

Title page

A randomized controlled trial to assess the safety and tolerability of the nutritional supplement, nicotinamide riboside, in systolic heart failure

Test drug: Nicotinamide Riboside (NR)

Study purpose: to assess the safety and tolerability of the nutritional supplement, nicotinamide riboside, in systolic heart failure

Clinical Study phase: I/II Date: 2/17/2016

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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: _____

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1. Introduction

1.1 Background

Medical care of heart failure (HF) has been stagnant for the past 20 years; innovative therapy is urgently needed. A novel therapeutic target, mitochondrial dysfunction, has been implicated in multiple diseases, including heart failure. However, there currently is no specific treatment for mitochondrial dysfunction in human heart failure or any other disease. Mitochondria-based therapy development has been hampered both by limited understanding of how mitochondrial impairment causes cardiac dysfunction, and by a lack of interventions shown to improve mitochondrial function.

1.2 Study rationale

Recently, we demonstrated in a murine model, that impaired mitochondrial oxidative phosphorylation led to an increased myocardial NADH/NAD⁺ ratio and increased mitochondrial protein acetylation, without affecting mitochondrial ROS production or ATP synthesis. These changes rendered the heart susceptible to chronic stresses, which accelerated the development of heart failure. We observed a similar increase of NADH/NAD⁺ ratio and increase in protein acetylation in animal models of heart failure due to chronic pressure overload with no prior mitochondrial dysfunction. Supplying the NAD⁺ precursor, nicotinamide mononucleotide (NMN), to these mice normalized the NADH/NAD⁺ ratio, prevented increased mitochondrial protein acetylation and improved cardiac function. 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. Thus, 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 therefore propose a safety and tolerability trial of the nutritional supplement, nicotinamide riboside (NR) in 30 participants with clinically-stable, systolic heart failure.

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. The application is in response to the program announcement of “Nutrition and Diet in the Causation, Prevention, and Management of Heart Failure” as NR has recently been approved for human use as a health supplement.

2. Study Objectives

The Study has three objectives: 1) to determine the safety and tolerability of NR in patients with clinically-stable, systolic heart failure (LVEF <40%); 2) to determine whether, at the doses employed, NR has measureable effects on whole blood NAD⁺ levels; and 3) to explore the potential range of effects of NR supplementation on heart failure surrogate endpoints.

3. Study design

3.1 Design Overview

This study is a single-center, prospective, double-blind, 2:1 randomization to NR or placebo, safety and tolerability trial of placebo versus nicotinamide riboside (NR) in participants with clinically stable (NYHA functional class I-IIIA) systolic heart failure (LVEF by echocardiography or radionuclide ventriculography <40%) due to non-ischemic or ischemic etiologies.

4. Study Population

4.1 Eligibility

4.1.1 Inclusion criteria

- Men and women aged 18 and older with systolic heart failure (LVEF by standard 2D echocardiography or radionuclide ventriculography of $\leq 40\%$) deemed, in the clinical opinion of their treating cardiologist to be non-ischemic or ischemic in origin.
- Clinically stable (no cardiac procedures or hospitalizations for hospitalizations for cardiac causes, including HF, ischemia or arrhythmia) within the previous 3 months
- Ability to undergo study procedures, including scheduled visits, blood draws and six-minute walk tests
- Willingness/ability to provide informed consent

4.1.2 Exclusion Criteria

- Heart failure with preserved ejection fraction (LVEF $>40\%$)
- Heart failure due, in the opinion of their treating cardiologist, to etiologies other than non-ischemic or ischemic. Examples of exclusionary heart failure etiologies include primary valvular disease, or infiltrative or inflammatory cardiomyopathies.
- Cardiac surgery, percutaneous coronary intervention (PCI) or cardiac device implantation within the previous 3 months
- Hospitalizations for cardiovascular causes, including heart failure, chest pain, stroke/TIA or arrhythmias within the previous 3 months
- Inability to perform Study visits or procedures (e.g., physical inability to perform 6MWT)
- Unwillingness/inability to provide informed consent
- ALT $> 3 \times$ upper limit of normal, hepatic insufficiency or active liver disease
- Recent history of acute gout
- Chronic renal insufficiency with creatinine $\geq 2.5 \text{ mg/dl}$
- Pregnant (or likely to become pregnant) women
- Significant co-morbidity likely to cause death in the 6 month follow-up period
- Significant active history of substance abuse within the previous 5 years
- Current participation in another long-term clinical trial
- History of intolerance to NR precursor compounds, including niacin or nicotinamide

