NHLBI Protocol: Exploratory Study of Nicotinamide Riboside on Mitochondrial Function in Li-Fraumeni Syndrome

Short Title: Nicotinamide Riboside in Li-Fraumeni syndrome

Keywords: p53, skeletal muscle, mitochondria

Principal Investigator:
*Paul M Hwang, MD, PhD (E)
Cardiovascular Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Building 10, Room 5-5-5330
Bethesda, MD 20892
Phone: 301-435-3068

Associate Investigators:
*Rebecca Huffstutler, CRNP CB, NHLBI (E)
*M. Michael Sack, MD, PhD CB, NHLBI (E)
Ping-yuan Wang, PhD CB, NHLBI (E)
*Investigators authorized to obtain informed consent

Research Coordinator:
*Rebecca Huffstutler, CRNP CPB, NHLBI (E)

Biospecimen Tracking: BSI
Study Site: NIH

Subjects in study at NIH: Number - 1; Gender - M/F; Age range - 18-70 years
Multi-center trial: No
Ionizing Radiation for Research: No
Off-Site Project: No
DSMB Involvement: No
Tech Transfer: No
IND/IDE: No
# Table of Contents

A. Precis .................................................................................................................. 3  
B. Background and Preliminary Results ................................................................. 3  
C. Hypothesis and Objectives .................................................................................. 6  
D. Recruitment and screening ................................................................................ 6  
E. Eligibility Assessment ....................................................................................... 7  
F. Study Design and Schedule ............................................................................... 7  
G. Procedures ......................................................................................................... 11  
H. Dietary Supplement ......................................................................................... 13  
I. Data and Biospecimen Management Plan ........................................................... 14  
   Data sharing and future use of data .................................................................. 15  
J. Statistical Considerations ................................................................................... 16  
K. Off Study Criteria .............................................................................................. 16  
L. Data and Safety Monitoring ............................................................................... 16  
   Safety monitoring ............................................................................................... 16  
   Adverse event reporting .................................................................................... 17  
M. Human Subjects Protections ............................................................................. 20  
   Rationale for subject selection ......................................................................... 19  
   Rationale for the exclusion of children .............................................................. 19  
   Rationale for the exclusion of pregnant women ............................................. 19  
   Rationale for the exclusion of cognitively impaired subjects ....................... 20  
   Risk/benefit assessment ................................................................................... 20  
   Risks and discomforts ....................................................................................... 20  
N. Conflict of Interest ............................................................................................ 23  
O. Reimbursement for Travel ................................................................................. 23  
P. Financial Compensation ..................................................................................... 24  
Q. References ......................................................................................................... 25
A. Precis

We have previously reported that inherited mutations of TP53, which causes the premature cancer disorder Li-Fraumeni syndrome (LFS), can promote mitochondrial function both in patients and mouse models (1). In the course of our follow up studies, we encountered a LFS patient with a long-standing history of fatigue and muscle weakness of unclear etiology. Notably, we observed in vivo evidence of markedly decreased mitochondrial function in her leg skeletal muscle during exercise using noninvasive phosphorus-31 magnetic resonance spectroscopy ($^{31}$P-MRS), a technique that has previously been used to study patients with primary mitochondrial disorders. The decrease in mitochondrial function was also confirmed by the patient’s skin fibroblasts in vitro using standard biochemical measurements.

There is growing evidence that nicotinamide adenine dinucleotide (NAD+) homeostasis plays a significant role in maintaining the mitochondria through various mechanisms and that it is possible to improve mitochondrial function by dietary supplementation with the vitamin B3 analogue nicotinamide riboside (NR), an intermediate in the NAD+ salvage pathway. Remarkably, we observed that culturing the LFS patient’s fibroblasts in medium containing NR rescued the severe deficit in mitochondrial respiration. While continuing our investigations into the molecular mechanism(s) underlying the mitochondrial dysfunction observed in this patient, the in vitro rescue of the respiratory deficiency by NR presents a unique opportunity to investigate whether it can also be observed in vivo using skeletal muscle $^{31}$P-MRS. We propose to explore the effect of NR, currently available as a dietary supplement, on in vivo mitochondrial function in this LFS patient.

