NCT04112043

Nicotinamide riboside as an Enhancer of Exercise Therapy in hypertensive older adults

- The NEET Trial

PI: Robert T. Mankowski, PhD

2-9-2023

Study Protocol and Statistical Analysis Plan

1. Project Title:

Nicotinamide riboside as an Enhancer of Exercise Therapy in hypertensive older adults – The NEET Trial.

2. Investigators:

Robert Mankowski, PhD
Stephen Anton, PhD
Christiaan Leeuwenburgh, PhD
Demetra Christou, PhD
Bhanuprasad Sandesara, MD
Peihua Qiu, PhD

3. Abstract:

More than 80% of older adults have hypertension, with higher prevalence of high systolic blood pressure (SBP) putting them at high risk for cardiovascular (CV) disease and death. Because drug therapy that lowers SBP is associated with side effects such as hypotension, syncope, and kidney dysfunction, there is a great need for effective lifestyle SBP-lowering interventions for the older population that can replace drug therapy. While aerobic exercise is a recommended lifestyle intervention for controlling SBP and preventing CV disease naturally, in older adults it has been shown to be less effective in vascular-tissue remodeling because of arterial stiffness, resulting in less efficient SBP control. Reduced bioavailability of nicotinamide adenine dinucleotide (NAD+), a cofactor for the deacetylase sirtuin1 (SIRT1), may contribute to age-related vascular dysfunction via oxidative stress and reduced nitric oxide (NO). Exercise-induced overexpression of NAD+-dependent SIRT1 improves the bioavailability of NO. Preclinical evidence suggests that poor vascular-function improvement in response to exercise in older mice is caused by insufficient NAD+ levels to stimulate SIRT1 activity. Importantly, replenishment of NAD+ levels induced vascular remodeling, improved vascular function, and reduced SBP in mice. An objective of this study, therefore, is to test a combination of aerobic exercise and nicotinamide riboside, a compound that replenishes NAD+ levels, to optimize exercise's SBP-lowering effect in hypertensive older adults. Initial human clinical trials demonstrated that nicotinamide riboside supplementation (1,000 mg/day) was safe and showed a higher potential to reduce SBP and arterial stiffness in participants with elevated SBP. As we have preclinical evidence that combining NAD+ replenishment with exercise is an ideal strategy for improving vascular function, our central hypothesis is that the intervention of aerobic-exercise training combined with nicotinamide riboside supplementation will reduce SBP in hypertensive older adults more effectively than will exercise alone. We will enroll 54 participants ≥ 55 years and older into either: (1) 1,000 mg/day of nicotinamide riboside plus 3 days/week of supervised, center-based walking exercise (n=18), or (2) the same exercise program combined with placebo (n=18), or (3) 1,000 mg/day of nicotinamide riboside alone (n=18). All participants will undergo daytime continuous SBP at baseline, 3 weeks, and 6 weeks, and arterial-stiffness measurements by pulse-wave velocity at baseline and at 6 weeks. Elevated SBP will be determined as daytime average equal to or above 130 mmHg, measured by the 24-hour blood-pressure device. To our knowledge, this study will be the first attempt to enhance exercise therapy with nicotinamide riboside in hypertensive older adults. We believe that nicotinamide riboside is "the missing piece of the puzzle" in improving vascular remodeling and SBP management in older adults. Preliminary evidence from this pilot study may support a full-scale Phase III clinical trial in hypertensive older adults. The ultimate goal of this line of research is to find adjuvant strategies to improve the exercise's SBP-lowering effects in older adults.

4. Background:

More than 80% of older adults are diagnosed with hypertension and thus are at high risk of atherosclerosis, cardiovascular (CV) events, and death [1]. Systolic blood pressure (SBP) is the most common form of hypertension and is considered more important than diastolic blood pressure (BP) as an independent CV disease risk factor [2]. SBP equal to or above 130 mmHg needs treatment, as even 10-mmHg elevation doubles the risk of a heart attack [3]. Because successful reduction of SBP with medication is often accompanied by side effects such as hypotension, syncope and kidney dysfunction [2], there is a great need for effective lifestyle SBP-lowering interventions in the older population.