4.2 Discontinuation of subjects from study treatment

This is a study of a nutritional supplement. For those participants taking nicotinamide riboside, a warm flushing sensation may occur when first taking this supplement. We will monitor for skin rash, increased blood sugar, or cause mildly abnormal hepatic function. In diabetic participants, it is possible that nicotinamide riboside may worsen glucose intolerance and necessitate an adjustment in medications for treatment of diabetes. 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. We will monitor blood levels of B-type natriuretic peptide (BNP), serum electrolytes, BUN and creatinine at Study visits 1, 3, 4, 7 and visit 6 according to the PI's judgement.

4.3 Subject identification

Each subject will receive a unique study ID. Once assigned to a subject, the subject's ID will not be re-used.

5. Treatments

Participants will be randomized to either NR at an initial dose of 250mg tablets or placebo two times per day. The NR dose then will be up-titrated weekly by 250mg/dose to a final dose of 1000mg two times per day at the end of Week 4. Participants will be continued on the 1000mg two times per day dose up to the final follow up visit (week 12). If, at any step, a dose increase is not tolerated, the maximum previously-tolerated dose will be continued through to week 12.

6. Procedures

Every attempt should be made to complete the follow-up visits during the defined window periods. A final follow-up visit is required for all participants. In the rare cases a final follow-up visit cannot occur within the 5-day timeframe following study end date, any attempt to contact must be recorded on a special contact form, until/unless appropriate information is obtained.

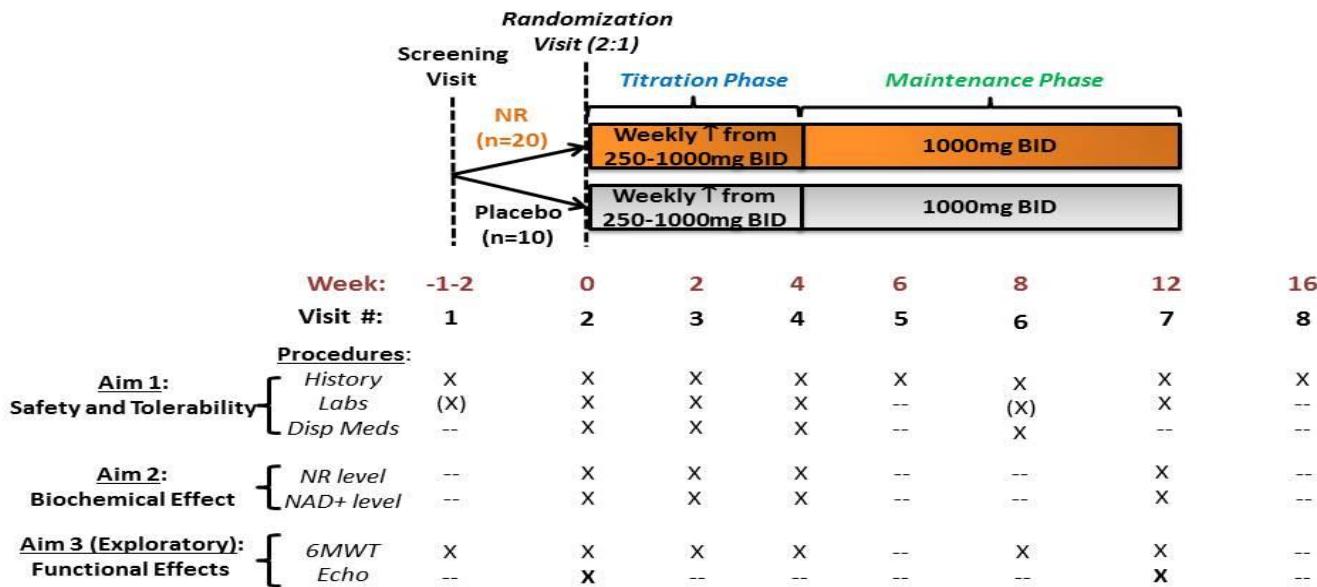
Laboratory results:

Participants will be blinded to the results of laboratory studies obtained as a part of this Study.