B. Background and Preliminary Results

Mitochondrial metabolism plays an essential role in both tumorigenesis and cardiovascular function. We showed that TP53, which encodes the p53 protein and is the most commonly mutated tumor suppressor gene in human cancers, can regulate mitochondrial respiration and impact aerobic exercise capacity (2) (3). Beside modulating genes important for cell cycle regulation and cell death, it is now clear that p53 can regulate mitochondrial biogenesis both by transcriptional mechanisms and by direct effects within the mitochondria (4). We subsequently provided evidence that patients with Li-Fraumeni syndrome (LFS), an early onset cancer disorder caused by germline mutations of TP53, have increased oxidative metabolism through our translational clinical protocol NHLBI 07-H-0030 (1). We have continued to study mitochondrial metabolism in LFS patients, because insights from our translational study have provided impetus for testing a new cancer prevention strategy in LFS (5). Furthermore, LFS is caused by many different germline mutations of the TP53 gene, so the specific metabolic effects of each unique p53 mutation remains to be clarified.

During the course of our studies, a LFS patient (Patient A) with prior history of
breast cancer participated in our clinical protocol with the chief complaint of life-long fatigue but with no clear diagnosis after medical evaluation. Although treadmill exercise testing can be used as a marker of mitochondrial function, there are many factors such as age, gender and exercise training that can contribute to aerobic fitness. To avoid these potential confounders, we adapted a previously developed non-invasive technique to directly measure mitochondrial function in the skeletal muscle (6). This method relies on the regeneration of phosphocreatine (PCr) that normally serves to shuttle high energy phosphate from the mitochondria to the cytosol in order to maintain skeletal muscle ATP levels during work performance. The measurement of PCr regeneration after exercise-induced depletion using phosphorus-31 magnetic resonance spectroscopy ($^{31}$P-MRS) has been reported to provide a sensitive measure of in vivo oxidative phosphorylation capacity (7). Consistent with her symptoms, we observed a profound delay in the phosphocreatine recovery time constant (PCr recovery Tc) of ~80 sec, indicating decreased mitochondrial function (Figure 1A).

This was in marked contrast to the PCr recovery Tc values of 30 sec and 37 sec in her family members (Patients B and C) carrying the same TP53 mutation, which were both within range of our previously published mean values of ~31 sec in a cohort of LFS patients and ~37 sec in non-mutation carriers (Figure 1B) (1). Further investigations in the laboratory using skin fibroblasts obtained from Patient A compared with Patient B confirmed decreased mitochondrial respiration (oxygen consumption) using standard biochemical techniques (Figure 2).
A consequence of impaired mitochondrial oxidative phosphorylation is decreased NAD+ levels, which in turn appears to exacerbate mitochondrial dysfunction by inactivating the NAD+ dependent deacetylase enzymes (sirtuins). Experimental models and human translational studies using NAD+ precursors (including nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR)) to increase NAD+ levels through the NAD+ salvage pathway have shown that normalizing the NADH/NAD+ ratio and activating sirtuin enzymes can improve mitochondrial function with beneficial effects in various systems including the skeletal muscle (8-10). We therefore examined the effect of culturing LFS Patient A’s fibroblasts in medium containing NR. Compared with the fibroblasts of her sibling Patient B which were relatively unresponsive, there was a significant improvement in the mitochondrial respiration of Patient A fibroblasts to NR exposure (Figure 2B).

