Study Title: Nicotinamide Riboside in LVAD Recipients (PilotNR-LVAD)

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Title:	**Preoperative nicotinamide riboside (vitamin B3) supplementation in patients undergoing elective LVAD implantation**
Short title:	NR-LVAD
Test product:	Nicotinamide Riboside (NR)
Study purpose:	To assess myocardial NAD+ levels and mitochondrial function in patients treated with NR prior to left ventricular assist device (LVAD)-implantation surgery
Sponsor:	Investigator-initiated study funded by the AHA

Signature of principal investigators

The signatories agree to the content of the final clinical study protocol as presented.

Name: _____ Role: _____

Date: _____ Signature: _____

Name: _____ Role: _____

Date: _____ Signature: _____

Table of Contents

1. Introduction

1.1 Background

Mitochondrial dysfunction has been implicated in aging as well as multiple diseases, including heart failure. Multiple dietary supplements aim at improving mitochondrial function, often without clear evidence of efficacy. In myocardium, mitochondrial dysfunction is characterized by increases in both the NADH/NAD+ ratio and mitochondrial protein acetylation. Recently, we have demonstrated in mice that intraperitoneal administration of nicotinamide mononucleotide (NMN), an NAD+ precursor, can increase myocardial NAD+ levels and normalize the NADH/NAD+ ratio, decrease mitochondrial protein acetylation, and protect against pressure overload-induced cardiac dysfunction..

Though NMN is not orally bioavailable, we and others have shown that oral supplementation with nicotinamide riboside (NR), the precursor from which NMN is produced, also decreases (normalizes) tissue NADH/NAD+ ratio and improves mitochondrial function in mouse models. These animal model results suggest a conceptually innovative mechanism linking mitochondrial dysfunction to the development and progression of heart failure that is distinct from the existing hypotheses of oxidative stress and energy starvation. We hypothesize that oral supplementation with NR will increase NAD+ levels, thereby normalizing the NADH/NAD+ ratio caused by mitochondrial dysfunction during chronic stresses, and improve functional capacity and ventricular function in systolic heart failure.

We have data suggesting that NR is safe and well-tolerated at a dose of up to 1000mg twice daily in humans:

1.1.1. We have completed an NIH-funded study of NR pharmacokinetics in 8 healthy volunteers, in which NR was up-titrated to a dose of 1000mg twice daily by Day 7. NR appeared to be safe and well-tolerated in this Study (Airhart PLOS ONE, 2017). Whole blood NAD+ levels increased by a mean of 100%

1.1.2. We currently are performing a safety and tolerability trial of NR in 30 participants with clinically-stable, systolic heart failure. In this Study, patients are randomized 2:1 to NR vs. placebo and followed on randomized Study drug for 12 weeks. Patients receive NR at 1000mg twice daily for the final 9 weeks of the Study. to date, 25 participants have completed the Study. While we, as investigators, are therefore still blinded to Study results, the Study's Data Safety and Monitoring Committee (DSMC) has completed interim reviews Study results after completion of 10 and then 20 participants. Following each interim review, the DSMC recommended Study continuation.

1.1.3. In a 2x6-week double-blind crossover study of NR at a lower dose of 500mg BID, NR was well-tolerated in 24 participants completing the protocol. This lower dose was associated with an approximately 60% increase in blood NAD+ levels—a lesser effect than seen with . While blood pressure (BP) was decreased with NR in those with "elevated" blood pressure (systolic BP $\geq$120mmHg or diastolic BP$\geq$ 80mmHg), it was unchanged in those with baseline BP <120/80.

