**CLINICAL RESEARCH PROJECT** **Protocol # 18-H-0107**
**Amendment B**
**IND #: NA**

**Note: Supplement, Nicotinamide Riboside, is not subject to IND regulations as covered by the Dietary Supplement Health and Education Act of 1994.**

**NHLBI Protocol:** Pilot Study to Evaluate the Effect of Nicotinamide Riboside on Skeletal Muscle Function in Heart Failure Subjects

Short Title: Skeletal Muscle Effects of NR in Heart Failure

Keywords: Heart Failure, Skeletal Muscle Function, Nicotinamide riboside, Mitochondrial Function

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| Subjects in study at NIH: | Number | Sex | Age range |
|---|---|---|---|
| | 25 | M/F | 18-75 years |

| | |
|---|---|
| Multi-center trial: | Yes |
| Ionizing Radiation for Research: | No |
| Off-Site Project: | Yes |
| DSMB Involvement: | Yes |
| Tech Transfer: | CRADA HL-CTCR-17-003 |
| IND/IDE: | No |

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**Précis**

As life expectancy increases and acute cardiac mortality decreases, the incidence of chronic heart failure (HF) continues to rise, and despite this, conceptual advances in the treatment of chronic heart failure have not increased substantially over last few decades. One intracellular component of heart failure progression is mitochondrial bioenergetic dysfunction. Although the mechanism underpinning this is not completely understood, recent metabolomics data demonstrated an incomplete flux of metabolites through oxidative phosphorylation (OX PHOS) in HF. In parallel, data has shown that hyperacetylation of mitochondrial bioenergetic enzymes, with the concomitant blunting of enzymatic activity is evident in HF. Putting these together, an emerging hypothesis implicates excessive acetylation of mitochondrial proteins with the subsequent blunting of bioenergetic enzyme function, as a mechanism underpinning incomplete flux through OX PHOS resulting in HF progression.

In parallel with cardiac bioenergetic deficiency chronic HF subjects display disrupted skeletal muscle OX PHOS, which is thought to contribute towards overall fatigue and reduced exercise tolerance. Interestingly exercise training in HF subjects improves skeletal muscle mitochondrial OX PHOS capacity and subject activity levels. Exercise training additionally increases activity of the mitochondrial regulatory deacetylase sirtuin enzymes SIRT1 and SIRT3, in parallel with improved skeletal muscle OX PHOS capacity. At the same time HF-associated disruption in skeletal muscle metabolic function activates skeletal muscle cytokine production. These inflammatory programs, in turn, are proposed to contribute towards impaired functional capacity in HF. Interestingly, and mirroring improved OX PHOS following exercise programs in HF studies, exercise training similarly reduces skeletal muscle inflammatory effects.

Biochemical and bioenergetic consequences of impaired mitochondrial OX PHOS leads to decreased $NAD^+$ levels, which exacerbate mitochondrial dysfunction by inactivating the $NAD^+$ dependent sirtuin enzymes. Experimental studies using $NAD^+$ precursors to increase $NAD^+$ production have been shown to normalize $NADH/NAD^+$ ratios and activate Sirtuin enzymes, resulting in enhanced OX PHOS with beneficial effects in numerous systems including skeletal muscle and in the blunting of inflammation.

In this pilot study we will **directly** assess the effect of the $NAD^+$ precursor, nicotinamide riboside (NR) on skeletal muscle mitochondrial OX PHOS in HF subjects using: skeletal muscle NMR spectroscopy assessment of the rate of high energy phosphate recovery in response to submaximal exercise; assessment of the effect of NR on functional capacity using cardiopulmonary exercise testing (CPET) to determine $VO_{2max}$ and anaerobic threshold; evaluation of the NR effect on serum metabolomics at rest and in response to CPET; and by measuring circulating cytokine levels pre- and post- NR administration. These studies would enable a more comprehensive assessment of the role for NR supplementation on skeletal muscle mitochondrial function in subjects with systolic HF.

## 1. Background

As a result of life expectancy increases and a reduction in acute cardiac mortality, the incidence of chronic heart failure is rising with approximately 900,000 new diagnoses per annum.[1] Despite this increasing burden of disease, conceptual advances in the treatment of chronic heart failure have not increased substantially over the last few decades.

One component of heart failure progression, which is also emerging as a biomarker of poor outcome, is the development of mitochondrial dysfunction with concomitant bioenergetic deficits.[2, 3] Prior therapeutic approaches to abrogate or delay bioenergetic deficiency focused on directing and augmenting specific substrate utilization in the heart.[2] However, studies to date have not shown convincing benefit and/or gave rise to unacceptable side effects.[2] A possible reason for the lack of efficacy of this approach may have been uncovered by metabolomics analyses, which have found that mitochondrial bioenergetic defects associated with chronic heart failure are associated with incomplete metabolism of substrates through metabolic pathways with evidence of a bottleneck of carbon flux through mitochondrial oxidative phosphorylation pathways.[4, 5] In parallel, data support that hyperacetylation of mitochondrial bioenergetic enzymes, with the concomitant blunting of enzymatic activity, may be a mechanism underpinning this incomplete metabolic flux.[6] At the same time biochemical data show that acetylation of mitochondrial proteins plays an important post-translational regulatory role in the blunting of mitochondrial energetic function and in exacerbating mitochondrial production of reactive oxygen species.[7, 8] Together, these data suggest that excess acetylation of mitochondrial proteins blunt bioenergetic enzyme function and may play a role in the progressive disruption in mitochondrial bioenergetic functional integrity.

In parallel with cardiac bioenergetic deficiency subjects with chronic heart failure also show disruption of skeletal muscle oxidative phosphorylation.[9] These skeletal muscle changes are thought to contribute towards overall fatigue and reduced exercise tolerance in heart failure.[9] Interestingly, using exercise training as an intervention to improve heart failure patient activity levels also improves muscle mitochondrial oxidative phosphorylation capacity.[9] Exercise training additionally increases the activity of the mitochondrial regulatory deacetylase sirtuin enzymes SIRT1 and SIRT3, in parallel with improved skeletal muscle oxidative phosphorylation capacity.[10, 11] At the same time, serum obtained from exercising individuals compared to sedentary individuals demonstrated augmented mitochondrial function in skin fibroblasts when their serum was used to condition the fibroblast growth media.[12] Additionally heart failure associated disruption in skeletal muscle metabolic function plays a role in activating skeletal muscle cytokine production. These inflammatory programs are proposed to contribute towards the detrimental effects on heart failure patient functional capacity. Interestingly, and mirroring improved oxidative phosphorylation following exercise programs in heart failure studies, exercise training has also been found to reduce skeletal muscle inflammatory effects.[13]

Biochemical and bioenergetic consequences of impaired mitochondrial oxidative phosphorylation leads to decreased $NAD^+$ levels, which in turn appears to exacerbate

mitochondrial dysfunction by inactivating the $NAD^+$ dependent sirtuin enzymes. Experimental studies using $NAD^+$ precursors (including nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR)) to increase $NAD^+$ production through the $NAD^+$ salvage pathway have been shown to normalize the $NADH/NAD^+$ ratio and activate Sirtuin enzymes, which in turn function to improve mitochondrial function with beneficial effects in numerous systems including skeletal muscle and in suppressing inflammation.[14, 15]

Our collaborators at the Univ. of Washington (UW - O'Brien and Tian) are currently enrolling stable heart failure subjects into an placebo-controlled NR study to assess the effect of NR on patient tolerability, on echocardiographic assessment of left ventricular function and on the effects of NR on the 6-minute walk test. However, none of those studies directly assay the effects of NR on skeletal muscle mitochondrial function.

