, phenotype and prognosis. No immortalized cell lines or animal studies are planned for this study.

6.3 Plan for collecting materials or the conditions under which material will be received: Subjects' genetic material will be collected and processed at YNHH Park Street clinical laboratory, our study's laboratory facilities at 300 George Street, or at YNHH-SRC campus. Each subject's specimens will be processed, labeled with unique study IDs and stored in cryovials in a -80˚C freezer.

6.4 Types of Protected Health Information (PHI) about the individual contributors that will be entered into a database: Name, address, telephone number, medical record number (MRN), medical history and allergies, current and past medications and therapies, vital signs, family medical history, results of blood, urine and imaging tests (ex: x-rays, CT scans, MRIs, or ultrasound) and pathology tests (biopsies), DNA, tissue and blood.

6.5 Conditions or procedures for sharing of materials and/or distributing for future research projects: Data collected by the PI and other study personnel listed in this protocol will be distributed for secondary research purposes only after the recipient investigator has obtained HIC approval for the proposed research objective, received an exemption or determination by the HIC the study is not considered human subject research. Data will be distributed for research projects of the same nature and similar purpose specified in this protocol, as agreed to by the subjects by signing the compound informed consent and HIPAA authorization form upon enrollment to the study. The PI is responsible for receiving appropriate attestation by recipient investigators prior to permitting access to the database for activities considered preparatory to research. Attestation will be obtained using the Request for Access to PHI for a Research Purpose Form found at http://www.yale.edu/hrpp/forms-templates/hipaa.html.

6.6 Widespread sharing of materials: Samples and data collected during the research study will be shared with other investigators for research projects of the same nature and similar purpose specified in this protocol, as described above.

6.7 Stripping materials of all identifiers: Samples will be immediately stripped of all identifiers, and given a unique study ID as soon as deposited in the AVA-HF bio-repository.

6.8 Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? Yes; researchers may still use the material or data that was collected before the subject withdrew permission/authorization in order to complete the research that has already commenced. All specimens and data retained in the AVA-HF biorepository will be destroyed upon receiving notice of a subject's withdrawal of permission for continued use of their specimens.

6.9 Provisions for protecting participants' privacy: The PI will ensure patients' anonymity is maintained. Each subject is assigned a unique study identification number and is tracked

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through this number. Subjects' clinical data will be entered into password protected software and stored on a secure server. A log of subjects' names, subject ID numbers and pertinent registration information (ex: address, phone number and emergency contact information) is maintained on a password-protected computer, to allow reidentification of subjects when necessary.

6.10 Methods for the security of storage and sharing of materials: Specimens are labeled only with unique study ID numbers. When certain samples are shipped and analyzed by appropriate collaborating laboratories, samples will be sent anonymously, ensuring laboratories will not have access to any clinical or phenotype data, thus assessments will be made in a blinded fashion. The specific laboratories will vary depending on the biochemical test performed; specimens may be analyzed at individual or core laboratories, academic institutions or industry collaborators.

7.1 Research Subject Population

Subject Recruitment and Screening: Patients with a clinical diagnosis of HF who have decompensated and require treatment with IV diuretics at YNHH (York St 5-1, 5-2, 5-3, or 5- 4, 5-7, 5-8 or St. Raphael's Campus Verdi 5 East and Verdi 3 East) will be identified from their electronic medical record by study coordinators in coordination with treating clinicians.

Patients identified from electronic medical records whose providers feel they are appropriate for the study will be approached and recruited to participate in this research study. Patients will be approached by doctors, cardiac surgeons, residents, fellows, nurse practitioners, physician assistants and registered nurses.

Research staff will have a one-on-one conversation with the patient regarding the study and what participating would involve. All patients approached will be 18 years or older and capable of providing written, informed consent. Pursuant to HIPAA regulations, a log of disclosures of protected health information for recruitment/screening purposes will be kept.

7.2 Subject Classification: Will subjects who may require additional safeguards or other considerations be enrolled in the study? **N/A** Is this research proposal designed to enroll children who are wards of the state as potential subjects? **N/A**

7.3 Subject Eligibility Determination: The study coordinator, working in conjunction with the PI, will determine subject eligibility.

7.4 Subject Inclusion and Exclusion Criteria

Inclusion Criteria

a. Clinical diagnosis of decompensated heart failure with at least one objective sign of volume overload (rales, edema, elevated JVP, or weight gain of at least 5 pounds)

b. A projected need by the treating clinician for continued treatment with IV diuretics

c. Chronic loop diuretic use **OR** no chronic loop diuretic use and serum chloride <100 mmol/l

d. 18 years old

Exclusion Criteria

a. Inability to commit to or comply with serial visits

b. History of severe metabolic or respiratory acidosis within 30 days of enrollment

c. Use of metformin, acetazolamide, or any other agent that could predispose to acidosis. Patients who are on metformin may be enrolled if their metformin can

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be discontinued safely for the duration of the study. Any participants who have consistently elevated Blood glucose readings > 200 mg/dL while inpatient will not be enrolled.

d. Serum bicarbonate level <20mmol/L
e. Estimated glomerular filtration rate <20 mL/min or renal replacement therapy
f. Urine output of <500 ml/24 hours despite use of IV loop diuretic therapy
g. Appears unlikely, or unable to participate in the required study procedures, as assessed by the study PI or research RN (ex: clinically-significant psychiatric, addictive, or neurological disease)
h. Inability to give written informed consent or follow study protocol

Exclusion criteria for randomization:
Prior to randomization, a venous pH level will be determined. If the venous pH is less than 7.25, the patient will not be randomized.