The major contributors to high SBP are decline in endothelial function, decline in capillary vessel density, and arterial stiffening, which are profound changes in vascular aging [4, 5]. Reduced bioavailability of nicotinamide adenine dinucleotide (NAD+), a cofactor for the deacetylase sirtuin1 (SIRT1), may contribute to age-related vascular dysfunction via oxidative stress and reduced nitric oxide (NO) [6]. Endothelial dysfunction reduces bioavailability of NO, which increases arterial stiffness and elevates SBP [6]. Interventions that elevate NAD+ levels and increase SIRT1 activity may be critical for improving therapies that target endothelial dysfunction and arterial stiffness to manage SBP and prevent CV disease in older adults.

While aerobic exercise is recommended for SBP control and CV disease prevention [7], it has been shown to be less effective in lowering SBP in older adults because of arterial stiffness [8]. Preclinical evidence suggests that age-related lower vascular function improvement in response to exercise may be caused by insufficient levels of NAD+ to stimulate SIRT1 activity and induce vascular remodeling for SBP reduction [9]. In particular, preclinical evidence showed that in old mice with replenished NAD+ by administration of nicotinamide mononucleotide (NMN), exercise was more effective in stimulating SIRT1 activity and restoring endothelial function and capillary density, compared to mice with lower NAD+ levels [9]. Such findings suggest that replenishment of NAD+ levels with metabolites (e.g., nicotinamide riboside (NR) or NMN) may be the key to enhancing vascular function in response to aerobic-exercise training and, thus, reducing SBP in older adults more effectively.

In healthy, men and women aged 55 to 79 years, 6 weeks of supplementation with 1,000 mg/day of NR was well-tolerated and increased NAD+ levels in peripheral blood [10]. Importantly, NR supplementation produced trends towards reductions of SBP as well as arterial stiffness compared to placebo [10]. A post-hoc analysis of the same study sample showed trends towards larger reductions after NR supplementation in individuals with higher SBP (9 mmHg lower after NR compared to placebo among participants with 120–139 mmHg SBP) and arterial stiffness (41.5 centimeters per second (cm/s)) [10]. We believe that promising results from Martens et al., study and promising preclinical evidence from Das et al., that showed a combination of NAD+ replenishment and exercise was an optimal strategy to most effectively improve vascular function and reduce SBP in older mice, support our novel proposal that a combination of NR and aerobic exercise (NREx) will be more effective than either NR or exercise alone (exercise and placebo - PLEx) in hypertensive older adults. Thus, our central hypothesis is that the intervention of aerobic-exercise training combined with NR supplementation will reduce SBP in older adults who have high SBP more effectively than will exercise alone.

To test our central hypothesis, we will recruit 54 older adults ≥55 years old with high 24-hour average SBP and follow them for 6 weeks in a double-blind fashion. Participants will be randomly assigned to receive either (1) 1,000 mg/day of NR plus 3 days/week of supervised, center-based walking exercise, or (2) the same exercise program combined with placebo, or (3) 1,000 mg/day of NR alone. Inclusion of all treatment groups will be important for detecting the potential benefit of NREx. High SBP will be determined at screening as daytime average above or equal to 130 mmHg and below 160 mmHg, measured by the 24-hour BP device. All enrolled participants will undergo daytime continuous SBP measurements at baseline, 3 weeks, and 6 weeks, and arterial-stiffness measurements by pulse-wave velocity (PWV) at baseline and 6 weeks.

5. Specific Aims:

The objective of this study is to conduct a pilot study to refine and finalize elements critical to conducting a future, definitive randomized, controlled trial (RCT). We propose to recruit 54 persons aged ≥ 55 years with SBP ≥130 mmHg and <160 mmHg for 6 weeks. Participants will be randomly assigned to receive either **(1)** 1,000 mg/day of NR plus 3 days/week of supervised, center-based walking exercise, or **(2)** the same exercise program combined with placebo, or **(3)** 1,000 mg/day of NR alone. Inclusion of all treatment groups will be important for detecting the potential benefit of NREx. High SBP will be determined at screening as daytime average above or equal 130 mmHg and below 160 mmHg, measured by the 24-hour BP device. All enrolled participants will undergo daytime continuous SBP measurements at baseline, 3 weeks, and 6 weeks, and arterial-stiffness measurements by pulse-wave velocity (PWV) at baseline and 6 weeks. This study will enable us to address the following aims, all of which are of critical importance for designing a full-scale trial.