In this pilot study we propose to **directly** assess the effect of NR on mitochondrial oxidative phosphorylation by: skeletal muscle NMR spectroscopy assessment of the rate of high energy phosphate recovery in response to submaximal exercise; assessment of the effect of NR on functional capacity using cardiopulmonary exercise testing (CPET) to determine $VO_{2max}$ and anaerobic threshold, to evaluate the NR effect on serum metabolomics at rest and in response to CPET and to measure circulating cytokine levels pre- and post- NR administration. We will also evaluate whether serum-conditioned media from individuals subjects drawn at 12 weeks of NR supplementation compared to baseline show improved mitochondrial function in skin fibroblasts. These studies would enable a more comprehensive assessment of the role for NR supplementation on mitochondrial function and metabolic flux through oxidative phosphorylation in subjects with systolic heart failure.

## Preliminary Results

Recently, our collaborators at the University of Washington (UW) demonstrated in a murine model, that impaired mitochondrial oxidative phosphorylation led to a decreased myocardial $NAD^+/NADH$ ratio and increased mitochondrial protein acetylation. These changes rendered the heart susceptible to chronic stresses, which accelerated the development of heart failure. They observed a similar decrease in $NAD^+/NADH$ ratio and an 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 $NAD^+/NADH$ ratio, prevented increased mitochondrial protein acetylation and improved cardiac function.[16] 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 $NAD^+/NADH$ ratio and improves mitochondrial function in mouse models.[16-18] Together, these animal model results suggest a conceptually innovative mechanism linking mitochondrial dysfunction to the development and

progression of heart failure. Additionally, NR may be a useful nutritional supplement to increase $NAD^+$ levels, thereby normalizing the $NAD^+$/NADH ratio caused by mitochondrial dysfunction during chronic stresses, and potentially improve functional capacity and ventricular function in systolic heart failure.

Our UW collaborators completed a pharmacokinetics study (NCT02689882). NR was supplied as 250 mg capsules by the manufacturer (Niagen®, ChromaDex, Irvine, CA). NR was manufactured in a GMP-compliant facility according to ISO/IEC 18025:2005 standards. Two Certificates of Analysis provided by the manufacturer and performed on separate lots reported ~99% purity of the NR preparation. In addition, an independent analysis was performed at the University of Washington on NR (Niagen®) purchased online (Amazon.com, Inc., Seattle, WA) by the investigators; 1H-NMR spectroscopy of this NR demonstrated 98-99% purity (Figure 1).

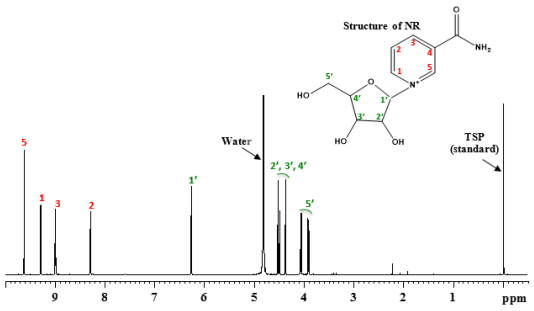


Figure 1. **Typical $^1$H NMR spectrum of an aqueous extract (in deuterated water) of a NR capsule obtained on a Bruker AVANCE III 800 MHz nuclear magnetic resonance (NMR) spectrometer**. NMR signals that arise from NR are labeled with the corresponding location of the hydrogen atom(s) as shown in the molecular structure of NR (inset). TSP [3-(trimethylsilyl)propionic acid-2,2,3,3-$d_4$ sodium salt] was used as an internal standard to quantify the amount and purity of NR in the capsules. The purity of NR was calculated based on integration of all the peaks and was in the range of 98-99%.

Eight subjects participated in this PK study and NR in incremental doses up to 1 gram bid was administered over 9 days. The mean basal (Day 1) circulating level of $NAD^+$ was 18 ± 4 µg/mL, consistent with values reported previously in healthy subjects.[19] Nicotinamide adenosine dinucleotide (NADH) was not detected in blood. Nicotinamide mononucleotide (NMN), a known immediate precursor of $NAD^+$, was detected in Baseline (Day 1) blood samples of all subjects but was at or just above the assay lower limit of quantitation. Notable elevations in blood NAD+ concentrations were seen on Day 9 compared to Baseline on Day 1 in every subject (Figure 2). Blood $NAD^+$ was, on average, about 2-fold higher than Baseline (range 1.34- to 2.66-fold), with a mean increase of 17.7 µg/mL ($p = 0.001$). As a group, the rise in blood $NAD^+$ following NR treatment did not correlate with the basal blood level on Day 1 ($R^2 = 0.27$, $p = 0.2$).

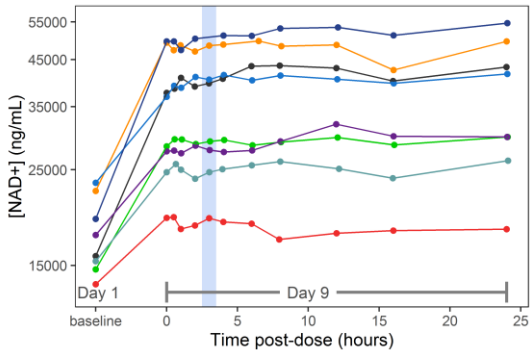


Figure 2. **Concentration-time curves for NAD$^+$.** Each subject is depicted in a different color with time points connected by a line. The y-axis depicts NAD+ concentrations in ng/ml, and the x-axis depicts values on Day 1 (baseline) and then time post-dose in hours on Day 9. The baseline time point was collected pre-dose on Day 1 of the trial.

**This study revealed no significant side effects and the primary pre-specified safety data showed no changes in levels of potassium, glucose, uric acid, creatine kinase or alanine aminotransferase levels (data not shown). The subjects also displayed no changes in hemodynamics, although the hematocrit dropped from 40±3 to 39±3 (percent) and the platelets dropped from 220±40 to 200±30 (x10$^3$/µL), both ±SD with a p value < 0.05. The modest reduction in hematocrit, hemoglobin and platelet count were suspected to result from the repeated daily laboratory blood draws over the nine-day study.**

At the same time we recently found that NR improves mitochondrial oxidative phosphorylation capacity in parallel with suppressing in-vitro inflammation in human peripheral blood cells.[15] Hence, given the role of skeletal muscle in heart failure inflammation, we will also measure serum cytokine levels in response to NR in this protocol.

Additional studies have been completed or are ongoing using NR to study effects on mitochondrial biology related conditions including aging (at the University of Colorado in Boulder – PI - Drs Christopher R. Martens - actively recruiting) and in heart failure (University of Washington in Seattle – Dr Kevin O'Brien - actively recruiting). Based on their prior pharmacokinetics study described above the University of Seattle team are using 1000mg BID in their Heart Failure protocol. To date, they have recruited 15 subjects and the dose of 1000 mg BID has been well tolerated. We therefore propose to use the same, 1000mg BID dosing.

**Hypothesis**

NR supplementation in stable NYHA FC II-III heart failure subjects will improve skeletal muscle mitochondrial function.

**Proposed Scientific/Clinical Innovations and Advances of the Study**

- Evaluate whether pharmacologic activation of Sirtuin enzymes by NR will enhance mitochondrial oxidative phosphorylation in skeletal muscle of heart failure subjects.
- Use metabolomics to evaluate whether NR can ameliorate the bottleneck in metabolic flux through the oxidative phosphorylation pathway in HF subjects.
- Expand our understanding of the role of disrupted oxidative phosphorylation on inflammation in heart failure.

