Title: NAD therapy for improving memory and brain blood flow in older adults with mild cognitive impairment

NCT Number: NCT03482167

Document Date: 11/14/2024

HUMAN SUBJECTS PROTOCOL
University of Delaware

NAD therapy for improving memory and brain blood flow in older adults with mild cognitive impairment

Principal Investigator:

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Investigator Assurance:
By submitting this protocol, I acknowledge that this project will be conducted in strict accordance with the procedures described. I will not make any modifications to this protocol without prior approval by the IRB. Should any unanticipated problems involving risk to subjects occur during this project, including breaches of guaranteed confidentiality or departures from any procedures specified in approved study documents, I will report such events to the Chair, Institutional Review Board immediately.

1. **Is this project externally funded?** X YES □ NO

National Institute on Aging (K01-AG054731)

2. Research Site(s)

X University of Delaware
□ Other (please list external study sites)

3. **Project Staff**
Please list all personnel, including students, who will be working with human subjects on this protocol (insert additional rows as needed):

[REDACTED]

4. **Special Populations**
Research with any other vulnerable population (e.g. cognitively impaired, economically disadvantaged, etc.)?

The subjects in this study must have cognitive function scores that are consistent with amnestic mild cognitive impairment (aMCI), which is defined by a lack of dementia and mild impairment in memory with or without impairments to other domains of cognitive function. Given the mild impairment of the prospective subjects, a legally authorized representative (LAR) is not required to obtain informed consent.

The decisional capacity of study subjects and their ability to provide informed consent on their own will be evaluated at the time of initial consent and confirmed at each visit to ensure continued ability to provide consent. The following questions will be used to assess whether or not subjects can consent for themselves:

1. Describe in your own words the purpose of this study?
2. What are you being asked to do today?
3. What are the side effects/risks of being in the study?
4. Is this voluntary?
5. What do you do if you have questions or possible side effects?

Individuals who cannot answer these questions will be asked to take MMSE test of dementia (described below) before being allowed to continue in the study. Any subject who lacks the ability to provide consent and/or is determined to have developed dementia at any point during the study will be withdrawn from the study and will be referred to their Primary Care Practitioner (PCP).

5. **RESEARCH ABSTRACT** Please provide a brief description in LAY language (understandable to an 8th grade student) of the aims of this project.

Increased blood pressure and stiffening of the large elastic arteries (i.e., the aorta and carotid arteries) occurs with aging and likely contributes to memory loss and risk of Alzheimer's disease (AD) by impacting blood vessels within the brain[1–4] . Healthy lifestyle strategies, including regular calorie restriction (CR), have been shown to improve blood vessel function in the brain and slow the progression of AD[5]; however, long-term adherence to CR is very difficult and may lead to unsafe weight loss in normal weight older adults[6]. The purpose of this study is to determine whether a dietary supplement that mimics the beneficial effects of CR (nicotinamide riboside; "NR") restores memory and other cognitive functions (aim 1), reduces arterial stiffness and blood pressure and improves arterial function within the brain (aim 2), and improves neurovascular coupling and neuronal activation (aim 3) in patients with "amnestic" mild cognitive impairment (aMCI), a condition associated with impaired memory increased risk for AD. To accomplish these aims, we will conduct a randomized, double-blind, placebo-controlled clinical trial of NR supplementation vs. placebo. This project has the potential to identify a novel, safe and cost-effective strategy for decreasing age-related memory loss and will help us understand how impaired blood vessel function may contribute to risk for AD.

6. **PROCEDURES**

Study Design. A randomized, placebo-controlled, double blind, parallel group clinical trial will be conducted to assess the efficacy of 12-weeks of oral nicotinamide riboside capsules (NR; 500mg, 2x/day, trade name NIAGEN®, ChromaDex Inc., Irvine CA) for improving memory and other cognitive functions in adults with cognitive scores indicative of aMCI. Secondary outcomes will include measures of vascular/cerebrovascular and neuronal function and brain structure. This design and duration has been used previously to study the efficacy of interventions for improving vascular function in older adults[7]. Study participants and investigators involved in data acquisition and/or analysis will be blinded to treatment condition. All primary and secondary outcome measures will be made at baseline and after 12 weeks of treatment. Visits will take place in the Neurovascular Aging Laboratory (NOVA Lab) on the STAR Research Campus at the University of Delaware, with the exception of MRI measures which will be made at the UD Center for Biomedical Brain Imaging (CBBI). Details pertaining to the safety and toxicity of the intervention and rationale for the proposed frequency and duration are presented at the end of this section.