Published human studies have shown that NR supplementation increases cellular NAD+ content and can be tolerated up to 2000 mg daily without adverse side effects (11-13). A pharmacokinetics study (NCT02689882) of NR with dose escalation over 9 d up to 1000 mg twice daily increased blood NAD+ concentrations over pre-treatment levels in every subject, on average about 2-fold (range 1.34- to 2.66- fold, $P = 0.001$) (12). This study revealed no significant side effects, and the primary pre-specified safety data showed no changes in the levels of potassium, glucose, uric acid, creatine kinase or alanine aminotransferase levels. The subjects also displayed no changes in hemodynamics, although the hematocrit dropped from 40 ± 3% to 39 ± 3% and the platelets dropped from 220 ± 40 to 200 ± 30 (x10³/µL), both with $P < 0.05$. The modest reduction in hematocrit, hemoglobin and platelet count were suspected to result from the repeated daily laboratory blood draws over the 9-day study. Additional studies are actively recruiting human subjects to study the effects of NR on mitochondrial biology in aging (PI: Dr. Christopher R. Martens, University of Colorado, Boulder) and in heart failure (PI: Dr Kevin O’Brien, University of Washington, Seattle). Based on their prior pharmacokinetics study, the University of Washington team is using 1000 mg twice daily in their Heart Failure protocol (12). Within the NHLBI DIR, more than 30 human subjects have received NR 1000 mg daily without adverse side effects through protocol 16-H-0129, and heart failure patients will received NR 2000 mg daily through the recently approved protocol 18-H-0107 (Dr. M. Sack, CB/NHLBI-NIH, PI on both protocols).

The significant improvement in mitochondrial function in the fibroblasts of Patient A fibroblasts.

Figure 2. Nicotinamide mononucleotide (NMN) rescues the decreased mitochondrial respiration of Patient A fibroblasts. Fibroblasts obtained from the indicated patients were grown under standard tissue culture conditions with or without 100 µM NMN, a metabolite of NR, prior to measuring whole cell respiration (O2 consumption) using a Seahorse Metabolic Analyzer.
A by NR treatment and its benign nature as a commercially available dietary supplement make it compelling to examine whether the improvement in fibroblast mitochondrial function can be replicated in vivo. To date, this is the only LFS patient in whom we have observed significant mitochondrial dysfunction, representing an opportunity to further expand our understanding of the biology. While continuing our experimental investigations into the molecular mechanism(s) underlying the mitochondrial dysfunction observed in Patient A, we propose to perform a translational study using skeletal muscle $^{31}$P-MRS to investigate whether NR can improve the in vivo mitochondrial function of Patient A.

C. Hypothesis and Objectives

We hypothesize that NR supplementation in this LFS patient with evidence of mitochondrial deficiency will result in improvement of mitochondrial function in vivo.

Primary objective
Investigate the effect of NR supplementation on the PCr recovery Tc of skeletal muscle after exercise as a marker of mitochondrial oxidative phosphorylation capacity.

Secondary objectives
Examine whether NR supplementation affects the:
1) respiratory capacity of blood mononuclear cells; and
2) cardiopulmonary exercise test (CPET) performance as a measure of aerobic capacity.

Exploratory consideration
Self-reported symptoms of fatigue

Proposed scientific/clinical innovations and advances of the study
1) Scientific innovation of demonstrating improved mitochondrial function in vivo with NR supplementation using noninvasive PCr $^{31}$P-MRS.
2) Clinical innovation of using novel treatments such as NR to improve the symptoms of LFS patients with mitochondrial insufficiency.
3) Advance our understanding of the molecular mechanisms of mitochondrial regulation in LFS patients and the effects that novel treatments may have on this regulation.

D. Recruitment and screening

This is a one-subject study so there are no recruitment or screening of other subjects. The subject has confirmed Li-Fraumeni syndrome and TP53 mutation. The subject is currently enrolled in study # 07-H-0030 and is referred to this protocol by Dr.
E. Eligibility Assessment

The PI or Al may assess eligibility using results from Li-Fraumeni syndrome natural history protocol (07-H-0030).

