Diagnosing Natriuretic Peptide Deficiency: A Pilot Study

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1.0 Background

The cardiac natriuretic peptide (NP) system may protect against the development of cardiometabolic risk. [redacted]

2.0 Rationale and Specific Aims

NP hormones may protect against cardiometabolic risk. [redacted]

Studies in animals suggest that glucocorticoids potently stimulate NP production. There are limited studies in humans showing that glucocorticoids stimulate NP production;[3] however, the NP response specifically to dexamethasone, the glucocorticoid which has been shown in animal data to potently stimulate NP production, has not been defined in humans. Moreover, the range of normal responses to glucocorticoids in healthy individuals is not well-defined. [redacted]

The goal of the proposed project is to generate **preliminary data** that will be used to develop power calculations, inform cutoff ranges, and inform the timing of the NP response for larger subsequent studies [redacted]." In this pilot study, we will quantify the distribution of NP responses and characterize the time course of NP responses to a highly standardized dosing protocol of dexamethasone. This pilot study will allow us to determine the range and standard deviation of NP responses to dexamethasone in healthy controls.

Aim: To determine the range of distribution and time course of NP responses to a single dose of dexamethasone IV 4 mg in healthy lean individuals.

Hypothesis: Determination of the NP responses (the range and time course of changes in NP levels) to dexamethasone in 11 healthy individuals will inform the time course and frequency of blood sampling in a definitive prospective study [redacted], as well as enable us to perform a sample size calculation for a definitive prospective study.

3.0 Animal Studies and Previous Human Studies

Studies in animals suggest that glucocorticoids potently stimulate NP production. Administration of dexamethasone to rats caused an increase in atrial natriuretic peptide (ANP) expression in the heart, where ANP is produced.[4] [redacted].[5]

There are limited studies in humans showing that glucocorticoids stimulate NP production. [redacted].

4.0 Inclusion/Exclusion Criteria

Inclusion Criteria:

- Men and women ages 18-50 years
- BMI 18.5 to <25 kg/m^2

Exclusion Criteria:

- Current use of glucocorticoids, or significant use of systemic glucocorticoids for an extended period of time during the prior 6 months
- Current use of antihypertensive medications
- Current use of metformin, or any antidiabetic medications (which could affect glucose and insulin levels)
- Current use of medications known to affect dexamethasone metabolism, including phenytoin, rifampin, carbamazepine, troglitazone, and barbiturates
- Active, clinically significant infection at time of visit
- History of adrenal insufficiency or Cushing's syndrome
- Prior or current cardiovascular disease, renal disease, or liver disease
- Diabetes mellitus, pre-diabetes, impaired fasting glucose, or impaired glucose tolerance
- Atrial fibrillation

- Bleeding disorder or anemia
- Elevated LFTs > 2 times upper limit of normal
- eGFR < 60 ml/min
- HbA1c > 5.7
- Abnormal sodium or potassium level
- Positive pregnancy test, women of child-bearing age not practicing birth control, women who are breastfeeding

5.0 Enrollment/Randomization

Subjects will be recruited via the Vanderbilt Broadcast email system (research.notifications@vanderbilt.edu).

ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (see IRB #090207).

6.0 Study Procedures

Screening Visit

After informed consent has been obtained, the subject's medical history and medications will be discussed and documented. A physical exam, including measurement of height, weight, and vital signs, will be performed. Blood will be collected for a comprehensive metabolic panel, CBC, and Hemoglobin A1c, and a urine pregnancy test will be done on female subjects of child-bearing age. Inclusion/exclusion criteria will be reviewed to confirm that the subject meets study eligibility requirements.

Eligible subjects who wish to participate in the study will be scheduled for 4 additional outpatient visits at the CRC. Subjects will be instructed to subjects to continue their habitual sodium intake for several days prior to the study and throughout the study. The Study design and CRC Protocol Overview are shown in the **Figure** and **Table** below, respectively.