Aim 1. To obtain critical data necessary to project the sample size needed for a full-scale trial using the change in SBP, as the primary outcome and;

Aim 2. Using the change in arterial stiffness, as the secondary outcome.

6. Research Plan:

We propose to conduct a randomized, controlled pilot trial to test if nicotinamide riboside improves an effect of aerobic exercise training on cardiovascular function among hypertensive older adults. Following study entry, participants (N = 54) will be randomly assigned to either NREx, NR or PLEx groups for 6 weeks. Exercise groups will be involved in three center-based walking training sessions per week. We anticipate that up to an additional 20 individuals enrolled may discontinue participation in the project prior to completion, and that we may have to screen up to 250 additional individuals to reach our recruitment goal of 54 subjects.

6.1 Participants: Eligible participants will be males or females ≥ 55 years of age with daytime average SBP ≥ 130mmHg and < 160 mmHg.

Table 3.1.1. Inclusion and exclusion criteria.

Inclusion Criteria

- Age 55 years and older
- Daytime average of systolic blood pressure of ≥ 130 mmHg and < 160 mmHg.
- Sedentary lifestyle, defined as < 150 min/wk of moderate physical activity as assessed by the CHAMPS questionnaire.
- Willingness to be randomized to either treatment group
- Willingness to participate in all study procedures

Exclusion criteria

- Failure to provide informed consent.
- Pregnant
- Change in blood pressure therapy (type or dose) within the last 3 months- Temporary Exclusion
- Daytime average of systolic blood pressure ≥160 mmHg.
- Regular consumption of nicotinamide riboside supplement
- Current involvement in supervised rehabilitation program

- Absolute contraindication(s) to exercise training according to American College of Sports Medicine guidelines [11]
- Daytime average of systolic blood pressure below 130mm Hg or Diastolic BP ≥ 100mm Hg.
- Peripheral vascular disease; peripheral neuropathy; retinopathy
- Severe cardiac disease, including NYHA Class III or IV congestive heart failure, clinically significant aortic stenosis, history of cardiac arrest, use of a cardiac defibrillator, or uncontrolled angina;
- Myocardial infarction or stroke within past year
- Significant cognitive impairment, including known dementia diagnosis or a Mini-Mental State Examination exam score < 24
- Progressive, degenerative neurologic disease, e.g., Parkinson's Disease, multiple sclerosis;
- Severe rheumatologic or orthopedic diseases, e.g., awaiting joint replacement, active inflammatory disease;
- Severe pulmonary disease, requiring steroid therapy or the use of supplemental oxygen;
- Hip fracture, hip or knee replacement, or spinal surgery within past 4 months;
- Other significant co-morbid conditions that would impair ability to participate in the exercise-based intervention
- Simultaneous participation in another intervention trial

6.2 Recruitment: Participant recruitment will be coordinated in conjunction with the *OAIC Clinical Research Core*, led by co-investigator Dr. Stephen Anton. We will develop a targeted recruitment approach based on previous experiences utilizing methods of recruitment that include direct mailings, newspaper classified and print ads, community luncheons and health fairs, as well as clinic referrals. We have been extremely successful in implementing this approach in past studies

6.3 Screening and Study Entry: Participants deemed eligible based on an initial phone screening by our front desk manager, will be encouraged to consult with their physician and invited to an in-person screening visit. During this visit, potential participants will first be asked to give their informed consent and then will be screened for study entry criteria. Initial screening procedures will include a review of their medical history, medication use, a physical exam or nursing assessment, physical activity levels, cognitive function (assessed by the Mini-Mental State Exam), measurement of vital signs, height and weight, BMI (calculated in database only), collection of demographics information, single 24-hour ambulatory SBP monitoring (daytime average SBP≥130 mmHg and <160 mmHg – qualifying criterion), and urine collection for a pregnancy test. If all study entry criteria are met, participants will be scheduled to return to the clinic for baseline assessments. In addition, the study participants will be asked to sign Medical Records Release Authorization if they qualify. This Authorization will be used exclusively to obtain medical records to acquire facts, details, and outcomes of Serious Adverse Events that occurred during the study and have to be reported to the sponsor and/or IRB.