Inclusion criteria:
• At least 18-years of age and able to give informed consent
• Have delayed PCr recovery time constant >45 sec by $^{31}$P-MRS testing and a history of fatigue symptoms
• Ability to undergo study procedures, including scheduled visits, blood draws and skeletal muscle exercise NMR spectroscopy
• Have Li-Fraumeni syndrome and confirmed TP53 mutation by genetic testing
• Committed to using reliable contraception which may include abstinence during study participation
• Female participants of child-bearing ability and potential willing to commit to reliable contraception while participating in the study

Exclusion criteria:
• Current systemic treatment for cancer
• Unable to perform required study visits or procedures
• MRI incompatible hardware
• Pregnant or breastfeeding women
• History of intolerance to NR precursor compounds, including niacin or nicotinamide

F. Study Design and Schedule

Participant with documented LFS meeting study criteria will have baseline tests performed only after providing consent. The study design is an open label exploratory pilot study with the subject serving as own control with testing at baseline, week 6 and week 12 of NR supplementation. The research subject will maintain routine diet and not make changes in dietary supplements for the duration of the study. If the primary endpoint of improved skeletal muscle mitochondrial function using $^{31}$P-MRS is met with NR supplementation, there will be a 6-wk washout period followed by re-testing to confirm the specificity of the NR effect. If the primary endpoint is not met or there is no self-reported improvement in fatigue symptoms, the patient may choose to continue NR supplementation for a 12-wk extension period. If the primary objective is met after this extension period, it will be followed by a washout period. The outline of the protocol is shown in tabular form.
<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>4**</th>
<th>5**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4*</td>
<td>4**</td>
<td>5**</td>
<td></td>
</tr>
<tr>
<td>Subject contact***</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Physical exam</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Start NR supplementation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR dose adjustment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop NR supplementation</td>
<td>X*</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine pregnancy test</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Clinical blood tests</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>P-31 MR spectroscopy ($^{31}$P-MRS)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CPET (cardiopulmonary exercise test)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research blood</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>AE assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>NR compliance</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X¹ For subject with childbearing potential only
X² For subject who has not had a prior echocardiogram in past 5 years
X³ For subject who does not have skin fibroblasts available
* For subject who meets primary endpoint at Week 12
** For subject who does not meet primary endpoint at Week 12 and elects treatment extension
(For scheduling flexibility, the visits and contacts may be performed ± 5 working days.)
*** Subject contact can be done by phone, email or other electronic means

**Week 0/Visit 1 (Screening and Baseline)**
The following will be obtained at the screening/baseline visit.

**Screening**
- Demographic information, medical history and physical examination
- Pregnancy test if indicated

**Baseline**
- Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK, lactate and pyruvate
- Research blood draw
- Skeletal muscle exercise NMR spectroscopy
- Cardiopulmonary exercise test
- Echocardiogram (if a baseline study is not available)
- Administer SF-36
- NR Supplement will be dispensed for the study duration.
  - Week 0: 250 mg twice a day (Level 1, 500 mg daily, po, to initiate dietary supplementation in evening after Visit 1)
  - Weeks 1-5: 500 mg twice a day (Level 2, 1000 mg daily, po, to initiate on Week 1)
  - Week 6: 750 mg twice a day (Level 3, 1500 mg daily, po, to initiate after Visit 2 if tolerated)
  - Weeks 7-12: 1000 mg twice a day (Level 4, 2000 mg daily, po, to initiate on Week 7 if tolerated).