## 2. Objectives

A pilot study will be undertaken where up to 25 participants with clinically stable, systolic heart failure (LVEF $\leq$45%) will be enrolled with a goal of having 15 subjects complete the treatment. NR will be increased at two weekly intervals by 250 mg/dose (500 mg/day) to a final dose of 1000mg PO BID (2000 mg/day). Clinic visits with bi-weekly labs during dose escalation will assess HF symptoms and monitor proBNP, CBC, HbA1C, ALT, CK, insulin/glucose, uric acid, electrolytes, BUN/Cr levels. Serum $NAD^+$ levels will be measured at baseline, 12 and 16 weeks and baseline (within 3 months of enrollment) and 12 week echocardiograms will be performed to measure left ventricular systolic and diastolic function.

**Scientific Aims: Determine the effects of NR on skeletal muscle oxidative phosphorylation capacity in heart failure:**

A) Baseline, 12-week (± 5-days) (on NR) and 16-week (± 5-days) (4-week washout) skeletal muscle fixed-load sub-maximal exercise NMR spectroscopy to assay skeletal muscle phosphocreatine recovery time as a measure of mitochondrial oxidative phosphorylation.
B) Baseline and 12-week (± 5-days) cardiopulmonary testing to assay $VO_{2max}$ and anaerobic thresholds.
C) Baseline and 12-week(± 5-days) serum quantitative metabolomic profiling, pre- and post-CPET to evaluate whether NR increases the rate of oxidative phosphorylation.
D) Baseline and 12-week(± 5-days) quantitative serum cytokine immunoassay profiling to assess whether NR blunts HF linked inflammation.
E) Skin biopsy at baseline and research blood at baseline and 12 weeks (± 5-days) to explore the effects of NR on oxidative phosphorylation and inflammation in respective subject primary skin fibroblasts.

## 3. Study Design and Procedures

The study design is an open label pilot study with subjects serving as their own controls. The outline of the clinical protocol is shown in a tabular schematic below.

| Visit # | 1 | 2 | 3 | 4 | 5 | 6 | 7A/B | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Weeks | -1 to -3 | 0 | 0+ 1-2 days | 2 | 4 | 6 | 12 | 12 + 1-2 days | 16 |
| | | | | | | | | | |
| **Procedures** | | | | | | | | | |
| History | X | X | | X | X | X | X | | X |
| Physical exam | X | | | X | X | X | X | | X |
| Initiate NR/dispense meds | | | X | | | | | | |
| Dose adjustment | | | | X[1] | X[1] | X[1] | | | |
| Stop NR | | | | | | | | X | |
| Urine pregnancy test | X[2] | X[2] | X[2] | | | | X[2] | X[2] | X[2] |
| Clinical blood tests | (X) | X[4] | X[4] | X | X | X | X[4] | X[4] | X |
| NMR spectroscopy | | X[4] | X[4] | | | | X[4] | X [4] | X |
| CPET | | X[4] | X[4] | | | | X [4] | X[4] | |
| Echo | X* | | | | | | X | | |
| Research blood | | X[4] | X[4] | | | | | X[4] | |
| Skin biopsy | | X[3] | X[3] | | | | | | |
| AE assessment | | | | X | X | X | X | X | X |
| Concomitant meds/tx | X | | | X | X | X | X | X | X |
| Study drug adherence | | | | X | X | X | X | | |

Key:

(X) Performed only if baseline laboratory studies not performed during routine testing within 6 months

X Procedure performed

[1]Increase NR dose by 250mg BID

[2]Pregnancy test will only be performed in women with childbearing potential. It can be completed at Visit 1, 2 and/or 3 and Visit 7 or 8

[3]Skin biopsy will be completed at either Visit 2 or 3.

[4]NMR spectroscopy, clinical and research blood, and CPET testing can be performed at either Visit 2 or 3 for baseline timepoint and Visit 7 or 8 for end of treatment timepoint.

* Procedure is optional (Echo is optional on Visit 1 if PI feels there is an adequate and timely historical Echo)

Visits 1, 4, 5, 6, and 7 may take place at either WRNMMC or NIH Clinical Center.

Visits 2, 3, 7B, 8, and 9 will take place at the NIH Clinical Center

Subjects will be recruited either at the WRNMMC Heart Failure Clinic under the Direction of Dr.'s Flanagan (Site PI) and Welch or by physician or self-referral through the NIH Clinical Center. When a subject agrees to participate in this study from either site, the study referral contact information will be provided to arrange the appropriate study visits. The screening and clinic visits and all dose escalation visits will be performed at either the WRNMMC ambulatory clinic or the NIH CC depending on the enrollment site. Subjects recruited from the WRNMMC Heart Failure Clinic will undergo all screening and clinic/dose escalation visits (Visit 1,4,5,6, and 7) at the WRNMMC Heart Failure Clinic unless there is a scheduling conflict at WRNMMC Heart Failure clinic that would result in a delay in dose escalation. In this case, the visit may take place at NIH CC. Subjects recruited and enrolled from the NIH CC will complete all study visits at the NIH CC only. The data end-point analysis visits (Visits 2, 3, 7, 8 and 9) for all patients will take place only at the NIH Clinical Center. WRNMMC enrolled subjects may visit both sites for Visit 7.

**Visit 1 (Screening visit, WRNMMC or NIH CC)**

Prospective, eligible participants with documented systolic heart failure meeting study inclusion criteria will undergo a screening visit. All screening tests will be performed only after obtaining consent.

**The following procedures will be performed at the screening visit:**

- Demographic information, medical history, physical examination, current medical treatments will be reviewed
- Blood draws will analyze: CBC+Diff, BNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels.
- Urine pregnancy test at screening in women of child-bearing age
  Baseline echocardiogram (optional at Visit 1 if PI feels there is an adequate and timely historical echo)

If eligible, consent to protocol

* Blood draw not required at screening visit for patients who have historical blood work (within the last 6 months from the time of consent).

If enrollment takes place at WRNMMC, the signed consent form will then be faxed to the NHLBI Investgator team and subjects will be remotely registered at the NIH Clinical Center (Off-site registration) to obtain an NIH CC medical record number.

**Visit 2 (Week 0, NIH)**

**The following procedures will be performed at Visit 2:**

- Brief history to review heart failure symptoms
- NIH consent
- Blood draws will analyze: CBC+Diff, BNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels. This blood draw will not be performed if subject has normal clinical blood work within the last two weeks.
- Baseline skeletal muscle exercise NMR Spectroscopy
- Urine pregnancy test for women with childbearing potential
- Skin biopsy will be done at Visit 2 or Visit 3

**Visit 3 (Week 0 + 1-2 days, NIH CC)**

**The following procedures will be performed at Visit 3:**

- Dispense NR: Level 1 ( 250mg PO BID to initiate supplementation in evening after visit 3).
- Dispense Level 2-4 bottles of NR: Level 2 (500mg PO BID – to initiate after visit 4), Level 3 (750mg PO BID – to intiate after visit 5) and Level 4 (1000mg PO BID – to initiate after visit 6 and maintain through visit 7).