6.4 Baseline Assessment and Randomization: If all study entry criteria are met, participants will be scheduled to return to the clinic for baseline assessments prior to randomization. Participants who have not yet had a physical evaluation or nursing assessment will complete this before undergoing physical tests. Participants will be asked to provide a fasting blood sample for evaluation of clinical safety lab values. We will also collect urine samples (first sample collected between 8 am to 11 am and another sample collected between 5 pm to 8 pm) at this visit, which will be slow frozen in a -80 freezer and analyzed after data collection is complete. The first sample will be collected when the participant comes in the morning to participate in the in-person visit. Then, the study coordinator will provide the urine sample collection cup for the participant to bring back home to collect the second urine sample. The study coordinators can help transport the second urine sample or, if the participant prefer, the participant could bring the urine sample back to the study coordinator. In addition, vital signs and weight

will be measured, as well as exercise capacity with the 6-Minute Walk Test and self-assessed functionality with the Late Life Function and Disability Instrument (LLFDI). Participants will also be given the 24-h BP device to complete the 24-hour ambulatory recording and will have their arterial stiffness assessed. We will also collect adverse events and update medication changes. Finally, the study coordinator will provide participants with a supplement intake form to take home to record when the supplement is taken. Staff members will then discuss randomization procedures with participants. The participants will receive a return envelope/box to return the ambulatory BP device after the 24-hour recording.

6.5 Exercise Intervention: After random assignment to either the NREx or the PLEx groups, participants will engage in a three-days a week, center-based walking exercise intervention for 6 weeks. Following a brief warm-up, participants will be instructed to walk for 30 minutes at a 5-6 on the CR10 scale with encouragement for 10 minutes to be vigorous (7-8 on CR10 scale). Sessions will also include balance training to promote cool-down. Participants will be introduced to exercises in such a way that they begin with lighter intensity and gradually increase. The progressive nature of the intervention is designed to minimize discomfort and prevent injury. Blood pressure will be measured prior to each exercise session; the participant will not complete the session that day if he or she has an in-person SBP >200 mmHg or DBP >110 mmHg (AHA guidelines for exercise contraindication) measured at the exercise center.

6.6. Supplementation: Participants will be randomly assigned to receive, in a double-blind fashion, either 1,000 mg/day of encapsulated NR (Niagen®) or placebo. The company ChromaDex (Irvine, CA) will provide both the NR and placebo capsules for the proposed study. Placebo capsules will be identical to those including NR. As noted above, a 6-week NR study of Martens et al. demonstrated that NR supplementation at a dose of 1,000 mg/day was safe in rather healthy middle-aged and older adults. Subjects will ingest NR (NIAGEN®; 500 mg, twice per day; ChromaDex, Inc.) or alike looking placebo capsules [10]. The participants will be provided with capsules sufficient for at least 3 weeks. The second 3-week portion of the capsules will be dispensed at 3-week follow-up visits by a study coordinator. A study coordinator will be given a jar with the study product by an unblinded staff person not-involved in this study. The not-involved staff member will be is possession of the identified randomization list and will provide this list after completing the enrollment and outcome measure analyses. The participants will receive a supplement intake diary to note the taken pills and a study coordinator will count the remaining pills at 3 and 6-week visits.

Data from the exercise interventions will be collected and stored in the study database. Following each session, participants will be asked to provide a rating of perceived exertion (RPE) for the session according to the Borg CR10 scale.

Table 1. Data collection summary by study visit.

Study phase	**Pre-randomization**		**Randomization**	
Visit description (FU=follow-up, CO=close-out)	Screen	Baseline	FU	CO
Visit number	1	2	3	4
Week number	-2	0	3 (+ or - 7 days)	6 (+ or - 7 days)
Informed consent, review of inclusion/exclusion criteria	**X**			
Medical history and medications, MSSE	**X**			
Height	**X**			
Weight	**X**	**X**	**X**	**X**
BMI (calculated in database only)	**X**			
Demographics information	**X**			
Physical Exam/nursing assessment	**X***	**X***		
Monitor vital signs	**X**	**X**	**X**	**X**
Ambulatory BP recording	**X**	**X**	**X**	**X**

