
PROTOCOL SYNOPSIS

Study Title	NAD⁺ Precursor Supplementation with Exercise Training to Increase Aerobic Capacity in Freidreich's Ataxia
Funder	National Institutes of Health (NIH)
Clinical Phase	Mechanistic clinical trial (Phase II)
Study Rationale	Decreased aerobic capacity (VO ₂ max on exercise testing) is common in FA, and may contribute to decreased insulin sensitivity (S _i). Decreased muscle mitochondrial oxidative phosphorylation (OXPHOS) capacity is a potential cause of decreased VO ₂ max in FA. Preclinical and clinical studies suggest that exercise training and NAD ⁺ precursor supplementation may each produce increases in OXPHOS that translate into increased VO ₂ max. Also, the effects of exercise and NAD ⁺ precursor supplementation may be additive.
Study Objective(s)	<p>Primary</p> <ul style="list-style-type: none"> To measure the effect of a combination intervention (NR + exercise) on VO₂max in FA. <p>Secondary</p> <ul style="list-style-type: none"> To measure the effect of combination administration (NR + exercise) on S_i in FA.
Supplement	<p>Tru Niagen ® Nicotinamide Riboside (NR) (300 mg capsules)</p> <p>Placebo for NR</p>
Study Design	<p>Randomized, placebo-controlled trial with a 2x2 factorial design testing the effects of an NAD⁺ precursor (NR) and exercise on VO₂max and S_i health in FA.</p> <p>Participants will be assigned to 1 of 4 groups:</p> <ul style="list-style-type: none"> - NR Supplement, Plus Exercise - NR Supplement, NO Exercise - Placebo for NR Supplement, Plus Exercise - Placebo for NR Supplement, NO Exercise <p>The 12-week in-home intervention will include recumbent cycling, an activity with demonstrated safety, feasibility, and appeal in this cohort.¹¹</p>

Subject Population	Inclusion Criteria
key criteria for Inclusion and Exclusion:	<ol style="list-style-type: none"> 1) Molecular diagnosis of Friedrich's Ataxia (FA). 2) Males and Females, Age 10 to 40 years (inclusive). 3) Girls ≥ 11 years of age must have a negative urine/serum pregnancy test and must use an acceptable method of contraception, including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive, for the duration of the study. 4) Not currently meeting exercise guidelines as outlined by The Physical Activity Guidelines for Americans. <ul style="list-style-type: none"> - Children and Adolescents should do 60 minutes (1 hour) or more of moderate-to-vigorous physical activity daily. - As a part of their physical activity, children and adolescents should include muscle-strengthening physical activity on at least 3 days a week. - Adults should do at least 150 minutes (2 hours and 30 minutes) to 300 minutes (5 hours) a week of moderate-intensity, or 75 minutes (1 hour and 15 minutes) to 150 minutes (2 hours and 30 minutes) a week of vigorous-intensity aerobic physical activity. - Adults should also do muscle-strengthening activities of moderate or greater intensity that involve all major muscle groups on 2 or more days a week. 5) Cardiac echocardiogram or cardiac MRI, performed within 1 year of enrollment, showing a LVEF $\geq 45\%$ 6) ECG, performed within 1 year of enrollment, without clinically significant arrhythmia. 7) Weight > 24 kg 8) Parental/guardian permission (informed consent) and if appropriate, child assent.
	Exclusion Criteria
	<ol style="list-style-type: none"> 1) Known sensitivity to NR. 2) Concurrent use of any medications, including statins, likely to increase risk of NR toxicity. 3) HgbA1c $\geq 8.5\%$ and/or DM requiring insulin or insulin secretagogue.