Week 1
- Level 2 dose escalation
- Patient contact through telephone call or secure email for AE assessment, concomitant treatments or interventions, and NR compliance

Week 6/Visit 2
- Medical history and physical examination
- Skeletal muscle exercise NMR spectroscopy
- Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK, (lactate and pyruvate only if baseline levels were abnormal)
- Research blood draw
- Pregnancy test if indicated
- AE assessment
- Record of concomitant treatments or interventions
- NR compliance
- Communicate the initiation of Level 3 NR after Visit 2

Week 7
- Level 4 dose escalation
- Patient contact through telephone call or secure email for AE assessment, concomitant treatments or interventions, and NR compliance

Week 12/Visit 3
- Medical history and physical examination
- Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK (lactate and pyruvate only if baseline levels were abnormal)
- Research blood draw
- Pregnancy test if indicated
- Skeletal muscle exercise NMR spectroscopy
- Cardiopulmonary exercise testing
• SF-36 form
• AE assessment
• Record of concomitant treatments or interventions
• NR compliance
• The results of the PCr recovery Tc will be discussed with the subject and whether the 12 week NR extension period is a consideration.

Week 18/Visit 4*
• NR washout visit if primary endpoint is met at Visit 3
• Medical history and physical examination
• Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK (lactate and pyruvate only if baseline levels were abnormal)
• Research blood draw
• Pregnancy test if indicated
• Skeletal muscle exercise NMR spectroscopy
• SF-36 form
• AE assessment
• Record of concomitant treatments

Week 24/Visit 4**
• Extension period visit if primary endpoint is not met at Visit 3 and the subject elected to do the extension of NR supplementation for 12 wk
• Medical history and physical examination
• Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK (lactate and pyruvate only if baseline levels were abnormal)
• Research blood draw
• Pregnancy test if indicated
• Skeletal muscle exercise NMR spectroscopy
• Cardiopulmonary exercise testing
• SF-36 form
• AE assessment
• Record of concomitant treatments or interventions
• NR compliance

Week 30/Visit 5**
• Extension period NR washout visit
• Medical history and physical examination
• Clinical blood tests: chemistry panel, CBC+Diff, hsCRP, HbA1C, uric acid, ALT, CK (lactate and pyruvate only if baseline levels were abnormal)
• Research blood draw
• Pregnancy test if indicated
• Skeletal muscle exercise NMR spectroscopy
• SF-36 form
• AE assessment
• Record of concomitant treatments

Dispensing of NR supplement
NR supplement will be stored and dispensed by the NIH pharmacy.

G. Procedures

Skeletal muscle phosphocreatine P-31 magnetic resonance spectroscopy (31P-MRS) (primary endpoint):
To assess whether NR enhances mitochondrial function in skeletal muscle, 31P-MRS will be performed at baseline, 6 wk and 12 wk after NR supplementation using a protocol developed at the NIH (1). If the patient meets the primary objective of demonstrating improved PCr recovery Tc after NR supplementation, the test will be repeated 6 weeks after NR washout to determine the specificity of its effect.
A foot-exercise apparatus is employed to deplete PCr levels in the tibialis anterior, a muscle in the superficial anterior lateral aspect of the leg mainly composed of mitochondria-enriched oxidative type I and type IIA fibers. The participant engages in a submaximal exercise by dorsiflexing one foot against 30% of the maximum weight lifted before testing. The phosphocreatine level will be measured using 31P-MRS during the following sequence of 3-minute rest, 2-minute exercise, and 6-minute recovery periods. The 31P spectra will be obtained during these periods and analyzed with the use of SAGE 7 (GE Healthcare) and IDL, version 6.4 (Exelis Visual Information Solutions), software. The single exponential recovery time constant (Tc) is calculated from the post-exercise recovery period data.

Cardiopulmonary exercise testing:
As the metabolic response to exercise can reflect mitochondrial function, metabolic exercise testing will be conducted with 12-lead EKG monitoring at baseline and at the end of 12 weeks of NR administration. Cardiopulmonary exercise testing will be performed using a conservative ramping protocol while wearing a facemask enabling breath-by-breath analysis of inspired and expired air (SensorMedics, CA) as previously performed (14) (15). 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.
The 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 clinically indicated per testing criteria or if the patient wishes to stop exercise. Analysis of VO2max and the anaerobic threshold will be measured and compared to each subject at baseline and after completion of the NR supplementation protocol. Subject will be asked not to significantly modify activity levels for the duration of the study.
Echocardiography:
Cardiac function is a major determinant of aerobic exercise capacity, so a resting clinical echocardiogram may be performed if no baseline studies are available. Cardiac morphology and function will be assessed through standard clinical ultrasound imaging and doppler techniques.