CHAMPS Questionnaire	X			
Pregnancy test	X			
6 minute walk test		X		X
Late Life Function & Disability Instrument		X		X
Randomization		X		
Blood collection		X	X	X
Urine collection		X	X	X
Arterial stiffness		X		X
Dispense Supplement Intake Diary		X	X	
Dispense Study Supplement (NR or Placebo)		X	X	
Pill counts, collect diaries, assessment of adverse experiences			X	X
Collection of AEs and update medication changes		X	X	X
*The physical exam/nursing assessment can be completed at either Screening Visit 1 or at the Baseline Visit as long as it is prior to randomization.				

6.7

Adherence to interventions. To enhance adherence to the study interventions (compliance), we will utilize empirically-supported techniques which we have successfully utilized in prior studies. Our first step will be to fully inform participants of study requirements before randomization and enroll only those persons who are willing to complete all study procedures. Undoubtedly, however, issues arise after randomization with the potential to limit compliance. Common causes of poor compliance include vacations, spouse care, fatigue, and fluctuations in motivation, perceived lack of benefit, physical discomfort, and adverse health experiences. First, we have designed the trial, including the inclusion/exclusion criteria and physician monitoring, to limit the likelihood of experiencing adverse health events in response to the interventions. In addition, care will be taken by the interventionists to promote the safe engagement in the exercise intervention. For other issues of non-compliance, we will approach this potential problem using a social problem solving model ("toolbox") approach in dealing with individual adherence problems.[28] This approach assumes that the process of changing behavior is a collaborative effort between the participant and the interventionists, and that behavior change can be readily undertaken during the regular weekly contacts that intervention staff will initiate throughout the course of the intervention, as well as during face-to-face visits. Our team has successfully applied this type of problem-solving approach in promoting adherence to behavioral interventions in prior studies.

6.8 Follow-up and Close-out visits. In addition to baseline and screening visits, assessment visits will be conducted at approximately 3 weeks (follow-up) and approximately 6 weeks post-randomization. During the 3-week assessment visit, the research team will evaluate participant's vital signs, measure weight, update medical history, collect blood samples for safety markers and inquire about any adverse experiences since the last visit. We will also collect urine samples (first sample collected between 8 am to 11 am and another sample collected between 5 pm to 8 pm) at these visits, to be analyzed after data collection is complete. The first sample will be collected when the participant comes in the morning to participate in the in-person visit. Then, the study coordinator will provide the urine sample collection cup for the participant to bring back home to collect the second urine sample. The study coordinators can help transport the second urine sample or, if the participant prefer, the participant could bring the urine sample back to the study coordinator. Blood pressure will be measured prior to completing physical function tasks at these visits; the participant will not complete the physical function tasks for that visit if he or she has an in-person SBP >200 mmHg or DBP >110 mmHg (AHA guidelines for exercise contraindication) at the clinic. The coordinator will collect and count return pills and dispense a new supply. At the end of the 3 week visit, the participant will be provided with a 24-Hour Ambulatory Blood Pressure Monitor and the measurements will be recorded as in previous visits. During the close-out (6-week) visit, the team will perform the same evaluations as during the baseline visit, as well as collect and count returned pills, and collect supplement intake information from participants. We will also collect adverse events and update medication changes.

6.9. Primary and Secondary Outcome Measures.

24-h BP	All blood pressure recordings will be performed with validated Oscar 2 monitors, under the supervision of trained personnel and according to the manufacturer's recommendations. SBP will be measured every 20 minutes (min) during the day (from 0700 to 2200 hours), and every 60 min overnight (from 2200 to 0700 hours) [12].
Arterial Stiffness	Aortic pulse-wave velocity (PWV), commonly measured as carotid to femoral PWV (cfPWV), is considered to be the gold standard method of assessing arterial stiffness in humans. cfPWV will be measured using the SphygmoCor XCEL system (AtCor Medical, New South Wales, Australia). Briefly, cfPWV will be determined by recording pressure pulse waves at the carotid and femoral arteries using a high-fidelity micromanometer (Millar Instruments, Houston, TX) and calculating the distance between the recording sites divided by the time delay between the carotid and the femoral pulse waves [13].