8.1 Risks and Minimizing Risks

8.2 Risks

a. **Data collection:** We anticipate minimal risk related to collection of data from the clinical record, including paper charts, EMR, physical exams, and blood and urine samples. We also anticipate minimal risk associated with data entry into the electronic database, which will be password-protected, and stored on a secure server.

b. **Emotional, psychological, economic risk:** In terms of economic expenditure, patients who are admitted to the hospital will not have to drive to the study site or pay for parking to participate in the study. Patients who are not currently inpatient will have to drive to the study site, park at their own expense, and spend up to 8 hours there for the study protocol and thus the research study will only modestly increase the inconvenience for inpatients.

c. **Urine collection and bladder scanning:** There are virtually no risks to the patient related to urine collection and bladder scanning, other than mild discomfort when the ultrasound probe is briefly pressed on the abdomen.

d. **Blood draws:** Blood drawing is generally associated with minimal risk, such as bleeding at the site, pain, bruising at the site, rare transient dizziness, anemia, infection, emboli or phlebitis.

e. **IV drug administration:** IV injections generally present minimal risk to the patient, though the injection site can become infected, there can be bruising, inflammation of the vein, leaking of fluid into the surrounding tissues, or an injection site reaction depending on the type of medication injected.

f. **Lysine Chloride:** Lysine Chloride is freely available to the public as a dietary supplement and food additive and is on the FDA Generally Recognized as Safe (GRAS) list. Every day we eat large quantities of lysine and chloride in our diet. No significant side effects are anticipated to occur from lysine chloride based on a multitude of animal and human studies done with this supplement and the brevity of this intake (8 days). There was a rare incidence of minor GI upset associated with taking lysine chloride as a supplement in some of these studies, including nausea, abdominal pain and diarrhea. However, no side effects necessitating the discontinuation of lysine chloride supplementation were reported.[a, b, c, d] This research group previously performed a preliminary study with 11 patients involving supplementation of lysine chloride at 21 grams per day, with no adverse events reported (HIC #1308012508, minimal risk study). The doses of lysine chloride

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administered in this study are higher than the majority of prior studies, which did not study patients with heart failure. As a result, new, unexpected, or exaggerated side effects are possible.

i. **Acidosis:** One possible side effect that we will follow closely will be the buildup of acid in the patients' blood, a condition that in very severe cases can cause illness or even be fatal. This condition would slowly develop if it were to happen and thus we will closely monitor serum pH and bicarbonate over the course of the study to be sure this does not occur. Importantly, we did not see any evidence of acid buildup in our pilot study of heart failure patients were similar doses were used.

ii. **Volume depletion:** An additional possibility is that lysine chloride will work "too well" in removing fluid which could cause dehydration and possible kidney damage. As a result, we will closely follow kidney function throughout the course of the study and adjust diuretics dosage and the study medication if too much fluid is removed.

References

a. Rubin, A.L., et al., The Use of L-Lysine Monohydrochloride in Combination with Mercurial Diuretics in the Treatment of Refractory Fluid Retention. Circulation, 1960. 21(3): p. 332-336.

b.Spencer, H., Samachson, J., 1963. Effect of lysine on calcium metabolism in man. J. Nutr. 81:301-6.

c. Griffith, R. S., D. E. Walsh, et al. (1987). "Success of L-lysine therapy in frequently recurrent herpes simplex infection. Treatment and prophylaxis." Dermatologica 175(4): 183-190.

d.Thein, D J, and W C CHurt. "Lysine as a prophylactic agent in the treatment of recurrent herpes simplex labialis." Oral surgery, oral medicine, oral pathology 58.6 (1984):659-666.

g. **Iothalamate meglumine (for patients without a contrast allergy):** The most common adverse reactions to iothalamate meglumine are allergic reactions (which can be severe), headache, dizziness, low BP, nausea, vomiting, flushed sensation, transient taste perversion, and local injection site reactions. While iothalamate carries the risk of contrast associated kidney injury, the risk with this very-low dose (1cc) is exceedingly small; doses in this range have been proven to be exceptionally safe, even in the highest risk patients.