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- 4) Use of supraphysiologic steroids
 - 5) Laboratory abnormalities that indicate clinically significant anemia or bleeding risk.
 $\text{Hgb} < 10 \text{ g/dL}$ or $\text{Platelets} < 100\text{K}$
 - 6) Laboratory abnormalities that indicate clinically significant kidney disease using serum creatinine and MDRD equation.
 $\text{eGFR} < 60 \text{ ml/min/1.73 m}^2$
 - 7) Laboratory abnormalities that indicate clinically significant liver disease.
 $\text{AST/SGOT} \geq 3.0 \times \text{ULN}$
 $\text{ALT/SGPT} \geq 3.0 \times \text{ULN}$
 - 8) Uncontrolled and persistent arrhythmias that are felt to be clinically significant
 - 9) Known history of moderate or severe left ventricular systolic dysfunction ($\text{LVEF} < 45\%$)
 - 10) Standard contraindications to exercise testing
 - 11) Inability to sit and pedal unassisted, at a cadence of at least 55 rpm during unloaded warm up, in a cycle ergometry chair and complete a maximal Cardio Pulmonary Exercise Test (CPET)
 - 12) Inability to sit and pedal unassisted in a recumbent tricycle
 - 13) For individuals completing the MRI: Any contraindication to MRI Including:
 - ANY intra-luminal implant, filter, stent or valve replacement
 - ANY type of life assist device, pump, or prosthetic
 - ANY vascular clip or clamp
 - ANY surgically placed clips or clamps or bands on visceral organs
 - ANY intracranial implants of any type other than dental fillings
 - ANY non-removable piercings, jewelry, or medicinal patch
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	<ul style="list-style-type: none"> – ANY personal history of intraocular injury or fragment in or around the orbit that cannot be cleared through radiologic examination. – ANY personal history of bullet, shrapnel, or stabbing wounds that cannot be cleared through radiologic evaluation. – Inability to lie flat in the MRI scanner for 60-90 minutes. * participants who cannot complete the MRI will not be excluded from participation in the remainder of the study procedures if they meet those inclusion and exclusion criteria <ol style="list-style-type: none"> 14) Use of any investigational agent within 4 weeks of enrollment, except open-label extension phase 15) Females: Pregnant, lactating, or planning to become pregnant during their participation. 16) Any medical condition, in the opinion of the investigator that will interfere with the safe completion of the study. 17) Parents/guardians or participants who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.
Number Of Subjects	We anticipate enrolling 80 participants (~40% pediatric, ~60% adult) in order to obtain complete data on 64 participants. Pediatric participants (n = 32) will complete study procedures at The Children's Hospital of Philadelphia. Adult participants (n = 48) will complete study procedures at The Children's Hospital of Philadelphia and The Hospital of the University of Pennsylvania.
Study Duration	Each individual's participation will last up to 36 weeks (+/- 1 Week).
Study Phases Screening Intervention Follow-Up	(1) Screening: Participants will undergo a telephone screening to assess eligibility and then, within ~12 weeks, complete an in-person baseline visit to confirm eligibility. (2) Baseline Visit: Participants confirmed to be eligible after completion of screening procedures will be assigned to 1 of 4 arms and begin the 12-week intervention. (3) Follow-Up: Participants will return after the 12-week intervention to complete follow-up procedures.
Outcomes	VO ₂ max (primary outcome); S _i (key secondary outcome); muscle mass (key secondary outcome); muscle mitochondrial OXPHOS capacity via CrCEST MRI (key secondary outcome); NAD ⁺ from

	muscle biopsy (key secondary outcome); nutrient-specific mitochondrial OXPHOS capacity from muscle biopsy (key secondary outcome)
Safety Evaluations	Safety will be monitored through the collection of laboratory assessments at baseline, interim, and follow-up. Participants will also be monitored using a standardized assessment of symptoms. We will use the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0, U.S. Department of Health and Human Services) to grade adverse events. Individuals who experience a new Grade 3 or higher AE may need to stop study participation at the discretion of the investigative team.
Statistical And Analytic Plan	Linear mixed-effects modeling will be used to evaluate effect of interventions over time on the primary and key secondary outcomes. The models incorporate subject-specific random effects to account for within-subject correlation due to repeated measures. Group averages, as well as subject-specific intercepts and slopes, will be estimated to capture potential variations in the baseline values and slopes among individuals. Linear mixed-effects models accommodate missing data due to dropout, such that all randomized participants can be included. We will compare participants who complete the study with those who do not, and identify potential factors that are associated with dropout. In the mixed-effects models, we will adjust for time-invariant covariates (age at randomization, sex) and time-varying covariates (lean mass, muscle OXPHOS capacity, muscle NAD ⁺ content). By including these indices of “muscle quality” as covariates in the models, we will be able to assess whether intervention effects are explained by these effects. To assess other potential factors influencing outcomes, we will also analyze the incremental effect of adding each of the other measured covariates (final dose of NR achieved, final training intensity achieved, FA severity) to the models.
DATA AND SAFETY MONITORING PLAN	The PI will designate an independent Data Safety Monitoring Board (DSMB) to perform an independent review of ongoing study progress and safety. The DSMB will likely consist of a cardiologist, neurologist, and endocrinologist with expertise in FA, and also a statistician. The cardiologist will serve as chair, and will facilitate meetings as per the DSMB charter.