Echocardiography results:

Participants will be blinded to the results of echocardiograms obtained as a part of this Study.

6.1 Schedule of procedures



6.1.1 Visit 1 (Screening visit)

Prospective, eligible participants with documented systolic heart failure meeting study inclusion criteria will undergo a screening visit.

The following procedures will be performed at the screening visit:

- Demographic information, medical history (including NYHA class and most recent creatinine value), physical examination, current medical treatments
- Minnesota Living with Heart Failure quality of life (MNQOL) score
- Fasting blood draw: ONE 5mL pearl top tube (for BNP), TWO 3mL lavender top tubes (for CBC, HbA1C); ONE 4mL lime top tube [for uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine)], ONE 4mL gold SST tube (for insulin) ONE 2.7 mL “blue top” tube for whole blood NR and NAD+ levels and FOUR 4.5 mL GLASS blue top tubes (for mitochondrial function)
- Urine pregnancy test at screening in women of child-bearing age
- Six-minute walk test (6MWT)
- Dispense placebo to take twice daily

6.1.2 Visit 2 (Randomization visit)

After successful completion of the Screening visit, the participant will return for the Baseline Visit, at which participants would be randomized in a 2:1 allocation to receive blinded study medication with nicotinamide riboside or matching placebo. All participants who are randomized are irrevocably in the study, whether or not they are subsequently found to be eligible or actually receive the allocated treatment, and they should be followed until the study end date or death. Study drug administration should be initiated as soon as possible after treatment allocation.

The following procedures will be performed at the randomization visit:

- Update as needed demographic information, medical history, current medical treatments
- Vital signs and weight
- Randomization by investigational drug services.
- BASELINE MNQOL score
- BASELINE 6MWT
- BASELINE echocardiography (TTE and TDI) This may also take place at screening if needed due to scheduling
- Dispense blinded study medication:
 - 250mg PO BID or Placebo for one week
 - 500mg PO BID or Placebo for one week

6.1.3 Follow-up visit 3 (week 2)

The following procedures will be performed at the follow-up visits (Day 14±3):

- Update as needed demographic information, medical history, current medical treatments
- Vital signs and weight
- MNQOL score
- Fasting blood draw: ONE 5mL pearl top tube (for BNP), TWO 3mL lavender top tubes (for CBC, HbA1C); ONE 4mL lime top tube [for uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine)], ONE 4mL gold SST tube (for insulin), and ONE 2.7 mL “blue top” tube for whole blood NR and NAD+ levels
- 6MWT
- Record of adverse events, if any
- Study drug adherence
- Record of concomitant medications or interventions, if any
- Dispense blinded study medication:
 - 750mg PO BID or Placebo for one week
 - 1000mg PO BID or placebo for one week

6.1.4 Follow-up visit 4 (week 4)

The following procedures will be performed at the follow-up visits (Day 28±3):

- Update as needed demographic information, medical history, current medical

- treatments
- Vital signs and weight
- MNQOL score
- Fasting blood draw ONE 5mL pearl top tube (for BNP), TWO 3mL lavender top tubes (for CBC, HbA1C); ONE 4mL lime top tube [for uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine)], ONE 4mL gold SST tube (for insulin), and ONE 2.7 mL “blue top” tube for whole blood NR and NAD+ levels
- Additional blood draw 3 hours after AM NR dose: ONE 2.7 mL “blue top” tube for whole blood peak NR and NAD+ levels
- 6MWT
- Record of adverse events, if any
- Study drug adherence
- Record of concomitant medications or interventions, if any
- Dispense blinded study medication:
 - 1000mg PO BID or Placebo

6.1.5 Follow-up telephone visit 5 (week 6)

This visit will be conducted by study staff via telephone interview. The assessments include:

- Record of concomitant medications or interventions, if any
- Record of adverse events, if any

6.1.6 Follow-up visit 6 (week 8)

The following procedures will be performed at the follow-up visits (Day 56±5):

- Update as needed demographic information, medical history, current medical treatments
- Vital signs and weight
- MNQOL score
- * Fasting blood draw: ONE 5mL pearl top tube (for BNP), TWO 3mL lavender top tubes (for CBC, HbA1C); ONE 4mL lime top tube [for uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine)], ONE 4mL gold SST tube (for insulin),
- 6MWT
- Record of adverse events, if any
- Study drug adherence
- Record of concomitant medications or interventions, if any
- Dispense blinded study medication:
 - 1000mg PO BID or Placebo

* Fasting blood draw optional in case of safety concerns

6.1.7 Final Follow-up visit 7 (week 12)

The following procedures will be performed at the final follow-up visit (Day 84 ± 5):

- Update as needed demographic information, medical history, current medical treatments
- Vital signs and weight

- MNQOL score
- Fasting blood draw: ONE 5mL pearl top tube (for BNP), TWO 3mL lavender top tubes (for CBC, HbA1C); ONE 4mL lime top tube [for uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine)], ONE 4mL gold SST tube (for insulin), and ONE 2.7 mL “blue top” tube for whole blood NR and NAD+ levels and FOUR 4.5 mL GLASS blue top tubes (for mitochondrial function)
- 6MWT
- Echocardiography
- Record of adverse events, if any
- Study drug adherence
- Record of concomitant medications or interventions, if any
- Record of efficacy endpoints, if any

6.1.8 End of washout telephone visit (30 days post Final Follow-up Visit)

The washout visit will be conducted by study staff via telephone interview. Washout visit assessments include:

- Record of concomitant medications or interventions, if any
- Record of adverse events, if any

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 a multidimensional database at the UW Clinical Atherosclerosis Research Laboratory (CARL). All lab samples are identified by a study number only. The two echocardiograms will be recorded on MOD disks during the study. Individually identifiable data is recorded in individual study charts for each participant, and in a password-protected 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.

There are no known hazards to exposure of ultrasound waves, however some participants may experience discomfort at lying still.

This is a study of a nutritional supplement. For those participants taking nicotinamide riboside, a warm flushing sensation may occur when first taking this supplement. We will monitor for skin rash, increased blood sugar, or cause mildly abnormal hepatic function. In diabetic participants, it is possible that nicotinamide riboside may worsen glucose intolerance and necessitate an adjustment in medications for treatment of diabetes. 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. Only if the participant expresses any interest will we continue study recruitment. To the participants who are interested in the study, study staff will explain the study's purpose and design and what would be required of participants. A series of questions will be asked to make sure the participant meets eligibility requirements. Then, the informed consent 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. Study participants and their primary care or vascular care physicians will be notified if there is clinical worsening of their heart failure. 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

Individual participants may benefit from this study by being on a nutritional supplement that has shown some promise in animal models of heart failure.

11. Importance of the Knowledge to be Gained

If the Study can demonstrate that nicotinamide riboside is safe and well-tolerated in participants with systolic heart failure, this will form the basis of a follow-on study to assess the efficacy of nicotinamide ribose on surrogate endpoints in heart failure, such as functional capacity and ejection fraction. Thus, the Study may provide information necessary to further explore the clinical utility of this nutritional supplement in the treatment of systolic heart failure, a large and growing population of high risk participants for whom treatment, at present, is less than adequate.

12. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be instituted for this Study in order to ensure its ongoing safety and to oversee the Interim Analyses. Recommendation for trial continuation will be guided by monitoring boundaries at interim analyses at which formal efficacy analysis is performed as well as safety evaluations at all safety data reviews. Safety review meetings will be held by the DSMB prior to Study initiation, at which time the DSMB will develop any formal "stopping rule(s)" for the Study. The DSMB will meet three

additional times: after completion of 10, 20 and 30 Study participants. 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 such as gout, as requested by the DSMB. Enrollment to the study will continue throughout the scheduled meetings of 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