h. **Iohexol:** The most common adverse reactions are allergic reactions (which can be severe), contrast induced kidney injury, pain, vision abnormalities including blurred vision, headache, taste perversion, arrhythmias including premature ventricular contractions (PVCs) and premature atrial contractions (PACs), angina/chest pain, nausea

i. **D_2O:** Deuterium oxide is a naturally-occurring, stable, non-radioactive isotope of hydrogen that is well-tolerated by humans. Also known as "heavy water," D_2O has twice the mass of a hydrogen atom and occurs naturally in human blood at concentrations of ~150 ppm. D_2O has been widely used to measure water space in both animals and humans. For an adult human, 10mg of D_20 is swallowed, increasing the blood's D_2O content from 150 to 300 ppm. There is thought to be essentially no risk with ingesting this amount of D2O.

j. **Volumex (radiolabeled albumin):** Volumex is albumin labeled with the iodine isotope I-131 and is an FDA-approved method used to determine total blood

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volume. The labeled albumin is available from Volumex, Daxor Corporation, NY, in a specialized, pre-filled flow chamber syringe designed to ensure >99.8% delivery of the radioisotope dose. A single 1cc dose of Volumex contains an ultra-low-dose of up to 25 micro-curies of activity. Although each organ will receive a different dose, the amount of radiation exposure the patient will receive from 2 doses of Volumex over the course of this study will be equivalent to the whole body being uniformly exposed to 0.2 rem of radiation. This amount of radiation is well below the dose guidelines established by the federal government and adhered to by the YNHH Radiation Safety Committee for research subjects. To give you an idea about how much radiation the patient will be exposed to, we will compare it to an every-day situation. Everyone receives a small amount of unavoidable radiation each year. Some of this radiation comes from space and some from naturally- occurring radioactive forms of water and minerals. The Volumex given during this study will give patients the equivalent of about 8 extra months' worth of natural radiation.

k. **Potassium Iodide (KI):** While the risks of 8 days of chronic exposure have been deemed greater than the potential benefits, with respect to acute administration, there may be some potential value for younger patients, given the FDA recommendations on the use of KI. As such, patients under the age of 40 who do not have a known iodine hypersensitivity will be given the option of taking a one- time dose of KI to further reduce the risk of radiation exposure from Volumex. The major exposure of the thyroid to unbound radioactive iodine will be the day the dose of Volumex is injected. As such, the option will be given to patients to take the KI after explaining that taking a dose of KI could potentially reduce the already low chances of damage to the thyroid by radiation, at the expense of the known, short-term effects of the KI. While side effects are unlikely when Potassium Iodide is used at the recommended dose and for a short period of time, the following are possible side effects: cardiac arrhythmias, myxedema, confusion, fatigue, metallic taste, numbness, tingling sensation, skin rash, goiter, hyperthyroidism, hypothyroidism, diarrhea, salivary gland swelling or tenderness, gastric distress, gastrointestinal hemorrhage, nausea, stomach pain, vomiting, adenopathy, thyroid adenoma, hypersensitivity reaction (angioedema, cutaneous and mucosal hemorrhage, serum-sickness-like symptoms), arthralgia, weakness, pharyngeal edema, fever, iodism.

l. **Dextrose 5% Fluid:** There is risk of fluid retention, hyperglycemia or hyperosmolar hyperglycemic state, hyponatremia, hypophosphatemia, hypersensitivity reactions and refeeding syndrome. We anticipate these risks to be minimal as the Dextrose 5% infusion will be used during the I 131 administration and the thirty-six-minute blood collection for the blood volume to flush the IV line. The Dextrose 5% may also be used as a KVO fluid should the participants IV line require this to maintain patency. The maximum infusion amount is 10ml/hr over the 8 hour study period(for KVO) and IV line flushes or 100 ml total per visit 1 and 2. Although the participant will be receiving 5% Dextrose during the I 131 administration and some participants may also receive 10 ml/hr to keep their IV patent, there is a minimal risk of hyperglycemia in diabetic and non-diabetic patients as the amount received will be a maximum of 100 ml over 8 hours which equates to only 5 grams of glucose.

m. **Imodium AD or Imodium (Loperamide):** There are risks of allergic reaction to this loperamide hydrochloride. Use cautiously in patients with heart arrhythmias, liver impairments, mucus in their stool and fever. Contraindicated in patients that have abdominal pain without diarrhea. May cause drowsiness, dizziness, and tiredness, may need caution when operating heavy machinery. If clinical improvement is not observed in 48 hours or they develop abdominal pain or bulging, the Imodium may be stopped by the study PI or sub I.