6.10 Sample size justification. This pilot study will help generate precise estimates of effect variance in the population in which a subsequent larger-scale study will be conducted. Published recommendations for the design of pilot studies indicate that a sample of 18 participants per randomized arm is typically sufficient to estimate a parameter for a future trial [14-16]. The enrollment sample of 54 (n=18 x 3 arms) participants is intended to provide preliminary data necessary to indicate feasibility of a larger trial and to provide descriptive estimates of effects. Based on our experience from the past and current clinical trials conducted at our institute, we do not expect an attrition higher than 15%, therefore, we increased a group size to n=18, which will give us sufficient sample sizes for the pilot purposes after drop-out (n=15 x 3 groups). The data will also provide for nominal estimation (using a 95% CI) of mean changes in dependent variables within each arm. By assuming the mean decrease in SBP after a 6-week of intervention to be 20 mmHg, 10 mmHg and 15 mmHg in the three groups of NREx, PLEx and NR, respectively, and the standard deviation of the SBP decreases to be 10 [16], the sample size of 15 participants for each group can guarantee a power of 85% to detect the difference between the NREx and PLEx groups and a power of about 80% to detect the differences among the three groups. We expect that the differences of the arterial-stiffness measurements among the three groups are larger than those of SBP. Thus, the power numbers for that measure will be larger as well.

6.11 Safety monitoring plan. To ensure participant safety in the proposed study, we will adapt procedures that we have implemented successfully in other studies including LIFE,[46,47] Weight Loss + Exercise,[48] and Task Specific Exercise.[49] As he has for a number of our prior studies, Dr. Bhanuprasad Sandesara will serve as the Study Physician who is responsible for assessment, monitoring and interventions for health events. To maximize participant safety we will follow a standardized screening protocol. Accordingly, all potential participants will undergo screening for cardiovascular and other major diseases by means of a health questionnaire, medication inventory, and physical exam/nursing assessment. Those with conditions that meet the exclusion criteria described in section 6.1 as determined by Dr. Sandesara will be excluded. Dr. Sandesara will also review clinically significant abnormal laboratory values and indicate the need for study withdrawal if he deems it necessary. Each participant will be instructed to report the occurrence of an adverse event at scheduled data collection times (scheduled clinical exams or phone interviews). Participants also have access to study clinic personnel at other times to report serious adverse events or concerns about the safety of participating in the study. If necessary, Release of Medical Records Authorizations signed by the study participants will be used to obtain medical records to collect details and outcomes of SAEs for safety and reporting purposes.

Venipuncture will be performed by a trained and experienced phlebotomist using standard techniques. Participants will always have venipuncture performed while seated upright with both feet on the floor.

Center-based assessments and interventions will be conducted and supervised by trained staff who will monitor potential adverse experiences and symptoms. All assessors and interventionists receive CPR training and training on management of acute events including syncope, chest pain, acute dyspnea, focal neurological symptoms and abnormal vital signs. Portable defibrillators are available at each intervention and assessment site and all study staff have on-call access to the study physician and contact numbers for emergency services. Institutional and community EMS services will be activated if needed. As indicated previously, participants will be taught the importance and proper method of warming-up prior to and cooling-down following structured activity sessions. If at any point during an exercise session, participants develop chest pain, shortness of breath, or dizziness, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur with further exercise.
Blood pressure and heart rate will be measured before and after the resistance training at each center based session for pre-exercise safety. The participant will not complete the session that day if he or she has an in-person SBP >200 mmHg or DBP >110 mmHg (AHA guidelines for exercise contraindication) measured at the exercise center.

Procedures to minimize discomfort include warm-up and cool-down activities. The participants will also be introduced to the intervention exercises in a structured way, such that they begin with lighter intensity and gradually increase over the course of the first week. During the intervention visits, participants will be supervised at all times. Participants will also be instructed to move slowly when rising from a seated or lying position to reduce the risk of experiencing orthostatic hypotension. Study staff will be instructed to stand in a supportive position when participants are rising to prevent a fall in case of syncope. Exercise will be stopped if the participant reports pain, tightness or pressure in the chest, significant shortness of breath, feeling faint, lightheaded or dizzy, or significant other medical problems.