1.2 Study rationale

While preliminary data show that oral NR supplementation increases myocardial NAD+ levels in mice, there has been no direct evidence that suggests oral NR increases NAD+

levels or improves mitochondrial function in *human* hearts. *Our colleague, Dr. April Stempien-Otero, currently studies left ventricular myocardial samples obtained from advanced heart failure patients at the time of implantation of left ventricular assist devices (LVADs).* We recently submitted an R01 application (1 R01 HL144937-01) to the NHLBI to test the hypothesis that oral NR supplementation will increase myocardial NAD+ levels and improve cardiomyocyte mitochondrial function in participants with advanced heart failure planned for elective LVAD implantation. The NHLBI CICS Study Section recently reviewed this R01 application, giving it a Priority Score of 42 and a Percentile Score of 24%. However, each of the three Reviewers gave individual review category scores of 1-2 for Significance, Investigator(s), 2-3 for Innovation, but 3-6 for Approach, primarily for lack of preliminary data on feasibility. Therefore, to demonstrate feasibility, we propose to enroll 5 participants planned for LVAD implantation in a Pilot Study of NR in which they will receive NR, up-titrated over 3 days to a final NR dose of 1000mg BID. Blood and myocardial tissue analyses collected previously from age- and gender-matched LVAD recipients will serve as controls. We propose the following Aims:

Aim 1: Enroll 5 participants scheduled for elective LVAD placement into an open-label study of NR.

a. Participants will have labs (including safety panels) drawn at baseline (Day 1), then receive escalating doses of NR to a maximum dose of 1000mg twice daily by Day 3. Participants will be continued on NR at 1000mg twice daily until LVAD implantation surgery.

On the morning of LVAD implantation Surgery (Day 5 or later), participants will have final labs drawn. Samples of fresh cardiac tissue removed from the left ventricular apex during LVAD implantation surgery will be collected in the operating room. The primary analyses will be performed on NR-treated participants who were on the maximum NR dose of 1000mg twice daily for at least 2 days prior to LVAD implantation surgery. We will cap the maximum day of NR administration to 21 days. If the surgery doesn't happen by then, we will exclude the patient from the study.

Aim 2: Determine the effect of NR (as compared to historical controls) on NAD(H) levels, mitochondrial function and its regulation through epigenomic modifications in the failing myocardium.

a. Measure NAD^+ and NADH levels in the blood and myocardium of the participants.
b. Assess mitochondrial morphology and function in cardiac tissue using, respectively, EM and isolated mitochondria.
c. Determine protein acetylation in the mitochondrial and non-mitochondrial compartments and changes in nuclear gene regulation.

Aim 3: Test the hypothesis that NR improves mitochondrial function and reduces inflammatory response in HF patients receiving NR (as compared to historical controls).

a. Measure mitochondrial function in peripheral blood mononucleated cells (PBMC).
b. Determine the inflammatory response in PBMC.
c. Compare effects on the circulating inflammasome vs. myocardial inflammation.

1.3 Nicotinamide Riboside

NR is a relative of niacin, but a closer relative of nicotinamide which, unlike niacin, does

not induce flushing or pruritis and has no effect on lipid levels. NR does not induce insulin resistance or dysglycemia in mouse models. Thus, NR would potentially be cheap, safe, well-tolerated and readily available.

2. Study Objectives

Enroll 5 participants undergoing elective LVAD placement to study the effects of NR on whole blood and myocardial NAD+ levels, as well as on measures of PBMC and cardiomyocyte mitochondrial function. The effects of NR on blood and myocardial markers of inflammation also will be assessed. Blood and myocardial tissue collected previously from individuals at the time of LVAD implantation will serve as historical controls.

3. Study design

3.1 Design Overview

This study is a single-center study to compare myocardial NAD+ level and other mitochondrial endpoints from NR administration with baseline untreated patients.

4. Study Population

4.1 Eligibility

4.1.1. Inclusion Criteria:

- Diagnosis of advanced heart failure.
- Planned elective LVAD implantation surgery with patient agreements for candidacy in place as required by UWMC.
- Hospital inpatient at time of enrollment.
- Ability to undergo Study procedures.
- Willingness/ability to provide informed consent.

4.1.2. Exclusion Criteria:

- Current smoking
- Receiving certain concurrent supplements (to be determined at discretion of the PI). Note that UWMC Nutrition Care standards call for a general multivitamin (1 tab PO daily) as part of the advanced heart failure therapy (AHFT) work-up.
- Known allergies to niacin or nicotinamide.
- Hepatic, renal, endocrine, or neurological disease that disqualify them from consideration for LVAD implantation.
- Inability to perform Study visits or procedures.
- Unwillingness/inability to provide informed consent.
- Women who are currently pregnant or who wish to become pregnant over the course of the study follow-up are not allowed to join this study. This exclusion is built into the LVAD candidate selection process.