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8.3 Minimizing Risks

a. **Data collection:** Identifying data will be available only to the study PI and study coordinator, and will be used only to facilitate medical record review, and prevent duplicate screenings of patients while looking for subjects to enroll in the study. All data sheets, electronic data entries, and laboratory samples will be labeled with a unique identifier code. These numbers will be unique to the patient and will be the common link between specimens and the data. No identifying data will be reported to the public or in any publications that may result from this research.

b. **Emotional, psychological, economic risk:** Blood samples will be drawn off of the IV line to reduce patient discomfort, and to avoid the patient being subjected to multiple straight needle sticks.

c. **Urine collection and bladder scanning:** Collecting urine and performing bladder scans during the study presents minimal risk to the patient.

d. **IV Insertion & Blood draws:** The risks of blood draws will be minimized by drawing the minimum volume necessary for study purposes. The risks of pain and infection will be minimized by having blood drawn by qualified, experienced staff who have achieved competency with phlebotomy. All standard precautions will be maintained during the procedure. Patients' discomfort will be minimized by drawing blood from the IV line when possible, instead of subjecting the patient to multiple straight sticks.

e. **IV drug administration:** Injections will be performed by qualified, experienced staff who have demonstrated competency with the technique. The sterility of the IV line will be diligently maintained by research staff to help prevent infections. Sites will be closely monitored for warmth, redness, swelling, pain and leaking. The IV line will be flushed before and after each medication is administered

f. **Lysine Chloride:** Patients will be seen every other day (except for weekends) for laboratory monitoring of electrolytes and renal function to ensure the absence of any adverse effects, and to ensure that the supplement is being taken in the correct manner.

- **i. Acidosis:** If laboratory results provide any indication of acidification, the dose of lysine chloride will be adjusted (either 2 doses/day, 1 dose/day or 0 dose/day) at the discretion of the study physician and study nephrologist.
- **ii. Volume depletion:** Daily lab values will be monitored by the study team. In addition, vital signs will be measured daily. Patients who are admitted at YNHH will additionally be monitored by their treatment teams. Daily lab values will be reviewed by the study physician and nephrologist and, at their discretion, study medication or diuretic dosage may be adjusted.

g. **Iothalamate meglumine (for patients without a contrast allergy):** The dose administered is the smallest effective dose possible to minimize any medication side effects.

h. **Iohexol (for patients without a contrast allergy):** The dose administered is the smallest effective dose possible to minimize any medication side effects.

i. **D_2O:** Deuterium oxide is a safe, natural isotope found in everyone's tap water. It is a frequently used tracers in studies determining TBW. We do not anticipate any risk to the patients from its use.

j. **Volumex (radiolabeled albumin):** We do not anticipate any significant risks from patients receiving 2 doses of Volumex. This will expose patients to 0.2 rem of radiation, which equals about 8 extra months' worth of natural radiation exposure. Patients under the age of 40 will be given the option of taking a one-time dose of Potassium Iodide to further reduce the risk of radiation exposure from Volumex.

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k. **Potassium Iodide (KI):** To reduce the risks of taking KI, the lowest effective, one-time dose (135 mg) will be administered to patients under the age of 40 who have opted to take this medication to further protect against radiation exposure from Volumex.

l. **Dextrose 5% Fluid:** The volume of Dextrose 5% will be administered during the blood volume procedures to flush the line and given continuously throughout the study visit only if a KVO fluid is required to keep the IV-line patent. At these volumes, we anticipate the potential of these risks to be minimal. In addition, the participant's vital signs will be measured.

m. **Imodium AD or Imodium(Loperamide):** This medication is generally well tolerated; it will be administered in smallest effective dose to maximize the benefit of the medication and to minimize any medication side effects. We expect minimal risk by giving the smallest effective dose, initial dose is 4mg and maximum daily dose is 16mg.

9.0 Moderate Risk Data and Safety Monitoring Plan (DSMP) with Data Safety Monitoring Board (DSMB)

Personnel responsible for the safety review and its frequency: The PI will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when re-approval of the protocol is sought). During the review process, the PI (monitor) will evaluate whether the study should continue unchanged, require modification &/or amendment, or close to enrollment. Either the PI, the IRB, or the Yale Data Safety Monitoring Board (DSMB) have the authority to stop or suspend the study or require modifications.

The overall risk associated with the proposed study is deemed moderate for the following reasons: We believe there are minimal risks to the subjects from the lysine chloride and D2O administered during the study. Lysine chloride is freely available to the public as a dietary supplement. No significant side effects are anticipated to occur from supplementing lysine chloride based on a multitude of animal and human studies done with this supplement and the brevity of this intake (8 days). This research group previously performed a preliminary study with 11 patients involving supplementation of lysine chloride at 21 grams per day, with no adverse events reported (HIC #1308012508). D2O occurs naturally, is widely used in various water space studies, and is very well tolerated, especially in such small, one-time doses. Iothalamate and Iohexol have been used extensively in diagnostic radiology, and while it can cause transient nausea, vomiting, facial flushing and a feeling of body warmth, these side effects are typically only seen in much higher doses than that proposed for this study. We view the risks associated with collecting up to 105cc of blood during each Study Visit as moderate, as the proposed volume to be drawn is larger than the amount traditionally drawn for a basic complete count and chemistry. The IV radiolabeled albumin presents moderate risk to the study subjects. Each ultra-low dose proposed will be equivalent to whole body, uniform exposure of 0.1 rem of radiation, which is well below the dose guidelines established by the federal government, and adhered to by the YNHH Radiation Safety Committee for research subjects.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events (AE), serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