**The following procedures will be performed at either Visit 2 or Visit 3:**

- Baseline Cardio-Pulmonary Exercise Testing
- Research Bloods
- Skin biopsy
- Blood draws will analyze: CBC+Diff, proBNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels. This blood draw is not required if subject has normal clinical blood work within the last three weeks.
- Baseline skeletal muscle exercise NMR Spectroscopy
- Urine pregnancy test for women with childbearing potential

**Visit 4 (Week 2, WRNMMC or NIH CC)**

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

- Medical history, physical examination, current medical treatments
- Blood draws will analyze: CBC+Diff, proBNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels
- Record of adverse events, if any
- Study drug adherence with pill count and collect Level 1 NR 250mg PO BID bottle.
- Record of concomitant medications or interventions, if any
- Communicate the initiation of Level 2 NR 500mg PO BID in evening after visit 4

**Visit 5 (Week 4, WRNMMC or NIH CC)**

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

- Medical history, physical examination, current medical treatments
- Blood draws will analyze: CBC+Diff, proBNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels.
- Record of adverse events, if any
- Study drug adherence with pill count and collect Level 2 NR 500mg PO BID bottle
- Record of concomitant medications or interventions, if any
- Communicate the initiation of Level 3 NR 750mg PO BID in evening after visit 5

**Visit 6 (Week 6, WRNMMC or NIH CC)**

**The following procedures will be performed at the follow-up visits (Day 42±5):**

- Medical history, physical examination, current medical treatments
- Blood draws will analyze: CBC+Diff, BNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels.
- Record of adverse events, if any
- Study drug adherence with pill count and collect Level 3 NR 750mg PO BID bottle
- Record of concomitant medications or interventions, if any
- Communicate the initiation of Level 4 NR 1000mg PO BID in evening after visit 6

**Visit 7 (Week 12, WRNMMC and/or NIH CC)**

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

- 7A WRNMMC (Day 84 +/-5)Medical history, physical examination, current medical treatments
- Blood draws will analyze: CBC+Diff, proBNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels.
- Record of adverse events, if any
- Study drug adherence with pill count
- Record of concomitant medications or interventions, if any
- Continue Level 4 NR - 1000mg PO BID
- Echocardiography

7B NIH (Day 84+/-5)

- Pregnancy test on women with childbearing potential (may be performed at Visit 7 or 8)

- Skeletal muscle exercise NMR spectroscopy or CPET
- Research blood if CPET performed at this visit

**Visit 8 (Visit 7B + 1-3 days, NIH CC)**

- CPET or Skeletal muscle exercise NMR spectroscopy (whichever was not done at Visit 7B)
- Research blood if CPET performed at this visit
- Pregnancy test on women with childbearing potential (may be performed at Visit 7 or 8)
- Record of adverse events, if any
- Study drug adherence with pill count
- Discontinue NR
- Record of concomitant medications or interventions, if any

**Visit 9 (Week 16 ± 1 week, NIH)**

The following procedures will be performed at the final study visit (Day 112 ± 5)

- Medical history, physical examination, current medical treatments
- Blood draws will analyze: CBC+Diff, proBNP, HbA1C, uric acid, ALT, CK, Chemistry panel (electrolytes, glucose, blood urea nitrogen and creatinine) and insulin levels.
- Pregnancy test on women with childbearing potential
- Record of adverse events, if any
- Record of concomitant medications or interventions, if any
- Skeletal muscle exercise NMR spectroscopy

**Dispensing of dietary supplement:**

The dietary supplement will be stored and dispensed of by the NIH pharmacy.

**Description of Study Population and Recruitment**

The WRNMMC has an active heart failure clinic where approximately 500 ambulatory heart failure patients are seen on a regular basis. The Cardiologists staffing the clinic review all heart failure patients the day prior to their appointments and they determine who potentially meets inclusion and exclusion criteria for studies. The patients who are eligible are then informed about the study at their appointments and asked whether they would be willing to be screened, consented and enroll in the studies.

We will also recruit subjects through NIH using traditional recruitment methods such as referrals from other protocols, outside physician referral and self-referral.
The study may also opt to use the following strategies for recruitment of patients :

- ClinicalTrials.gov
- Clinical Center Research Studies website
- National Heart, Lung and Blood Institute (NHLBI) patient recruitment website
- Twitter messages and chats with study investigators
- Social Media posts
- Use of CC Office of Patient Recruitment services including creation and distribution of study flyers and information through pre-existing recruitment avenues such as the NIH recruitment listserv.

Up to 25 participants may be recruited for this study with the objective of having 15 subjects completing the study.

**4. Eligibility Assessment**

The PI and/or AI at WRNMMC or NIH will assess eligibility. Screening will be performed under this protocol only. Screening studies for subject enrollment:

i. History and physical examination.
ii. Screening and Research laboratory tests may include:
    - Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
    - Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
    - Hepatic Panel (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
    - CBC + Differential
    - Insulin level
    - βHCG (females of childbearing potential)
    - proBNP
    - Echocardiogram

**Eligibility**

**Inclusion criteria**

- Men and women between the ages of 18 and 75 years with NYHA Class II-III systolic heart failure (LVEF by standard echocardiography or radionuclide ventriculography of ≤45%) deemed 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, skeletal muscle exercise NMR spectroscopy and CPET testing
- Willingness/ability to provide informed consent
- Must be DEERS eligible to be enrolled in a research protocol at WRNMMC

**Exclusion Criteria**

- Heart failure with preserved ejection fraction (LVEF >45%)
- Change in heart failure medications due to deterioration of function with the exception of up- or down-titration of diuretic dose up to 100% of baseline dose.
- Heart failure due 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 exercise testing)
- Unwillingness/inability to provide informed consent
- ALT > x3 upper limit of normal, hepatic insufficiency or active liver disease
- Recent history of acute gout
- Chronic renal insufficiency with creatinine > 2.5mg/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 drug study
- History of intolerance to NR precursor compounds, including niacin or nicotinamide
- MRI incompatible hardware including pacemakers or ICD's
- Study adherence concerns
- Individuals with diabetes type 1 and 2 who use insulin
- Women of child-bearing potential unwilling to use contraception or unwilling to practice abstinence
- Breastfeeding women unwilling to stop breastfeeding

## 5. Procedures

**Skeletal muscle exercise NMR spectroscopy (primary outcome):**

To measure whether NR enhances mitochondrial function in skeletal muscle NMR spectroscopy will be performed at baseline, at the end of the 12-week (± 5-days) NR supplementation period and repeated 4 weeks (± 5-days) post-NR washout, using a protocol developed at the NIH.[20] Here, a foot-exercise apparatus is employed to deplete phosphocreatine levels in the tibialis anterior, a muscle in the superficial anterior lateral aspect of the leg mainly composed mitochondrial enriched oxidative type I and type IIA fibers. Each participant engages in submaximal exercise by dorsiflexing one foot against 30% of the maximum weight lifted before testing. The phosphocreatine level will be measured using $^{31}$PMRS during a 3-minute rest period, a 2-minute exercise period, and a 6-minute recovery period, from which the single exponential

recovery time constant ($T_c$) is calculated with data obtained during the post-exercise recovery period. In accordance with the protocol, $^{31}P$ spectra will be obtained at rest and during exercise and recovery, and the results will be analyzed with the use of SAGE 7 (GE Healthcare) and IDL, version 6.4 (Exelis Visual Information Solutions), software.

**Cardiopulmonary exercise testing:**

As the metabolic response to exercise indirectly reflect mitochondrial function, metabolic exercise testing will be conducted with 12-lead EKG monitoring after an overnight fast at baseline and at the end of 12 weeks of NR administration. Cardiopulmonary exercise testing will be performed using a ramp protocol while wearing a facemask enabling breath-by-breath analysis of inspired and expired air (SensorMedics, CA) as performed previously in our laboratory.[21] EKGs are obtained at rest, after each minute of exercise, at end-exercise, and at each minute for 5 minutes after exercise. Blood pressure will be measured at rest, at the end of each stage of exercise (3 minutes in duration), at end-exercise, and at 3 minutes after exercise. Exercise will be symptom-limited (fatigue, shortness of breath or chest pain) and supervised by an experienced nurse and a LIP investigator of this study. Exercise will be terminated prior to this end-point if evidence of severe ischemia is noted (>2 mm ST segment depression in leads with isoelectric segments at rest, hypotension, ventricular tachycardia) or if the patient wishes to stop exercise. For patients with interpretable EKGs (absence of significant resting ST-T wave or major conduction abnormalities), EKG criteria for ischemia is ≥ 1 mm ST segment depression with a horizontal or down-sloping configuration in at least 2 contiguous leads. Analysis of $VO_{2max}$ and the anaerobic threshold will be measured and compared to each subject at baseline and after completion of the NR supplementation protocol. Subjects will be asked not to significantly modify activity levels for the duration of the 16-week study.