4.2 Discontinuation of subjects from study treatment

This is a study of a nutritional supplement. Two published trials of NR administration in healthy participants did not show any major adverse effects. We also will monitor for worsening in signs and symptoms of heart failure, by asking about worsening of dyspnea, dyspnea on exertion, orthopnea, paroxysmal nocturnal dyspnea, arrhythmia/palpitations or edema.

(Airhart S, PLOS ONE 2017, Mertens CE Nature Communications 2018).

4.3 Subject screening and identification

Prospective, eligible participants will be identified from the medical record from among patients with advanced heart failure who are planned to undergo LVAD implantation. If eligibility is confirmed, they will be contacted in person to determine their interest in enrolling into the Study. Each subject will receive a unique study ID. Once assigned to a subject, the subject's ID will not be re-used.

5. Treatments

At the AHFT Thursday meeting, if the committee decides that a participant is to receive an LVAD, he or she will be prescribed NR according to the following administration schedule:

Dose Escalation

Day 1: 250 mg (1 capsule) twice daily (total daily intake = 500 mg)

Day 2: 500 mg (2 capsules) twice daily (total daily intake = 1000 mg)

Day 3: 1000 mg (4 capsules) twice daily (total daily intake = 2000 mg)

Dose Maintenance

Day 4: 1000 mg (4 capsules) twice daily

Day 5-21 as applicable thru Day Before Surgery: 1000 mg (4 capsules) twice daily

Washout

Day of LVAD Surgery and/or Day 22: None

6. Procedures

6.1 – Identification of participants and consent

Based on the list of patients who are to be discussed at the Thursday Advanced Heart Failure Therapy (AHFT) meeting, potential research participants who are likely to receive an LVAD will be approached and, if interested, consented (please see separate consent form).

6.2 – Study enrollment and follow-up

In-hospital Visit (Day 1)

- Demographic information, medical history, physical examination, current medical treatments
- Labs obtained as a part of routine inpatient heart failure clinical care: CBC with WBC differential and platelets, serum chemistry panel (sodium, potassium, chloride, glucose, blood urea nitrogen and creatinine), AST and ALT
- Additional Study blood collection: (20mL):
  - 10mL EDTA tubes for PBMC isolation
  - 10mL acid-citrate-dextrose (ACD)-containing tube for NR and NAD+ assays
- Eligible participants will begin NR administration

In-hospital Visit (Day 2)

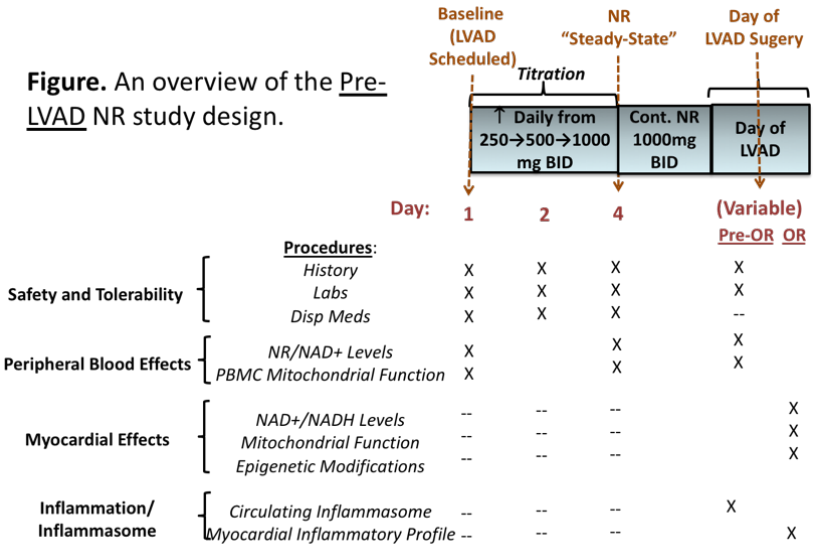
- Demographic information, medical history, current medical treatments