Any significant adverse events will be reported promptly to the IRB and to the NIA Data Safety Monitoring Board (DSMB). Otherwise, annual reports will be prepared for both.

Written informed consent will be obtained after explanation to subjects of all procedures and time commitments. The study interviewers will explain to prospective participants the purpose, methods and extent of the study. Potential participants will be asked to read the informed consent form and to ask questions. The form will be written in simple, easy-to-understand language. Staff members will also review all key aspects of the study verbally.

Confidentiality of data will be maintained by using research identification numbers that uniquely identify each individual. Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Safeguards will be established to ensure the security and privacy of participants' study records. The information collected from participants in this study has a low potential for abuse, since the data do not address sensitive issues. Nevertheless, appropriate measures will be taken to prevent unauthorized use of study information. The research records will be kept in a locked room at the Institute on Aging. The files matching participants' names and demographic information with research ID numbers will be kept in a separate room and will be stored in a locked file that uses a different key from that of all other files. Only study personnel will have access to these files, and they will be asked to sign a document that they agree to maintain the confidentiality of the information.

After the study is completed, local data will be stored with other completed research studies in a secured storage vault. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we will access personal health information and medical records only after receiving signed informed consent, as described above.

Finally, the study protocol will be registered at www.ClinicalTrials.gov before study enrollment begins.

7. Possible Discomforts and Risks:

The first clinical trial with a 6-week NR supplementation (1000 mg/day) in middle-aged and older adults showed that adverse events reported over the course of the study did not differ between the treatment group and the placebo. We will test the same supplementation dose and duration provided to us by the same company. A different study that tested an effects of a different product but containing NR (500 or 1000mg/day) and Pterostilbene showed no serious adverse effects among older adults other than nausea, fatigue, headaches, diarrhea, stomach discomfort and indigestion. We will inform the potential participants that supplementation with similar products containing NR in older adults was associated with these side effects and there is always a risk that they may experience these side effects.

Potential risks are those associated with health information privacy, blood pressure testing, venipuncture and participation in exercise training and testing.

The pregnancy test may provide a false-positive or false-negative result.

Venipuncture can be associated with pain, bruising, hematoma formation, superficial phlebitis, and rarely cellulitis or fainting. The risks of placing a blood pressure cuff on a participant are that it may cause pinching, slight bruising, discomfort, or skin irritation from the cuff. The primary risk associated with moderate-intensity exercise training is skeletal muscle soreness. There are also other risks that relate to falls and fractures, exacerbation of arthritis and other joint conditions, post-exercise hypotension, and cardiovascular events. There is a risk that a participant may trip, stumble, or fall during the exercise sessions and experience shortness of breath, dizziness, palpitations, chest pain or discomfort, heartburn, light headedness, or feeling about to faint. Exercise is contraindicated when resting SBP is >200 mmHg or DBP > 110 mmHg. We are recruiting participants with systolic hypertension of ≥ 130mmHg and < 160 mmHg, therefore, there is no specific increased risk to these participants over the general risks associated with moderate-intensity exercise training.

The DSMB will be set-up and run by the National Institute on Aging.

Confidentiality: Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Confidentiality of data will be maintained by using research identification numbers that uniquely identify each individual. Safeguards will be established to ensure the security and privacy of participants' study records. Appropriate measures will be taken to prevent unauthorized use of study information. Data other than demographic information will not use names as an identifier. The research ID number will be used. The research records will be kept in a locked room in the study site. Only trained and certified study personnel will have access to these files, and they will be asked to sign a document that they agree to maintain the confidentiality of the information. Electronic records will be stored on a password protected network server maintained by the university information technology department. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we access personal health information and medical records only after receiving signed informed consent. If a participant does not qualify based on the initial phone pre-screening, we will delete the individual's identifying information from the study record immediately, but we will keep the other information provided to track the outcome of our calls.

8. Possible Benefits:

Importantly, the proposed project should have tangible benefits for participants. These benefits include information about their health and assessments of their cardiovascular status. All study participants will be encouraged to communicate the results from the study to their primary care providers. Moreover, participants in exercise groups will receive supervised exercise training and instruction about how to maintain exercise habits at home. Participants in the NR alone group may experience potential benefits of the supplementation. We expect that these benefits will improve quality of life for all participants.