Blood and skin biopsy sample collection:
In addition to the clinical blood samples (estimated 40 mL), subject will have research bloods drawn within the blood withdrawal volume limits established by the Clinical Center. Blood NAD intermediate levels, respiration measurements, metabolomics, and cytokine profiling may be performed to assess the effect of NR on metabolism and overall systemic response to improved mitochondrial function. If skin fibroblasts are not already available from the subject, a skin biopsy sample may be obtained at the beginning of the study. 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. Mitochondrial biogenesis and function will be assessed in the primary skin fibroblasts to correlate with the $^{31}$P-MRS data.

SF-36 Health Survey:
The Short Form (36) Health Survey is a well-validated 36-item, patient-reported survey of patient health. It involves completing a set of generic, coherent, and easily administered quality-of-life measures. It includes one multi-item scale that assesses eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. We will use it to monitor perceived health status before and at the end of treatment with nicotinamide riboside and following the washout period.

H. Dietary Supplement
Niagen (trademark) is a commercially-available form of nicotinamide riboside (NR). The nucleoside NR is a single chemical moiety containing nicotinamide and ribose (16). NR is a form of vitamin B3 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+. 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 (250 mg/day). This recommended level is the equivalent of 3.8 mg/kg body weight/day, which is 1000-fold less than the highest dose determined to be safe and well tolerated in rats, and there is no limit on the duration of ingestion.
There are extensive literature on the beneficial effects of NR in animal models (17,18), and multiple lines of non-clinical data suggest that it should be well tolerated in human subjects. 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). The NR dose proposed for this study is within the doses tested in mice for up to 4 months and is 300-fold below the daily dose that was given in rats in a 14-day dose range finder study. There are also accumulating data on the pharmacokinetics and safety of orally administered NR in humans. Recently, a 2 x 6-week randomized, double-blind, cross-over study was performed to assess the tolerability of chronic NR supplementation and its efficacy for increasing NAD+ bioavailability by investigators at the University of Colorado, Boulder (13). They demonstrated that the oral administration of NR 500 mg twice daily (total 1000 mg daily, Niagen, ChromaDex, Inc.) was well tolerated, readily absorbed, and detectable in human plasma, white blood cells, and urine. In a pharmacokinetic study of 8 healthy volunteers at the University of Washington, Seattle, the oral dose of NR dose was escalated from 250 mg daily to 1000 mg twice daily over a 9 day period with a resultant 100% increase in blood NAD+ content that highly correlated to NR levels (12). No adverse events were associated with NR treatment in either of these two published human studies.

Niacin is a form of vitamin B3 and has been used to treat hypercholesterolemia and pellagra for many years. Niacin administration can lead to undesirable effects such as spontaneous flushing, but based on its pharmacology, NR would not be predicted to give this side effect (19,20). Indeed, the incidence of flushing side effect with NR treatment was not different compared with placebo in the double-blind cross-over study (13). Nicotinamide, an expected metabolite of NR, is considered to be 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). Clinical trials of nicotinamide up to 3000 mg daily and 3 years in patients with or at risk of developing Type 1 diabetes have not reported significant adverse effects (21,22). In addition, doses of 25 and 42 mg/kg body weight per day had no effect on a variety of biochemical parameters including those assessing liver and kidney function.