Attribution of Adverse Events (AE): AE will be monitored for each study participant and attributed to the study procedures / design by the PI according to the following categories:
-Definite: AE is clearly related to investigational procedures(s)/agent(s)

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-Probable: AE is likely related to investigational procedures(s)/agent(s)
-Possible: AE may be related to investigational procedures(s)/agent(s)
-Unlikely: AE is likely not related to the investigational procedures(s)/agent(s) -Unrelated: AE is clearly not related to investigational procedures(s)/agent(s)

Plan for Grading Adverse Events: AE noted during the study will be graded by severity as Mild, Moderate or Severe.

Plan for Determining Seriousness of Adverse Events: In addition to grading the AE, the PI will determine whether the AE meets the criteria for a Serious Adverse Event (SAE). An AE is considered serious if it:
-is life-threatening OR
-results in in-patient hospitalization or prolongation of existing hospitalization OR
-results in persistent or significant disability or incapacity OR
-results in a congenital anomaly or birth defect OR
-results in death OR
-based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, OR -adversely affects the risk/benefit ratio of the study

An AE may be graded as severe but still not meet the criteria for an SAE. Similarly, an AE may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

Plan for reporting Reportable AE and other unanticipated problems involving risks to subjects or others to the IRB and DSMB: The PI will report the following types of events to the IRB:

A. AE that are serious or life-threatening AND unanticipated (or anticipated but occurring with a greater frequency than expected) AND possibly, probably or definitely related to the drug/device/intervention; and

B. Other unanticipated problems involving risks to subjects or others. These AE or unanticipated problems involving risks to subjects or others will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website.

C. Any untoward or unexpected occurrence in this study (physical or psychological harm) temporally associated with the individual's participation in the research (whether or not considered related to participation in the research) will be reported to the IRB within 5 calendar days and would be presented to the DSMB meetings/calls. For the randomized portion of the study, the side effects described that were described previously in this submission that are untoward or unexpected will be reported within the window between randomization and the final visit or phone call at the end of the two weeks.

D. Reportable Events (which are events that are serious or life-threatening and unanticipated (or anticipated but occurring with a greater frequency than expected) and possibly, probably, or definitely related) or Unanticipated Problems Involving Risks to Subjects or Others that may require a temporary or permanent interruption of study activities will be reported immediately (if possible), followed by a written report within 5 calendar days of the Principal Investigator becoming aware of the event to the IRB (using the appropriate forms from the website) and any appropriate funding and regulatory agencies.

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Plan for reporting AE as appropriate to the following individuals or group entities: For this study, the following individuals and regulatory agencies will be notified: All coinvestigators listed on the study protocol and the Yale DSMB. The PI will conduct a review of all AE upon completion of every study subject. AE frequency and severity will be evaluated to determine if modifications to the protocol or informed consent form (ICF) are required.

For multi-site studies for which the Yale PI serves as the lead investigator:

A. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?

   Adverse events and unanticipated problems will be recorded and reported in accordance with local institutional and IRB policies.

B. What provisions are in place for management of interim results?

   Given the observational nature of this study, no interim data analysis is planned.

C. What will the multi-site process be for protocol modifications? When a protocol modification is suggested or required at any of the three clinical sites, each site will receive the proposed changes.

   Modifications will be assessed for relevancy to the clinical site and for their impacts on the study design. When necessary, protocol modifications will be submitted in accordance with local IRB policy.

PI's assessment of the overall risk level if children are participating in this study? N/A

Statistical and Analytical Considerations

Endpoints: Change in blood volume over the 7-day intervention will serve as the primary endpoint. Secondary endpoints will include the change in log NTpro-BNP, serum creatinine, cystatin C, chloride and bicarbonate.

Analysis plan: A linear mixed effect model will be used to analyze the trial with class variables of time and treatment group. The mixed model will be able to accommodate the incomplete data by using all available observations. In this model, the fixed effects include the main effect of the treatment, the main effect of time, and the interaction between the them and the random effects include random intercept. Residual diagnosis for the mixed model will be performed to check whether model assumption is seriously violated. Statistical significance will be set at an alpha of 0.05. Secondary endpoints will be analyzed with the same approach.