**Echocardiography:**

All patients will undergo baseline resting echocardiography optional at Visit 1 if PI feels there is an adequate and timely historical echo) and follow up echocardiography at 12 weeks. All studies will be performed at Walter Reed National Military Medical Center or the NIH Clinical Center. Two dimensional and three dimensional images may be acquired in real time through standard imaging acquisition techniques.

Parasternal long axis (2D) will be utilized to measure left ventricular chamber size at end systole and end diastolic, intraventricular and posterior left ventricular wall thickness, left ventricular outflow tract, aortic root, and ascending aorta size, and the morphological integrity of the mitral and aortic valve. Color Doppler will assess for mitral regurgitation and aortic regurgitation. The size and function of the right ventricle and the morphology of the tricuspid valve will be assessed through the parasternal long axis right ventricular inflow views. Color Doppler will assess for

tricuspid regurgitation and a continuous flow Doppler interrogation through the tricuspid valve will estimated pulmonary arterial systolic pressure.

Parasternal short axis (2D) images will be acquired to assess regional wall motion of the left ventricular starting first at the apex then proceeding cranially to include the mid ventricle and finally the base. At the base of the heart, 2D imaging will assess the morphology and function of the aortic, tricuspid and pulmonic valve. Color doppler interrogation of the three valves will assess for regurgitation. Continuous wave doppler through the tricuspid valve will estimate pulmonary arterial systolic pressure. Continuous wave doppler through the pulmonic valve will be used to assess for pulmonic stenosis.

A thorough interrogation of the left ventricle will be obtained through the apical four chamber view (2D). Left ventricular cavity size, wall thickness (inferoseptum and anterolateral walls), and end diastolic area and volume will be measured. Left and right atrial areas and volumes will also be measured in the apical four chamber view. Color doppler will assess the presence and degree of mitral and tricuspid regurgitation. Tissue doppler interrogation of the left ventricle will assess for left ventricular filling pressures. Pulse wave doppler interrogation of the mitral valve inflows will be recorded and, in conjunction with the tissue doppler data set, be employed to assess diastolic function. Apical two chambers views will be utilized to assess overall left ventricular systolic function as well as regional wall motion of the anterior and lateral walls. Color Doppler will be used to assess the function of the mitral valve in the apical two chamber view. After completion of two dimensional imaging, 3D echocardiography data sets may be obtained in the apical two and four chamber views.

Finally, subcostal images will be obtained. This dataset will provide information on the presence and size of a pericardial effusion, overall left and right ventricular size and systolic function as well as the degree of tricuspid and mitral regurgitation as assessed by color Doppler.

**Metabolomic analysis:**

Recently, the combined use of exercise testing and metabolomics have been used to uncover metabolic disturbances in diabetic subjects.[22] At the same time, metabolomics have identified that impediments in metabolic pathways prevent the full catabolism of fuel sources with worsening heart failure and that left ventricular assist device placement to unload the heart reverses accumulation of these metabolic intermediates.[4] Combining these concepts we plan to undertake metabolomic analysis of heart failure subjects at baseline and in response to NR both at rest and within 3 minutes of cessation exercise at the time of the CPET testing. Serum samples will be collected at the NIH for shipping to the metabolomics core at the UW (http://depts.washington.edu/nwmrc/). LC-MS will be performed on serum samples using an

Agilent LC-AB Sciex 5500 QQQ MS instrument to assess the relative abundance of ≈ 200 aqueous metabolites. In addition MTBSTFA derivation will be employed to extract polar compounds to measure TCA, glycolysis, amino acid and fatty acid intermediates by GC-MS using the Agilent GC-5975C MSD instrument. Analytes at baseline and in response to CPET will be compared pre- and post- NR supplementation and the data will be interrogated by the NHLBI Bioinformatics Core facility.

**Cytokine profiling:**

Circulating cytokine levels have been found to directly correlate with worsening functional capacity in heart failure patients and have been shown to have prognostic value as a biomarker.[23, 24] Emerging evidence additionally supports that exercise training and NR reduce cytokine production.[15, 25] Taken together, we would propose that NR supplementation may similarly reduce circulating cytokine production. To investigate this, serum samples from subjects will be collected at baseline and in response to peak exercise during the CPET procedure pre- and post-NR supplementation. Cytokine, chemokine and acute phase reactants will be quantified using the Luminex bead based multiplexing assay to measure ≈ 40 analytes simultaneously using the NIH Center for Human Immunology standardized protocols.

**Skin biopsy:**

A recent study has shown that human primary fibroblasts incubated with serum from individuals, pre- and post-exercise, showed an upregulation of mitochondrial regulatory genes following exercise.[12] We will employ this same approach using serum obtained from study subjects pre- and post NR administration. A single 3mm or less skin biopsy sample would be obtained at the beginning of the study. The assessment of mitochondrial oxidative phosphorylation in primary skin fibroblasts conditioned with serum from heart failure subjects pre- and post-NR would validate and augment the data acquired by NRM spectroscopy.

**6. Dietary Supplement:**

Niagen™ is a commercially-available form of nicotinamide riboside (NR). The nucleoside NR is a single chemical moiety containing nicotinamide and ribose.[26] NR is a form of vitamin $B_3$ present in trace amounts in foods like milk, yeast extract and beer. It is also postulated that NR is generated in the gastrointestinal tract as part of dietary NAD+ digestion. Thus, humans are constantly exposed to NR from the diet, albeit at low levels. Since 2013, Niagen™ has been sold as a dietary supplement in the United States. Labeling guidelines recommend consumers to limit their intake to 2 capsules/day, which amounts to 250 mg/day. This recommended level is the

equivalent of 3.8 mg/kg bw/day, which is 1000-fold less than the highest dose determined to be safe and well tolerated in rats, and a quarter of the dose proposed for this pilot clinical trial. There is no limit on the duration of ingestion. Limited animal model and human data exist on the pharmacokinetics and safety of orally administered NR. However, ChromaDex has conducted a recent randomized, double-blind, cross-over, pharmacokinetic study (14NBHC, unpublished results) which demonstrated that NR was readily absorbed and detectable in human plasma, white blood cells, and urine. In addition, NR was well tolerated and presented no toxicity following a single dose up to 1000 mg in human subjects [27]. Moreover, in an acute toxicology study, rats that were given a single oral dose (5000 mg/kg) of NR did not show clinical signs of toxicity or mortality (unpublished results).
Niacin is a form of vitamin $B_3$[28] and has been used for a long time treat hypercholesterolemia and pellagra. Niacin administration can lead to undesirable effects, such as spontaneous flushing. Studies in animal models suggest that flushing results from nicotinic acid-mediated activation of the G-coupled receptor, GPR109A.[29] NR has low affinity for GPR109A, and experiments in cell lines expressing GPR109A demonstrate that nicotinic acid, but not NR, activates the receptor. [17] Thus, based on these findings, oral ingestion of NR may not result the spontaneous flushing that has been associated with high doses of niacin administration.

Multiple lines of non-clinical data suggest that NR intake does not present any potential risks and should be well tolerated in human subjects. The dose proposed for this study is within the doses tested in non-clinical studies of mice for up to 4 months, and are 300-fold below the daily dose that was given in rats in a 14-day dose range finder study.