Potential benefits of NR alone may be lowering systolic blood pressure and improving vascular flexibility, but the combination of NR and exercise may have better effects than exercise or NR alone on systolic blood pressure reduction and improvement of vascular flexibility in hypertensive older adults.

9. Conflict of Interest:

None

10. Statistical Analysis Plan

The primary analysis will follow an “intent-to-treat” model. Because repeated measures are obtained on individual participants and they are potentially correlated, a mixed-effect linear regression model will be used. Differences in outcome measures at the 3-week follow-up (or 6-week close-out) from baseline for each individual participant will be used as responses, and two dummy variables representing the three treatment groups, age, and gender, and other baseline characteristics of participants will used as covariates. A random-effect term will also be included in the model to accommodate within-subject data correlation. This analysis will be performed for each outcome variable. The relatively small sample size may imbalance pre-randomization covariates. Thus, caution will be taken in the interpretation of hypothesis testing results.

Literature cited

1. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. *Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association.* Circulation, 2018. **137**(12): p. e67-e492.
2. Group SR, Wright JT, Jr., Williamson JD, Whelton PK, Snyder JK, Sink KM, et al. *A Randomized Trial of Intensive versus Standard Blood-Pressure Control.* N Engl J Med, 2015. **373**(22): p. 2103-16.
3. Whelton PK, Carey RM, Aronow WS, Casey DE, Jr., Collins KJ, Dennison Himmelfarb C, et al. *2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.* Hypertension, 2018. **71**(6): p. 1269-1324.
4. AlGhatrif M, Strait JB, Morrell CH, Canepa M, Wright J, Elango P, et al. *Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore Longitudinal Study of Aging.* Hypertension, 2013. **62**(5): p. 934-41.
5. Ungvari Z, Tarantini S, Kiss T, Wren JD, Giles CB, Griffin CT, et al. *Endothelial dysfunction and angiogenesis impairment in the ageing vasculature.* Nat Rev Cardiol, 2018. **15**(9): p. 555-565.
6. de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, et al. *Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice.* Aging Cell, 2016. **15**(3): p. 522-30.
7. Tanasescu M, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ, and Hu FB. *Exercise type and intensity in relation to coronary heart disease in men.* JAMA, 2002. **288**(16): p. 1994-2000.
8. Stewart KJ, Bacher AC, Turner KL, Fleg JL, Hees PS, Shapiro EP, et al. *Effect of exercise on blood pressure in older persons: a randomized controlled trial.* Arch Intern Med, 2005. **165**(7): p. 756-62.
9. Das A, Huang GX, Bonkowski MS, Longchamp A, Li C, Schultz MB, et al. *Impairment of an Endothelial NAD(+)-H2S Signaling Network Is a Reversible Cause of Vascular Aging.* Cell, 2018. **173**(1): p. 74-89 e20.
10. Martens CR, Denman BA, Mazzo MR, Armstrong ML, Reisdorph N, McQueen MB, et al. *Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults.* Nat Commun, 2018. **9**(1): p. 1286.
11. American College of Sports M, Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, et al. *American College of Sports Medicine position stand. Exercise and physical activity for older adults.* Med Sci Sports Exerc, 2009. **41**(7): p. 1510-30.
12. Goodwin J, Bilous M, Winship S, Finn P, and Jones SC. *Validation of the Oscar 2 oscillometric 24-h ambulatory blood pressure monitor according to the British Hypertension Society protocol.* Blood Press Monit, 2007. **12**(2): p. 113-7.
13. Hwang MH, Yoo JK, Kim HK, Hwang CL, Mackay K, Hemstreet O, et al. *Validity and reliability of aortic pulse wave velocity and augmentation index determined by the new cuff-based SphygmoCor Xcel.* J Hum Hypertens, 2014. **28**(8): p. 475-81.
14. Lancaster GA, Dodd S, and Williamson PR. *Design and analysis of pilot studies: recommendations for good practice.* J Eval Clin Pract, 2004. **10**(2): p. 307-12.
15. Browne RH. *On the use of a pilot sample for sample size determination.* Stat Med, 1995. **14**(17): p. 1933-40.
16. Hertzog MA. *Considerations in determining sample size for pilot studies.* Res Nurs Health, 2008. **31**(2): p. 180-91.