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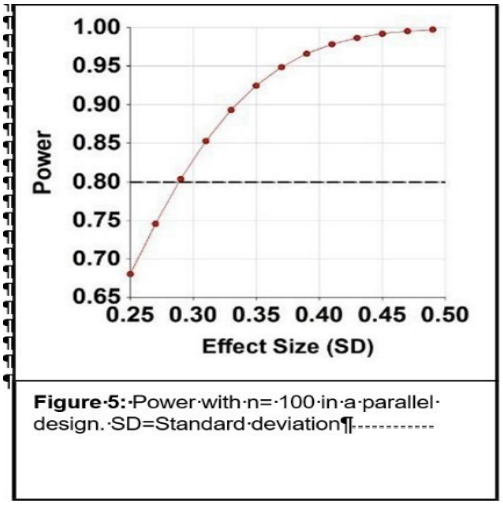

Figure 5: Power with n=100 in a parallel design. SD=Standard deviation

Power and sample size: The change in blood volume in hospitalized patients undergoing several days of IV diuresis was reported by Miller and Mullan to be on average -0.7L (a 9% reduction), with a standard deviation of 1.1L.107 The correlation between pre and post treatment blood volumes was high at r=0.87 (personal communication with Dr. Miller). Although some of this variability is driven by heterogeneity in treatment (which conducting the trial in the YTCC will address) some of it is also driven by heterogeneity in individual response to standard therapy and the relative importance of each is unknown.

Additionally, the variability in blood volume response to lysine chloride (if one exists) is unknown. As such, given the multiple sources of variance in order to detect a 0.5L difference in change in blood volume between lysine chloride and placebo, we need to power for a standard deviation as high as 1.5L. Figure 5 illustrates that we will have an 80% power to detect an effect size 1/3 the standard deviation with n=100 participants.

SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

11.1 RADIOTRACERS: FDA-approved Volumex (Iodinated I-131 Albumin)

If an **exemption from IND filing requirements** is sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies:

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Exempt Category 1: The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

1) The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. **YES**
2) The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. **YES**
3) The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. **YES**
4) The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). **YES**
5) The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. **YES**

11.2Drugs/Biologics: Background Information on Study Medications and Tracers

	Previous Human Use	**Known risks**	**Study Dose (x 2 Visits)**	**General Dose Range**	**Route of Admin**
Lysine Chloride	Lysine Chloride has been used in a multitude of human studies, including a preliminary study with 11 patients at Yale involving supplementation with 21 grams/day of Lysine Chloride, with no adverse events reported (HIC	May cause nausea, abdominal pain and diarrhea.	165 mmol/day (30.1 g/day)	1 gram 2X/day	PO (powder)

Volumex (Iodinated I-131 Albumin)	FDA-approved radio-pharmaceutical for the determination total blood & plasma volumes studies. & protein turnover	Immune virtually unaltered by iodination process, but an allergic reaction could occur after multiple dose	1.0cc (up to 25 microcuries)	blood & plasma volume studies = 0.2cc-2.0cc	IV-Push

Iothalamate Meglumine*	Non- isotopic (cold) contrast agent. Cost effective tracer to measure GFR, allowing researchers to avoid exposing patients and themselves to isotopes.	Most frequently: nausea, vomiting, facial flush and a feeling of body warmth but generally occur at far higher doses than used in current study.	1 ml	30-60 ml	IV
Iohexol*	Cost effective tracer to measure GFR, allowing researchers to avoid exposing patients and themselves to isotopes	Contrast induced kidney injury pain, vision abnormalities including blurred vision, headache, taste perversion, arrhythmias including premature ventricular contractions (PVCs) and premature atrial contractions (PACs), angina/chest pain, nausea	1 ml	50 ml-200 ml	IV
D2O	Standard reagent used in common clinical technique to determine TBW.	Naturally occurring, stable isotope of hydrogen found in all tap water. Known to be a safe, non-radioactive technique. Exact toxicity amount not determined; animal studies have shown D20 concentrations must reach 30-35% of TBW to exert toxic effects (required dose would be ~15L for a 70kg patient).	10.0 grams	9-11mg	PO (liquid)

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Potassium Iodide**	Used to block thyroidal uptake of radioactive isotopes of iodine.	Can cause arrhythmias, confusion, fatigue, fever, numbness, tingling, hyperthyroidism (prolonged use), hypothyroidism (prolonged use), diarrhea, nausea, vomiting, eosinophilia, lymphedema, hypersensitivity reaction, arthralgia, weakness, dyspnea, wheezing.	130 mg	130 mg	PO (Liquid)
Dextrose 5 % Fluid	FDA approved nonpyrogenic salt free solution for fluid and calorie replenishment for intravenous administration	Can cause fluid retention, hyperglycemia or hyperosmolar hyperglycemic state, hyponatremia, hypophosphatemia, hypersensitivity reactions and refeeding syndrome	200ml	500ml-1000ml	IV
Imodium AD or Imodium***	FDA approved to treat diarrhea	Can cause dizziness, drowsiness and Tiredness	Taken only is the participant experience diarrhea after taking Lysine Chloride/ placebo.	Maximum dose 16 mg /day	PO

* Iothalamate or Iohexol will only be administered to patients without a contrast dye allergy
**Potassium Iodide will be offered to patients under 40 to further reduce the radiation risks of Volumex to the thyroid.
*** Imodium will only be administered to participants who experience loose stools and are randomized on the study medication.