- Labs obtained as a part of routine inpatient heart failure clinical care: CBC with WBC differential and platelets, serum chemistry panel (sodium, potassium, chloride, glucose, blood urea nitrogen and creatinine), AST and ALT
- Record of adverse events, if any

<u>Pre-LVAD implantation Visits (Day 2 or later; starting at least 2 days prior to LVAD implantation surgery)</u>

- Day 2+:
  - Medical history, physical examination, current medical treatments
  - Labs obtained as a part of routine inpatient heart failure clinical care: CBC with WBC differential and platelets, serum chemistry panel (sodium, potassium, chloride, glucose, blood urea nitrogen and creatinine), AST and ALT
  - Record of adverse events, if any
- Day 3+:
  - Labs obtained as a part of routine inpatient heart failure clinical care: CBC with WBC differential and platelets, serum chemistry panel (sodium, potassium, chloride, glucose, blood urea nitrogen and creatinine), AST and ALT
  - Record of adverse events, if any
  - Subjects then will receive the last 1000 mg dose of NR at 8pm.
- Day 4 (If NOT Day of Surgery):
  - Medical history, physical examination, current medical treatments
  - Labs obtained as a part of routine inpatient heart failure clinical care: CBC with WBC differential and platelets, serum chemistry panel (sodium, potassium, chloride, glucose, blood urea nitrogen and creatinine), AST and ALT
  - Record of adverse events, if any
  - Additional Study blood collection (20mL):
    - 10mL EDTA tube for PBMC isolation
    - 10mL ACD tube for NR and NAD+ assays

Figure. An overview of the <u>Pre-LVAD</u> NR study design.



Day of Surgery

- Medical history, physical examination, current medical treatments
- Routine pre-operative labs
- Additional Study blood collection (20mL):
  - 10mL EDTA tube for PBMC isolation
  - 10mL ACD tube for NR and NAD+ assays
- In the operating room, myocardial samples from the LV apex (removed as an integral part of the LVAD implantation surgery:
  - Flash-frozen in liquid nitrogen for subsequent NAD+/NADH assays
  - Fresh samples for immediate processing for cardiomyocyte mitochondria isolation

If a subject has been enrolled as an inpatient, but needs to be discharged home due to a delayed LVAD surgery, IDS will dispense the study drug as discharge medication for a duration up to 7 days to bridge to the readmission. The patient will be called daily to assess for any adverse reactions to the study drug as an outpatient. Laboratory testing may be ordered per physician's discretion.

6.3 – Specimen Destinations

- Whole blood NAD+/NADH levels – Professor Danny Shen's Pharmacokinetics Lab at the UW Health Sciences Building.
- Fresh tissue mitochondrial purification and functional (Seahorse®) assay, Seahorse – Professor Rong Tian's Lab at South Lake Union (SLU).
- Frozen mitochondrial ROS and DNA copy number, protein acetylation pattern (myocardium and PBMC) – Professor Rong Tian's Lab at SLU.
- Inflammatory response in PBMC and effects on the circulating inflammasome vs. myocardial inflammation – Dr. April Stempien-Otero's Lab at SLU.

6.4 – Safety and adverse effect monitoring

Participants will have a structured history to enquire as to potential side effects and "safety" lab panel on Day 3. In addition, participants already hospitalized will have daily labs. Those not hospitalized at the time of enrollment also will have additional "safety" labs at the time of admission for the elective LVAD procedure (1-2 days prior to the LVAD implantation surgery). While the patient is inpatient, the chart will be reviewed daily to monitor for any potential adverse effects from NR administration. 4 weeks after the last dosing of the study drug, the subjects will be interviewed either in person or by phone to ask for any adverse effects or discomfort that they may have experienced during NR administration. The patient will be called daily to

7. Sources of Materials

Data will be collected throughout the study from participants on their health status and concomitant medications. Clinical, demographic and laboratory values collected as a part of the Study will be stored in the multidimensional, REDCap database. All lab samples will be identified by a study number only. Individually identifiable data is recorded in individual study charts for each participant, and in the password-protected REDCap study database. Individually identifiable data will be accessible only to the study investigators.