In summary, careful analysis of the available information on NR does not reveal any potential serious toxicity that would preclude its use in LFS subjects. Given the safety and tolerability of NR in published studies at two extramural institutions (University of Colorado and University of Washington) and the favorable experience with NR in Dr. Michael Sack’s NHLBI DIR protocol (16-H-0129, discussed in Background section) (12,20), we propose to test the effect of NR in LFS patients at a dose of 1000 mg twice daily, a dose that is currently approved for use in other active clinical studies of the aforementioned groups.

**Common name:** Nicotinamide Riboside Chloride  
**Product name:** Niagen  
**Chemical name:** 3-(Aminocarbonyl)-1-β-D-ribofuranosyl-pyridinium chloride (1:1)  
**Daily dose:** 500 mg x 1 wk, 1,000 mg x 5 wk, 1500 mg x 1 wk, up to 2000 mg as tolerated x 5 wk (plus an optional extension period of 12 wk)
Route of administration: oral  
Dosing instructions: twice daily dosing in 250 mg increments per dose  
Supply: supplements will be purchased from ChromaDex, Irvine, CA  
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.

I. Data and Biospecimen Management Plan

Primary research data will be coded by replacing personally identifiable 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 subject to parties not authorized to have access to individual subject identifiers.

Biospecimen management at the NIH:  
Samples will be de-identified prior to storage on the 5th floor of Building 10 in the laboratory of 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.

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.

Protocol: P184388  
Version date: October 23, 2018  
Paul M. Hwang, MD, PhD.
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:
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.

Human Data Sharing Plan: De-identified human data generated for use in future and
ongoing research will be shared through a NIH-funded or approved repository
(ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be
submitted to ClinicalTrials.gov either before publication or at the time of publication or
shortly thereafter.

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.

J. Statistical Considerations

Primary objective:
This is a pilot study translating experimental observations made in one LFS
patient. It is novel in nature with no prior data available on the effect of NR on \textit{in vivo} mitochondrial function, so statistical considerations are not feasible. The primary endpoint of this study will be intra-subject comparison of baseline to 12 wk NR supplementation PCr recovery Tc measurements using the $^{31}$P-MRS skeletal muscle submaximal exercise. This is a one-subject study so there are no statistical considerations.

**Additional analyses:**
Baseline and 12 wk respiratory capacity of blood mononuclear cells, cardiopulmonary exercise test performance, and self-reported fatigue symptoms will be analyzed.

**K. Off Study Criteria**

1) Subject taking less than 75% of the supplement
2) Completion of study visits in pre-specified time windows
3) Subject found to be pregnant or wishes to breastfeed during the study will automatically be withdrawn
4) Any other severe medical symptoms that may or may not be related to the NR supplement and as determined by contact with the physician.
5) If subject no longer wish to participate
6) Subject's non-compliance

**L. Data and Safety Monitoring**

\textbf{Safety monitoring}

\textbf{Principal investigator:} Accrual and safety data will be monitored by the PI.

\textbf{Adverse event reporting}

\textbf{Definitions:}

\textbf{Adverse Event (AE):} Any untoward medical occurrence in a human subject, including any abnormal signs (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.

\textbf{Abnormal laboratory values:} A moderate and severe abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
• Requires treatment or any other therapeutic intervention
• Is associated with death or another serious adverse event, including hospitalization
• Is judged by the Investigator to be of significant clinical impact
• If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the subject’s outcome.

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 (UP): 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.

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 investigator or designee 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 Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) for toxicity and adverse event reporting. A copy of the CTCAE v5.0 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 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**: 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

<table>
<thead>
<tr>
<th>Relationship to Intervention</th>
<th>Attribution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated to intervention</td>
<td>Unrelated</td>
<td>The AE is clearly <strong>NOT related</strong> to the intervention</td>
</tr>
<tr>
<td>Unlikely</td>
<td></td>
<td>The AE is <strong>doubtfully related</strong> to the intervention</td>
</tr>
<tr>
<td>Related to intervention</td>
<td>Possibly</td>
<td>The AE <strong>may be related</strong> to the intervention</td>
</tr>
<tr>
<td></td>
<td>Probably</td>
<td>The AE is <strong>likely related</strong> to the intervention</td>
</tr>
<tr>
<td></td>
<td>Definitely</td>
<td>The AE is <strong>clearly related</strong> to the intervention</td>
</tr>
</tbody>
</table>

### NIH-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 Clinical Director (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 CD 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.