Figure: Study Design

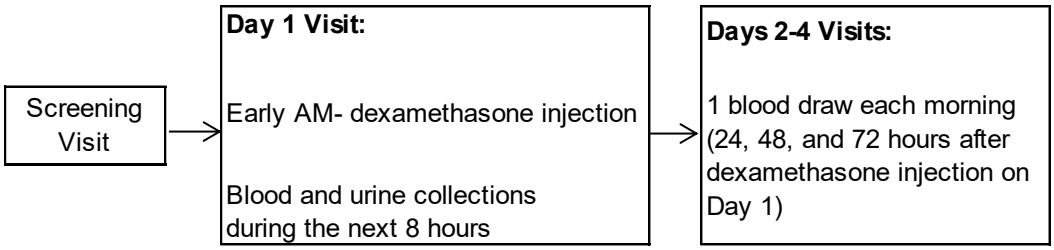

Table: CRC Protocol Overview – Day 1

Time	Procedures	NP levels	RAAS measurements	Urine	Glucose metabolism markers
7:00 AM (approx.)	IV placement; Lay supine for 60 minutes before baseline blood collection	Baseline collection after lying supine for 60 min	Baseline collection after lying supine for 60 min	Baseline collection	Baseline collection after lying supine for 60 min
8:15 AM-4:15 PM (approx.)	Inject dexamethasone	Q 30 min for first 3 hours; Then Q 60 minutes for next 5 hours	Q 30 min for first 3 hours; Then Q 60 minutes for next 5 hours	Q 120 min	Q 30 min for first 3 hours; Then Q 60 minutes for next 5 hours

CRC Visit - Day 1

Subjects will arrive at the CRC in the early morning (around 7 AM). Subjects will have fasted for at least 8 hours. Upon arrival to CRC, subjects will provide a urine sample. A urine pregnancy test will be done on female subjects of child-bearing age. Immediately after urinating, subjects will be instructed to lay in a supine position, and they will remain in this supine position during the remainder of the study visit, including during voiding (subjects will be instructed to use a bedpan or urinal jug when voiding, as they must remain supine). Supine positioning is crucial for reliable evaluation of RAAS measurements. One peripheral IV will be placed, which will be used for blood draws. Normal (0.9%) Saline (NS) will be infused at a rate of approximately 10 ml/hr to keep the IV line open and ensure successful subsequent blood draws.

After the subjects have been lying supine for 60 minutes, a baseline blood sample will be collected for NP, RAAS measurements (aldosterone, plasma renin activity), cortisol, basic chemistries, and markers of glucose metabolism (glucose, insulin level). Immediately afterwards, subjects will receive an intravenous injection of dexamethasone 4 mg. Blood will be collected every 30 minutes during the first 3 hours, and then every 60 minutes during the following 5 hours. Additional blood, at each time point, may be used to run other tests, including dexamethasone levels to help determine clearance rate of dexamethasone which may

differ between individuals. Subjects will remain fasting until 8 hours after the dexamethasone injection. At this time (8 hours after the dexamethasone injection), the study procedures for Day 1 will conclude. A meal will be provided at the completion of the visit.

All urine produced during the visit will be collected and some saved for future analysis. Subjects will be asked to produce a urine sample approximately every 120 minutes throughout the study visit; however, if the subject is unable to void at the pre-specified time points, urine will be collected when the subject is able to void and the time that the urine was produced will be recorded. Vital signs will be recorded every 30 minutes. During the visit, a dietician will speak with the subject and assess the subject's sodium intake during the 24-hours prior to the visit.

CRC Visits- Days 2, 3, and 4

Subjects will present to the CRC for brief visits on Days 2, 3, and 4. Subjects will arrive after having fasted for at least 8 hours. There will be one blood draw at each visit; the blood will be collected approximately 24 hours after, 48 hours after, and 72 hours after the dexamethasone injection was given on Day 1. Subjects will provide one spot urine collection at each visit.

Other study procedures

Subjects will be asked to discontinue use of NSAIDS, decongestants, and cold medicines one week prior to study and to remain off these agents until the study is completed.

Biomarkers

Blood and urine samples will be coded for subject confidentiality. Measurements of BNP, ANP, and their pro-peptides will be performed at Vanderbilt University Medical Center in the Cardiology Core Lab. Excess blood and urine samples will be frozen and stored for possible future investigation.