Nicotinamide, an expected metabolite of NR, is a molecule that is considered of low toxicity in food by several regulatory agencies including the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA).

Because nicotinamide is a putative metabolite of NR in humans, understanding the safety profile of nicotinamide is relevant when assessing the safety of NR in human subjects.

Based on the structural similarities between NR and nicotinamide, and that nicotinamide is a downstream metabolite of NR digestion, it is assumed that any unexpected adverse effects of NR may be similar to those associated with nicotinamide intake. However, no significant adverse effects have been reported in clinical trials which have used doses up to the equivalent of 3000 mg/day for up to 3 years to evaluate the possible benefits of nicotinamide administration to patients with or at risk of developing Type 1 diabetes [30, 31]. In addition, doses of 25 and 42 mg/kg bw/day had no effect on a variety of biochemical parameters, such as those that assessed liver and kidney function.

Additional studies have been completed or are ongoing using NR to study effects on mitochondrial biology related conditions including aging (at the University of Colorado in

Boulder – PI - Drs Christopher R. Martens - actively recruiting) and in heart failure (University of Washington in Seattle – Dr Kevin O'Brien - actively recruiting). Based on their prior pharmacokinetics study described previously the University of Seattle team are using 1000mg BID in their Heart Failure protocol [32]. We therefore propose to use the same, 1000mg BID dosing.

In summary, careful analysis of all the non-clinical information available on NR has not revealed any potential serious toxicity that would preclude its use in subjects with heart failure.

**Common name:** Nicotinamide Riboside Chloride

**Product name:** Niagen

**Chemical name:** 3-(Aminocarbonyl)-1-β-D-ribofuranosyl-pyridinium chloride (1:1)

**Daily Dose:** 500 mg daily x 2 weeks, 1,000 mg daily x 2 weeks, 1,500 mg x 2 weeks and 2,000 mg daily x 6 weeks.

**Route of administration:** oral

**Dosing instructions:** BID dosing starting at 250 mg bid with incremental uptitration to 1000 mg bid over the course of the study.

**Supply:** Supplement will be supplied by ChromaDex

**Manufactured by:** W.R. Grace & Co. 1290 Industrial Way, Albany, OR 97322, USA

**Toxicology:** None known.

**Drug Interactions:** None known.

**Off-label use:** Considering the clinical investigation is designed to study the relationship between a dietary supplement's effect on structure or function in humans or to characterize the mechanism by which a dietary supplement acts to maintain such structure or function this study would not need to be conducted under an IND. Under the Dietary Supplement Health and Education Act of 1994, a dietary supplement is not considered a drug and is not subject to the premarket approval requirements for drugs if the intended use for which it is marketed is only to affect the structure or any function of the body (i.e., not intended to be used for a therapeutic purpose). Similarly, whether an IND is needed for a clinical investigation evaluating a dietary supplement is determined by the intent of the clinical investigation. If the clinical investigation is intended only to evaluate the dietary supplement's effect on the structure or function of the body, an IND is ***not*** required.

**7. Data and Biospecimen Management Plan**
**Data Management and Access at the NIH:**

Primary research data will be coded by replacing individually identifying information (such as name) with a code that will enable the investigator to readily ascertain the identity of the subject through the use of a code-key, but will not reveal the identity of subjects to parties not authorized to have access to individual subject identifiers.

**Data Management/Monitoring and Access at participating sites:**

Paper case report forms (CRF) will be generated by the clinical team at WRNMMC for visits which take place there and information will be entered into an NHLBI-approved database selected by the NIH team. CRFs related to study visits conducted at the WRNMMC heart failure clinic will be completed and kept by the WRNMMC study team. Coded medical records generated at WRNMMC may be shared with the NIH team to confirm compliance with the protocol. CRFs related to NIH study visits will be completed and kept by Dr. Sack or his designee in an NHLBI-approved database. Monitoring will be done according to NHLBI Office of the Clinical Director schedule.

**Biospecimen Management at the NIH:**

Samples will be de-identified prior to storage on the 5th floor of building 10 in laboratory for the principal investigator following current NIH sample storage guidelines. Samples and data will be stored, using codes assigned by the investigators or their designee(s). Research samples will be stored using BSI in accordance with NHLBI DIR Biospecimen policy. Data will be kept on the NHLBI P:drive, accessible through password-protected computers. Only the members of the research team will have access to the samples and data. Coded biospecimens may be sent to collaborators outside of the NIH with IRB approval in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement.

**Biospecimen Management at participating sites:**

Clinical samples will be tracked through the electronic medical records system. No research biospecimens will be collected at the WRNMMC study site.

**End of study procedures:**

Data retained by the NHBLI will be stored in a password-protected database in conformity with NHLBI DIR policy until they are no longer of scientific value.

Destruction of research data collected on this protocol will be consistent with NIH policy and upon permission of the Clinical Director.

**Breach of Confidentiality:**

PIs will report any breach of subject confidentiality or trial data to the clinical director and IRB per NIH policy, including NIH HRPP SOP 16 - Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.

**Data Sharing and Future Use of Data**

**Data Sharing Plans**:

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB approval (coded data) or Office of Human Subjects Research Protections (OHSRP) approval (unlinked data). In both situations, a data use agreement between the sender and the recipient will be executed. Future research use of data not defined in the research protocol may occur only after IRB review and approval or a determination from the NIH OHSRP. Refusal of a research subject participant to permit future use of data-will be honored.

**Future Use of Biospecimens:**

Following analyses of biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB. Any future research use of biospecimens not defined in the research protocol will occur only after IRB review and approval, if the research holds the key that identifies research subjects, or determination from OHSRP (non-collaborative research). Biospecimens will not be sent outside of the NIH for future research use without IRB approval and an executed agreement. Refusal of a research subject participant to allow for future use of biospecimens will be honored.

**Loss or destruction of samples:** Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

**8. Statistical Considerations**

***Primary study outcome:***

Continuous data will be presented as mean ± standard deviation or median and range, as appropriate. The primary outcome of this pilot study will be the comparison of baseline to 12-week (on NR) skeletal muscle fixed-load sub-maximal exercise NMR spectroscopy skeletal muscle phosphocreatine recovery time. We are proposing a novel study with no data available on the effect of NR on mitochondrial function as measured in-vivo as measured by NMR spectroscopy. However, we appeal to data in Wang, et. al (ref. 20) to aid with a preliminary sample size calculation. In the Wang, et. al. study, the mean recovery time in leg muscle

phosphocreatine levels after foot-exercise in the control group was 36.7 ± 6.6 seconds. Based on the paired t-test, a sample of size 15 will allow our study to detect a 13% or greater relative reduction (4.8 sec or greater absolute reduction from 36.7 sec to 31.9 sec) in skeletal muscle recovery time with NR at 12 weeks compared to baseline with 80% power and a two-sided alpha of 0.05. The calculation assumes that the standard deviation of the recovery times of the paired baseline to 12-week differences on NR is the same as the standard deviation of the phosphocreatine recovery times in the control group (i.e. 6.6 sec) of the Wang, et. al. study. We request the enrollment of 20 subjects to allow for subject attrition and a margin of error for the study assumptions. The primary analysis of the primary endpoint will use a non-parametric Wilcoxon Signed Rank test for paired data.

***Additional Analyses:***

- Baseline and 12-week cardiopulmonary testing to assay $VO_{2max}$ and anaerobic thresholds.
- Baseline and 12-week serum quantitative metabolomic profiling, pre- and post-CPET to evaluate whether NR increases the rate of oxidative phosphorylation.
- Baseline and 12-week quantitative serum cytokine immunoassay profiling to assess whether NR blunts HF linked inflammation.
- Skin biopsy at baseline and research blood at baseline and 12 weeks explore the effects of NR on oxidative phosphorylation and inflammation in respective subject primary skin fibroblasts.