8. Potential Risks

Participants may experience discomfort and bruising from blood draws. Rarely, an infection could develop.
This is a study of a nutritional supplement. Two published trials of NR administration in healthy participants did not show any major adverse effects. We also will monitor for worsening in signs and symptoms of heart failure, by asking about worsening of dyspnea, dyspnea on exertion, orthopnea, paroxysmal nocturnal dyspnea, arrhythmia/palpitations or edema.

9. Adequate Protection Against Risk

Recruitment and Informed Consent. Recruitment for this proposed study will be conducted according to the IRB policies at the University of Washington, as well as HIPAA policies. Potential study participants will be identified under HIPAA waiver by chart review. Their cardiologist will approve the approach before researcher contact. Potential participants will be contacted by phone using the approved phone script, or seen in person if at the clinic or a hospitalized inpatient. Only if the participant expresses any interest will we continue study recruitment. Study staff will explain the study's purpose and design and what would be required of participants. Then, the informed consent process will be conducted by an investigator or a study coordinator. Participants will be encouraged to ask questions and to share the information with their regular doctor. A copy of this consent will be given to the participants; the original will be placed in the participant's study chart.

Protection Against Risks. All access to individually identified participant data is limited to the investigators who are directly involved in this research study, the clinical research staff of the Translational Research Unit during the subjects' visits, and the UWMC Research Testing Service. Study charts and password-protected databases are confidential and are not released to outside parties except at the participant's request. All blood samples for laboratory analysis outside UWMC are ONLY identified by a study number. The Study charts containing paper forms will be stored in the locked file cabinets in the research clinics or offices where they only can be accessed by the investigators. Each participant will receive a unique study number. The study number is linked to the participant's personally-Identifiable information in the study database and used to perform data entry and linking.

Notification of Participants and Their Physicians. A participant's primary cardiologist (or cardiology attending physician if hospitalized at time of discovery) will be any laboratory abnormalities develop. We will leave the further clinical follow-up and treatment plans to be made by the participants' physicians. Participants will not be notified of the results of other laboratory studies performed specifically for this Study.

10. Potential Benefits of Research to the Participant and Others

Minimal. Although individual participants may benefit from this study by being on a nutritional supplement that has shown some promise in animal models of heart failure, it is unlikely that a short-term NR administration will yield any substantial clinical improvement or worsening in their clinical status.

11. Importance of the Knowledge to be Gained

To our knowledge, no data exist regarding whether orally administered NR increases NAD+ levels or improves mitochondrial function in human myocardium. Therefore, we propose a trial to test the effects of NR on NAD+ levels and mitochondrial respiration in myocardium of advanced heart failure patients undergoing elective LVAD implantation

surgery. Secondarily, we plan to determine, using samples obtained at surgery, whether whole blood NAD+ levels and PBMC mitochondrial function correlate with, respectively, myocardial NAD+ levels and myocardial mitochondrial function.

12. Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) previously constituted for our on-going NR in Heart Failure Study *will amend its Charter* to provide oversight of this proposed Pilot Study. Safety reviews will be performed after Study completion by EACH of the 5 planned participants receiving NR. Safety data will include pre-specified evaluation of parameters for blood glucose, myopathy, hepatotoxicity, renal function as well as signs/symptoms of heart failure, or other possible clinical side effects, as requested by the DSMB. The three-member, Study DSMB Roster is provided below.

- **Jeffrey L. Probstfield, MD**. (Chair)
  Director, Clinical Trials Service Unit and Professor of Medicine (Cardiology)
  University of Washington School of Medicine
- **W. Robb MacLellan, MD**
  Professor of Medicine and Head, Division of Cardiology
  University of Washington
- **Kelley Branch, MD**
  Deputy Director, Clinical Trials Service Unit and Assistant Professor of Medicine (Cardiology)
  University of Washington School of Medicine