DNA Sample

For those subjects who provide consent, a DNA sample will be collected, frozen and stored to allow for future potential investigations of NP system by genotype. Samples will be coded for subject confidentiality.

7.0 Risks of Investigational Agents/Devices (side effects)

General: Risks related to overnight fasting include hypovolemia. Thus, subjects will have baseline blood pressure measured upon arrival at the CRC. The risks associated with phlebotomy in healthy individuals are minimal. Volume depletion and anemia are possible risks of phlebotomy; these risks will be minimized by excluding individuals with known anemia or other significant medical problems that may predispose to anemia, as well as by checking a CBC at the screening visit and by monitoring blood pressure at the study visits. Risks related to the placement of the peripheral intravenous lines include hematoma formation and phlebitis. In addition, there is a potential risk of hypoglycemia while fasting overnight and during the morning until study procedures are completed. The risk of hypoglycemia in healthy individuals while fasting for this amount of time is low. Subjects will be provided a meal at the conclusion of the infusion.

Dexamethasone: Potential adverse reactions to dexamethasone include infection, hypertension, increased blood glucose, headache, fatigue, emotional lability, increased appetite, nausea, abdominal discomfort, drug hypersensitivity, and increased bone turnover or bone loss. However, these potential adverse reactions are much more likely to occur with extended or chronic use of dexamethasone, and are much less likely with a single dose of dexamethasone. In the current study, we are giving a single IV dose of dexamethasone, which has a rapid half-life and thus is cleared rapidly from the body and therefore would be less likely to cause adverse reactions than with chronic use of dexamethasone.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse events will be reported to the IRB per Vanderbilt University Medical Center IRB policy.

9.0 Study Withdrawal/Discontinuation

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

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

- Development of an active infection at the time of the Day 1 visit
- Noncompliance with treatment or procedures
- Decision by participant/participant withdraws consent
- Lost to follow-up
- Starting a medication mentioned in exclusion criteria (glucocorticoids other than what is prescribed in the study, antihypertensive medications, metformin, or any antidiabetic medications, any medications known to affect dexamethasone metabolism)
- Development of a significant medical condition specified in the exclusion criteria
- In female subjects, becoming pregnant during study
- Significant adverse event deemed by investigator to preclude continued participation

10.0 Statistical Considerations

The two primary endpoints will be the NT-proANP trajectory (NT-proANP levels across time) and NT-proBNP trajectory (NT-proBNP levels across time). We will also measure ANP and BNP to fully define their kinetics. The descriptive statistics, such as mean, median, range, and standard deviation, will be examined at each time point. The mean levels of the endpoints will be plotted by time points to visualize their changes. The null hypotheses are that NT-proANP will not change over time (slope of NT-proANP trajectory= 0) and that NT-proBNP will not

change over time (slope of NT-proBNP trajectory= 0). For our analysis, we will use restricted maximum likelihood (REML)-based mixed effect model analyses for the two primary endpoints: 1) NT-proANP trajectory as the outcome and time points as covariates, and 2) NT-proBNP trajectory as the outcome and timepoints as covariates.

11.0 Privacy/Confidentiality Issues

Strict confidentiality will be maintained to the fullest extent by the research team, including keeping all data in a secure location. All specimens will be coded after they are obtained and the code key kept in a secure location. Blood samples will be coded anonymously to remain confidential and identifiers will be kept in a separate, secure location. Samples may be shared with third parties outside of Vanderbilt for future testing but will remain anonymous to the recipient. Subjects may contact the principal investigator at any time to request that samples be destroyed.

12.0 Follow-up and Record Retention

Anticipated study duration is 6 months. Research data will be maintained by the PI after study closure. After study closure, research data will be maintained for a minimum of 6 years and possibly indefinitely. Data will be stored on the Vanderbilt University computer network in a password-protected database. Only members of the study team will have access. Pertinent paper documentation will be kept in a locked office and only study personnel will have access. Only personnel directly involved with the study will have access to source data and the electronic database.

13.0 References

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4. Dananberg J, Grekin RJ. Corticoid regulation of atrial natriuretic factor secretion and gene expression. Am J Physiol 1992;263:H1377-81.
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