## 9. Off Study Criteria

- Subjects taking less than 75% of the supplement
- Completion of study visits in pre-specified time windows
- Subjects who are found to be pregnant or wish to breastfeed during the study will automatically be withdrawn.
- Heart failure decompensation resulting in hospitalization.
- Any other severe medical symptoms that may or may not be related to the NR supplement and as determined by contact with the physician. *Note: IRB will be notified of any removal of research subject for severe medical symptoms, including heart failure decompensation.*

## 10. Data and Safety Monitoring

### Safety Monitoring

**Principal Investigator:** Accrual and safety data will be monitored by the Principal Investigator at each site.

**NIH IRB:** Accrual and safety data will be monitored and reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed subject informed consent document will be reviewed and approved by the properly constituted IRB operating according to the 45 CFR 46. This committee must approve all amendments to the protocol or informed consent document, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects. The NIH IRB will have full oversight of all activities conducted at WRNMMC as the IRB of Record. A reliance agreement between NIH and WRNMMC has been executed.

**DSMB:** The NHLBI Data Safety and Monitoring Board (DSMB) will review the protocol at six or twelve month intervals. A progress report will be forwarded to the DSMB at these times.

**Adverse Event Reporting**

***Adverse Event (AE):*** Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

***Serious Adverse Event (SAE)*:** A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; 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.

***Unanticipated Problem*:** An UP is any incident, experience, or outcome that meets all of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to

a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and

b. the characteristics of the subject population being studied; and

2. **related or possibly related** to participation in the research; and

3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

**Reporting of Pregnancy:**
In the event a subject becomes pregnant while on study, this event will be reported to the IRB and Clinical Director as an unanticipated problem. Monitoring of the pregnancy will continue until conclusion of the pregnancy.

***Unanticipated Problem that is not an Adverse Event:*** An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

***Protocol Deviation (PD):*** Any change, divergence, or departure from the IRB approved research protocol.

***Non Compliance:*** The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

***Serious non-compliance:*** Non-compliance that:

a. Increases risks, or causes harm, to participants.
b. Decreases potential benefits to participants.
c. Compromises the integrity of the NIH HRPP.
d. Invalidates the study data.

***Continuing non-compliance:*** Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

***Minor (non-serious) non-compliance:*** Non-compliance that, is neither serious nor continuing.

**Adverse Event Management**

The principal investigators at WRNMMC or NIH, or designees, will be responsible for assessing adverse events. Information on adverse events will be solicited from subjects through questions from study personnel and information volunteered by the subject. Adverse events will be captured from the start of the first pill taken (day 0) until the completion of the washout phase of the study. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study supplement and/or disease. This study will utilize the CTCAE version 5.0 for toxicity and adverse event reporting. A copy of the CTCAE version 5.0 can be downloaded from the https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. AEs will be recorded, verified, and followed until satisfactory resolution.

In the event of any treatment-related SAEs, enrollment will be suspended until discussed with the IRB and Clinical Director.

## Grading and Attribution of Adverse Events

***Severity.*** Definitions found in the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) will be used for grading the ***severity*** (intensity) of AEs:

| | | |
|---|---|---|
| **1** | **Mild** | Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| **2** | **Moderate** | Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL* |
| **3** | **Severe** | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**. |
| **4** | **Life-threatening** | Life-threatening consequences; urgent intervention indicated. |
| **5** | **Death** | Death related to AE |

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

*Attribution of Adverse Events*

| Relationship | Attribution | Description |
|---|---|---|
| Unrelated to intervention | Unrelated | The AE *is clearly **NOT** related* to the intervention |
| | Unlikely | The AE *is **doubtfully related*** to the intervention |
| Related to intervention | Possibly | The AE ***may be related*** to the intervention |
| | Probably | The AE ***is likely related*** to the intervention |
| | Definitely | The AE ***is clearly related*** to the intervention |

## NHLBI-IRB and CD reporting

***Serious Events***

Reports to the IRB and CD: The PI must report Serious UPs, and Serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event.

***Non-serious Events***

Reports to the IRB and CD: The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event.

***Deaths***

Deaths possibly, probably, or definitely related to study procedures and interventions will be reported to the Clinical Director within 7 days after the PI first learns of the event.

***Reports at the time of continuing IRB review:*** At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs
- All AEs (except for those granted a waiver of reporting)

***Waiver of Reporting:***

***The following adverse events will be listed in the consent and not reported to the IRB:***

- Vasovagal symptoms during blood draws (expected frequency 50%).
- Transient bruising at the site of blood draws (expected frequency 50%).

***The following adverse events will not be recorded or reported to the IRB.***

- Grade 1 adverse events
- Grade 2 laboratory abnormalities not associated with clinical signs or symptoms except for grade 2 elevated liver enzymes, which will be recorded and reported.

## 11. Human Subjects Protections

### Rationale for Subject Selection

Subjects of both genders will be considered for inclusion in this study. There will be no racial, ethnic, or gender discrimination. Cognitively impaired and institutionalized persons will not participate in this study.

This study will enroll English-speaking, U.S. military individuals who are seen by Dr. Flanagan at WRNMMC. We do not anticipate enrollment of of non-English speaking subjects to this small pilot study.

### Rationale for the Exclusion of Children

Subjects under 18 years of age will not be considered for inclusion in this protocol because there is no direct benefit from participating in this study and the 24-hour fast and volumes of blood levels drawn exceed minimal risk for children.

**Rationale for the Exclusion of Pregnant Women**

Subjects must not be pregnant or actively seeking pregnancy in order to participate in this study. NR has not been determined to be safe in pregnancy or breastfeeding. A recognized form of contraception must be used by subjects while enrolled. Contraception use will be determined during telephone screening and confirmed at the screening visit.

**Rationale for the Exclusion of Cognitively Impaired Subjects**

Subjects with cognitive impairment will not be considered for inclusion because there is no direct benefit from participating in this study.

**Risk/Benefit Assessment**

The research involves greater than minimal risk to subjects, with no prospect of direct benefit, but is likely to yield generalizable knowledge (45 CFR 46.102).

**Risks and Discomforts**

- ***Phlebotomy:*** Standard precautions for obtaining human blood samples will be taken. Transient discomfort and minor bruising may occur at the phlebotomy site. Vasovagal symptoms can occur during blood drawing. Blood samples will be obtained by venipuncture. Blood samples will be obtained by a nurse, physician, or other skilled individual. The quantities of blood to be drawn for research purposes will be less than 300 mL, which is consistent with the CC policy as provided in Medical Administrative Series (MAS) 95-9 (revised 05/29/2012): for adults, no more than 10.5 mL/kg or 550 mL (whichever is smaller) will be drawn for research purposes over any 8-week period.
- ***Urine collection:*** There is no risk associated with this procedure.
- ***Nicotinamide Riboside:*** NR is a dietary supplement that is currently available for commercial use with no safety concerns noted to date. A recent PK study of 8 healthy volunteers at University of Washington gave incremental doses up to 1 gram bid over 9 days. The study showed no significant side effects or change in safety data. Consecutive doses of 1000mg BID of NR has not been reported in humans although there is currently an ongoing study at the University of Colorado using 1000 mg daily for 6 weeks. Based on the structural similarities between NR and nicotinamide, and that nicotinamide is a downstream metabolite of NR digestion, it is assumed that any unexpected adverse effects of NR may be similar to those associated with nicotinamide intake. The safety profile of nicotinamide has been well established in multiple species and provides reasonable certainty that the administration of doses of NR up to 1000 mg will not result in an adverse health effect. No significant adverse effects have been reported in clinical trials which have used doses up to the equivalent of 3000 mg/day for up to 3 years to evaluate the

possible benefits of nicotinamide administration to patients with or at risk of developing Type 1 diabetes.[30, 31] In addition, doses of 25 and 42 mg/kg bw/day had no effect on a variety of biochemical parameters, such as those that assessed liver and kidney function.