**** Unforeseen changes in the treatment plan may preclude the study team from continuing with study procedures after the iothalomate/iohexol and D2O are given. In this case the team may plan to initiate study on a subsequent day and administer an additional dose of iothalomate/iohexol and D2O on that day.

Iodide will be sourced from YNHH IDS. The Volumex will be sourced from the Daxor Corporation.

11.3 Is the drug provided free of charge to subjects? Yes, by the research group

11.4 Storage, Preparation & Use: Study medications will be stored by and obtained from YNHH **IDS.**

11.5 Investigational Drug Service utilized: YNHH IDS

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11.6 Use of a Placebo

a. **Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.** There are several other therapies available to help control volume status in patients with HF: diuretics, vasodilators (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, nitrodilators, etc.), cardiostimulatory or ionotropic medications (digitalis, beta-agonists, and phosphodiesterase inhibitors), and cardio-inhibitory medications (beta- blockers and calcium-channel blockers).

b. **Maximum time study subjects may receive placebo:** 8 days

c. **Greatest potential harm that may come to a subject as a result of receiving placebo:** Enrolled patients will receive either the study drug (lysine chloride) or the matched placebo for an 8-day period. Patients will continue taking their home loop diuretics, and will be monitored closely by YTCC and the research study for signs of volume overload.

d. **Procedures in place to safeguard subjects receiving placebo:** After transitioning to oral diuretics per the YTCC protocol, the patient will be seen every other day (except for weekends) for laboratory monitoring of electrolytes and renal function. Vital signs and weight will be measured. Patients will be monitored just as closely if taking the placebo as they would be monitored if taking the study medication (lysine chloride).

11.7 Continuation of Drug Therapy After Study Closure: No, subjects will not be provided the opportunity to continue receiving the study drugs after the study ends. After completing the research study, the patient will continue taking their regularly prescribed medications, including oral diuretics, if appropriate.

11.8 DEVICES: N/A

SECTION III: RECRUITMENT AND CONSENT PROCEDURES

12.1 Recruitment and Consent Procedures

12.2 Targeted Enrollment: 200 patients are anticipated to be enrolled across all study sites and complete all study procedures over the course of 6 years. Up to 5 additional patients enrolled into the open label arm are anticipated to complete all study procedures over the course of 1 year.

12.4 Recruitment Procedures

a. **Recruitment Methods to be used:** Medical Record Review

b. **Subject Recruitment and Screening:** Potential subjects will be identified by study coordinators in conjunction with treating clinicians by screening the EMR of patients with a clinical diagnosis of HF receiving outpatient loop diuretics. Patients whose providers feel they are appropriate for the study will be approached. Pursuant to HIPAA regulations, a log of disclosures of protected health information for recruitment/screening purposes will be kept.

c. **Who is recruiting potential subjects?** The PI or study coordinator will first approach the patient's treating clinician (ex: physician, nurse practitioner, or physician's assistant) regarding possible involvement in the study. If the provider feels the patient is appropriate, they will ask if the patient would be interested in hearing about a research project they might be able to

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participate in. If the patient expresses interest, the PI or study coordinator will then approach the patient

12.5 Assessment of Current Health Provider Relationship for HIPAA Consideration: Does the PI or any member of the research team have a direct existing clinical relationship with any potential subject? Yes, some of the subjects. Jeffrey Testani, Tariq Ahmad, Lavanya Bellumkonda, Henry Cabin, Matthew Griffin, Daniel Jacoby, Christopher Maulion, Edward Miller, Jeffrey Turner and Albert Sinusas are all physicians at YNHH, and therefore could potentially be the attending physician for some of the enrolled patients. Dr. Ralph Riello is a pharmacist at YNHH, and therefore could be the attending pharmacist for some of the enrolled patients.

12.6 Request for waiver of HIPAA authorization for Recruitment/Screening Purposes Only: Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: Due to the inclusion criteria that involves PHI, without a waiver of consent it would be extremely challenging to identify patients to approach for possible study enrollment.

The PI assures that the PHI for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAACovered entity must be accounted for in the "accounting for disclosures log," by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

12.7 Process of Consent: Proper written, informed consent and HIPAA authorization will be obtained prior to patient participation in this trial. The overall protocol (including objectives, procedures and duration), potential risks and benefits, voluntary nature and ability to withdraw will be discussed with each patient. The patient will be given a copy of the IRB-approved ICF to review and will have all questions answered before being asked to sign the ICF. Verification of comprehension of the consent will be obtained by asking the subject to describe in their words the purpose and risks of the study. The patient will be instructed that his or her care will not be affected by his or her decision to participate, or not. If the patient voluntarily agrees to participate in the trial, he or she will be asked to sign the ICF. The original ICF will be kept with the study documents and a copy will be made for the patient's chart. The subject will be given a signed copy of the ICF and HIPAA authorization form. All study subjects will be 18 years or older, with the capacity and ability to provide informed consent, thus making parental or surrogate permission not applicable.