#### 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 a protocol deviation. Monitoring of the pregnancy will continue until conclusion of the pregnancy, and then subject will be taken off study.

M. Human Subjects Protections

Rationale for subject selection
As described in the Background section, one subject has self-referred to us for inclusion in this study. There will be no racial, ethnic, or gender discrimination. This subject is not cognitively impaired, institutionalized, or non-English speaking.

Rationale for the exclusion of children
The one subject is over 18 years of age. We have no experience with $^{31}$P-MRS in children and the volumes of blood drawn exceed minimal risk for children.

Rationale for the exclusion of pregnant women
The subject 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 women. 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, follow the instructions.

Risk/benefit assessment
This is a single subject supplement study (interventional) which involves greater than a minimal risk procedure (skin biopsy), therefore 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 1000 mg twice daily over 9 d. The study showed no significant side effects or change in safety data. Longer duration of NR 1000 mg twice daily supplementation has not been reported in humans although there is currently an ongoing study at the University of Colorado using 1000 mg daily for 6 wk. 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 (21)(22). In addition, doses of 25 and 42 mg/kg/day had no effect on a variety of biochemical parameters, such as those assessing liver and kidney function.

Skin biopsy: 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 level 22 (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 8T.

**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 the NIH in the more than 5000 subjects that have been examined over the last 3 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 from noise in close to 20 years of experience at the NIH NMR center. In the past 3 years more than 5000 MRI subjects 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**
Informed consent shall be documented using the current IRB-approved consent form, which may be downloaded from the NIH Clinical Center active consent website. When consent is obtained, the consent document(s) must be signed and dated by the subject, and the person obtaining consent. For research conducted at the NIH Clinical Center a witness is also required to sign the document. Any adult other than the person obtaining or providing consent may serve as a witness. The witness attests only to the validity of the signature or mark (i.e., that the research subject signed the consent document), not to the validity or quality of the consent. The original, signed informed consent document will be placed in the medical record, and the subject will receive a signed copy of the informed consent document. Documentation of informed consent and the signed consent form will be maintained in CRIS.

**N. Conflict of Interest**

None of the members of the research team report 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](http://ethics.od.nih.gov/forms/Protocol-Review-Guide.pdf).

**O. Reimbursement for Travel**

Reimbursement for travel, food, and lodging will be provided according to NHLBI policy and rates.

**P. Financial Compensation**

The subject will be compensated for some of the procedures that are performed at the NIH since they may not provide direct benefit to the subject.
<table>
<thead>
<tr>
<th>Procedures</th>
<th>Inconvenience Units</th>
<th>Compensation per procedure</th>
<th>Frequency</th>
<th>Total Compensation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood draws</td>
<td>1</td>
<td>$10</td>
<td>6</td>
<td>$60</td>
</tr>
<tr>
<td>NMR spectroscopy (P-31 MRS)</td>
<td>10</td>
<td>$100</td>
<td>5</td>
<td>$500</td>
</tr>
<tr>
<td>CPET (cardiopulmonary exercise test)</td>
<td>3</td>
<td>$30</td>
<td>3</td>
<td>$90</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>5</td>
<td>$50</td>
<td>1</td>
<td>$50</td>
</tr>
<tr>
<td><strong>Maximum Compensation:</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$700</strong></td>
</tr>
</tbody>
</table>
Q. References

10. Katsyuba E, Auwerx J. Modulating NAD(+) metabolism, from bench to bedside. EMBO J 2017;36:2670-83