- ***Echocardiography:*** There are no known risks to the ultrasound exam.
- ***Skin biopsy:*** This procedure is performed using local anesthetic. After washing the skin with alcohol and numbing the skin, a 4 mm or smaller circle of skin is removed sterilely and the wound is dressed. The entire procedure takes approximately 5 minutes. Discomfort at the biopsy site is usually mild and transient. This can be treated with minor analgesics. Normally, the risks include a reaction to the local anesthetic and the slight possibilities of local bleeding or infection. Scarring always occurs at the biopsy site.
- **MR Spectroscopy:**

MRI uses non-ionizing radiation and is safe when used on subjects that are appropriately screened for the procedure. Subjects with any exclusionary criteria (Pregnancy, Aneurysm clip, implanted devices such as neural stimulators, cardiac pacemaker, defibrillator, cochlear implant, foreign body, such as, metal shavings, or insulin pumps, will be excluded from the study.

Potential risks of MRI relate to effects of the main, static magnetic field, the applied radiofrequency (RF) power, and the rapidly switching magnetic field gradients. The risks associated with each of these are described below. We will stay within all the FDA guidelines for the applied RF power, the rate of switching magnetic fields and the noise levels that these switching gradients produce (see Guidance for Magnetic Resonance Diagnostic Devices – Criteria for Significant Risk Investigations, issued July 14, 2003. http://www.fda.gov/cdrh/ode/guidance/793.pdf).

*Non-significant Risk (NSR) Device Determination:*
The use of the MRI scanner constitutes a non-significant risk device study, because it is performed within the FDA approved limits of main static magnetic field (<8T), specific absorptions rate (SAR), gradient field rates of change, and sound pressure level22 (Appendix 1). This protocol is therefore eligible for abbreviated IDE requirements of 21 CFR 812.2(b), in which IRB review constitutes the IDE.
The use of the MRI research coils, research pulse sequencing, and research image processing constitute the research component of this device which are not FDA approved. All research surface coils undergo safety testing and review by the NIH NMR Center Safety Committee. There is no potential for serious risk to the health, safety, or welfare of the subjects using the MRI scanner in these ways. *Magnetic field:* There is no data that shows any significant adverse effects of exposure to static magnetic fields. There are well known minor adverse effects associated with high

magnetic fields. These include nausea, metallic taste, and detection of flashes of light. All of these are associated with moving too rapidly in the magnetic field. To avoid these the subjects will be asked to walk slowly to the patient table and the table will be moved slowly into the magnet. Experiences at 3T show that these precautions eliminate these minor adverse reactions. Current FDA guidance allows for main static magnetic field less than or equal to 8 tesla.

*Radiofrequency power deposition:* The MRI will have safeguards which monitor and limit RF deposition within FDA guidelines. Only one adverse effect has been observed on the 3T MRI at NIH in the more than 5000 subjects that have been examined over the last three years. This one event was temporary eye discomfort reported by a normal volunteer. The eyes are sensitive to RF irradiation and the FDA guidelines take this into account.

*Switching magnetic field gradients:* In addition to the large, static magnetic field, MRI relies on rapidly switching magnetic field gradients of much lower strength than the main static magnetic field. Subjects who participate in MRI examinations may experience peripheral nerve stimulation in the form of involuntary skeletal muscle contractions and/or twitching due to these rapidly switching gradients. This peripheral nerve stimulation has also been described as a creeping sensation along the back or twitching of the nose or feelings of electrical shocks and has ranged from imperceptible to mildly painful sensations.

Current FDA guidelines do not specify a strict limit that magnetic field gradients can be switched in MRI studies. FDA guidelines state that the switching gradients should not cause severe discomfort or painful nerve stimulation. Currently the FDA approved 3T MRI can maximally switch field gradients up to 12 mT in 0.260 msec at a position 30 cm from isocenter. No adverse events related to nerve stimulation have been reported.

*Noise levels:* The switched gradients also generate noise in the scanner. Peak sound power produced in the magnet will be less than 140 dB and 119 dBA. The FDA limits constant noise exposure to 140 dB and 99 dBA for two hours. To stay within FDA guidelines on dBA limits all subjects will wear hearing protection in the form of ear plugs and/or headphones. This is the procedure that is routinely done on the 1.5T and 3T MRI and it is well established to decrease noise levels by 20 dbA. There has been only one reported adverse effect on hearing in close to twenty years of MRI in the NIH NMR Center from noise. In the past three years more than 5000 MRI subjects that have been examined. The one recent adverse event was temporary hearing loss reported by a patient after a MRI scan on a 3T MRI. It is not clear what caused this hearing loss. The patient had a previous stroke and an absent ear reflex to loud noises. Based on these experiences the risk of damage to hearing is very low.

- ***Exercise stress test:*** Stress tests are safe (a serious complication occurs in less than 1 in 10,000 tests) especially when the level of monitoring during exercise (ECG, blood pressure, physician supervision) proposed in the present study is employed. Shortness of breath and fatigue are often felt at the end of the exercise, and occasionally subjects may experience chest discomfort. Abnormal heart rhythms can be triggered by exercise but these are rarely persistent or severe. The mask used to measure exhaled gas is worn tightly on the face and may cause discomfort.

**Consent Processes and Documentation for Research Subjects**

Each participant will receive an oral and written explanation of the goals, procedures, and risks of this study. The Principal Investigator and those Associate Investigators who are listed on the cover page of the protocol with an asterisk next to their name may obtain informed consent from research participants. Consent will be obtained at WRNMMC and/or the NIH Clinical Center depending on which site recruited the subject.

The original, signed informed consent document will be placed in the medical record at the site where the consent was obtained, and the subject will receive a signed copy of the informed consent document. If consent is obtained at WRNMMC, a copy will be faxed to the Research Coordinator at the NIH Clinical Center and when subjects arrive at the NIH for visit 2 (week 0), they will then sign the NIH-specific consent for Clinical Center participation.

No member in a chain of command over a potential research subject at WRNMMC will be involved in recruitment or consenting process.

## 12. Conflict of Interest

None of the members of the research team reported a potential conflict of interest. The National Institutes of Health reviews NIH staff researchers at least yearly for conflicts of interest. The following link contains details on this process http://ethics.od.nih.gov/forms/Protocol-Review-Guide.pdf. This protocol has a CRADA with ChromaDex, Inc.

## 13. Reimbursement for Travel

As the study populations will be local, reimbursement for travel, food, and lodging will not be provided.

## 14. Financial Compensation

Subjects will be compensated for procedures that are performed at the NIH that are of no direct benefit to the subject.:

| Procedures | Inconvenience Units | Compensation per procedure | Frequency | Total Compensation |
|---|---|---|---|---|
| CPET | 5 | $50.00 | 2 | $100.00 |
| History and physical exam | 2.5 | $25.00 | 1 | $25.00 |

| | | | | |
|---|---|---|---|---|
| Outpatient Visit (first hour) | 2 | $20.00 | 10 | $200.00 |
| Research Blood Draw | 2.5 | $25.00 | 2 | 50.00 |
| MRS | 15 | $150.00 | 3 | $450.00 |
| Skin Biopsy | 5 | $50 | 1 | $50 |
| Drug administration, General | .5 | $5.00 | 84 | $420.00 |
| | | | **Maximum Compensation:** | $1295.00 |

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