12.8 Evaluation of Subjects' Capacity to Provide Informed Consent: Patients will be screened within EPIC for study eligibility, including mental status and ability to provide informed consent. Study coordinators will confirm with the patient's provider that the patient has the requisite ability and capacity to give informed consent prior to approaching the patient regarding the study. If at any time during the study there is concern regarding a patient's mental status, study staff will both notify the patient's provider, and re-evaluate whether the patient is still appropriate for participation in the study. After the study objectives and procedures are explained to the subject, they will be asked to describe in their words what the purpose and risks of the study are.

12.9 Non-English Speaking Subjects: N/A. Only English-speaking subjects will be enrolled. **12.10**

Requesting a Waiver of Consent for Recruitment/Screening only

a. **Does the research pose greater than minimal risk to subjects?** NO
b. **Will the waiver adversely affect subjects' rights and welfare?** NO

c. **Why would the research be impracticable to conduct without the waiver?** Determining which patients meet inclusion/exclusion criteria would be impossible without this waiver.

d. **Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?** All screened information will be present in the patient's electronic medical record (EMR), and thus the patient will already have full access to any information involved in the screening/recruitment by contacting YNHH's medical records department.

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

13.1 Confidentiality & Security of Data

13.2 Study subject PHI (medical information along with HIPAA identifiers) collected and used for the research: Name, address, telephone number, MRN, medical history, allergies, current and past medications or therapies, family medical history, information from a physical examination (ex: BP, heart rate, respiratory rate and temperature), results of blood and urine tests, pathology tests (biopsies), results of imaging tests (ex: x-rays, CT scans, MRI scans, or ultrasound), DNA, tissue and blood.

13.3 Research data collection, recording, and storage: Research data will be collected from patients' clinical record (paper charts and EMR, blood and urine samples and cardiac pressure monitoring data collected during the study). Data will be recorded in electronic databases (RedCap & Oncore), stored in password-protected software on a secure server. Yale University will serve as the Data Collection Center (DCC) for this study.

13.4 Digital data storage: ITS-approved computer on a secured server

13.5 What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study? Each subject's samples will be given a unique study ID number and cryovials will be labeled with barcodes. There will be no PHI or information directly linking the patient to these samples. Subjects' clinical data will be stored on password-protected software maintained on a secure server. A "linker file" with PHI will be maintained on a separate, password- protected system, which only the study PI and study coordinator will have access to.

13.6 What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured. After study enrollment is complete continued analysis of samples in the AVAHF biorepository will be undertaken, and thus destruction of identified data is not planned until completion of all analyses potentially requiring the linkers to remain in place.

13.7 PHI Access: The PI, co-investigators, and study coordinators will have access to the complete data set, including all PHI collected. Study documents and records may be accessed by the Food and Drug Administration.

13.8 Certificate of Confidentiality: N/A

13.9 Study procedures likely to yield information subject to mandatory reporting requirements: N/A

SECTION V: POTENTIAL BENEFITS

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14.0 Potential Benefits: Although there are no direct benefits that a participant can expect from this study, there are significant potential benefits to society at large. Given the tremendous public health importance of HF and the management of volume overload in HF, any incremental knowledge in this area has great potential impact.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

15.0 Alternatives: What other alternatives are available to study subjects outside of the research? Patients may choose to proceed with the standard of care for heart failure, without choosing to participate in the research study.

16.1 Payments for Participation (Economic Considerations): Patients will be compensated for each Study Visit, Safety Visit and Home Urine Collection. This compensation includes the inconveniences involved in serial collection of urine and blood samples, and home urine collections. Patients completing all study and safety visits and urine collections will receive a total of up to $575.

i. $500 for first Study Visit (8 hours)
ii. $20 for patients who must be withdrawn after IV placement and screening due to venous pH
iii. $25 for each Safety Visit (up to 5)
iv. $15 for each 24 Hr Urine Collection (x7)
v. $500 for last Study Visit (8 hours) if in the hospital, $1,000 if the study takes place outpatient
vi. $ 25 for follow up visit (1) or $10 for follow up phone call
vii. $50 if a study is initiated but cannot be completed

17.0 Costs for Participation (Economic Considerations): Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects. The following will be provided to the patient while participating in the study: blood draw supplies, urine sample collection supplies, and all study medications and placebo.

18.1 In Case of Injury: Required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

a. **Will medical treatment be available if research-related injury occurs?** Yes
b. **Where and from whom may treatment be obtained?** Patient's medical provider
c. **Are there any limits to the treatment being provided?** Unknown
d. **Who will pay for this treatment?** Patient's own insurance
e. **How will the medical treatment be accessed by subjects?** Through standard contact with their health care provider.

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