DETAILED OVERVIEW OF STUDY VISITS

PRE-SCREENING PHONE SCREENING

Prior to scheduling Visit 1 informed consent & screening, a member of the research team will complete a brief phone screen and will ask the subject the COVID-19 questionnaire in order to ascertain specific information regarding possible hospitalization as a result of COVID-19 diagnoses, and vaccination. If subject acknowledges any hospitalization as a result of COVID-19, the subject will be excluded and will not be permitted to enroll in the current study. If the subject discloses specific information as it relates to receiving the COVID-19 vaccine, the subject will not be permitted to participate in any data collection procedures until one week following any COVID-19 vaccination. These questions will also be asked during the week 10 phone check-in before scheduling week 12 visits.

VISIT 1: INFORMED CONSENT & SCREENING (2.0 hours)

Subjects will be instructed to fast for ≥ 12 hours before testing

- **Informed Consent** for participation in the clinical trial will be obtained by a trained member of the

Brief Overview of Study Visits

	Week	Visit #	Duration	Location	Visit Description	Blood
Screening	Week -1	*Screening for MCI will occur under a separate protocol and eligible participants will be brought in for informed consent and study-specific screening*				
		Visit 1	2 hours	Virtual*	Informed Consent & General Screening	
		Visit 1.1	30 minutes	NOVA Lab or LabCorp	Screening Blood Draw	74 mL (~5.0 TBL)
		Randomization *Group A: NR or Group B: Placebo*				
Baseline Testing	Week 0	Visit 2	3 hours	NOVA Lab	Vascular Testing	---
		Visit 3	1.5 hours	Virtual*	Cognitive Testing	---
		Visit 4	2 hours	CBBI	Neuronal Testing & Receive Capsules	---
Intervention Period	Week 2	*10 min Phone Check-In*				---
	Week 4	Visit 5	0.5 hours	Virtual*	Zoom Check-In	---
	Week 6	*10 min Phone Check-In*				---
	Week 8	Visit 6	0.5 hours	Virtual*	Zoom Check-In	---
	Week 10	*10 min Phone Check-In*				---
Follow-Up Testing	Week 12	Visit 7	3 hours	NOVA Lab	Vascular Testing	60 mL (~4.0 TBL)
		Visit 8	1.5 hours	Virtual*	Cognitive Testing	---
		Visit 9	2 hours	CBBI	Neuronal Testing	---
	Total Time		**16 hours**		**Total Blood**	134 mL (~9.0 TBL)

** Virtual visits may be completed in person at the Neurovascular Aging Laboratory if the participant does not have access to a computer with video conferencing capabilities or upon the participant's request.*

research team. All procedures and aspects related to the study including any potential risks will be

described to the subject. The investigator will read the informed consent form to the subject and will answer any questions that the subject may have prior to obtaining the subject's written consent. As documentation of the informed consent, the subject and the investigator performing the consent will sign the consent document and a copy of the signed consent form will be given to the subject. Alternatively, the consent may also be performed online via a private video conference and using the eConsent framework in REDCap. The video conferencing software is HIPAA-compliant and each scheduled online meeting will be secured with private meeting access controls, password protection, and end-to-end encryption so data in transit cannot be intercepted. The ability to provide informed consent will be monitored by the research team throughout the trial. In addition to obtaining written informed consent, all experimental procedures will be re-explained to the subjects at the time that they are performed, and verbal consent will be obtained.

- **Family history questionnaire**: A family history questionnaire will be used to obtain information regarding family history of cardiovascular diseases, cancer, diabetes, and Alzheimer's disease in order to determine if family history may account for any differences in the efficacy of NR supplementation in improving cerebrovascular, cognitive and/or neuronal functions. This questionnaire can be completed in-person or online via REDCap.
- **Patient-Reported Outcomes (PROMIS) Measures.** Subjects will answer a brief battery of self-guided questionnaires (administered using an iPad, or online via REDCap) to determine their own perception of their health and well-being. Outcome measures will include: fatigue, pain, physical function, sleep disturbance, anxiety, depression, alcohol use, and social abilities.
- **Menstrual History Questionnaire.** A menstrual history questionnaire will be administered to all female subjects to confirm postmenopausal status. This questionnaire can be completed in-person or online via REDCap.
- **Medical History.** We will ask subjects about their medical history to ensure that subjects meet the inclusion criteria and do not have an underlying condition that would disqualify them based on stated exclusion criteria. This can be completed in-person or online via REDCap. This information will be shared with the NMPCC Nurse Practitioner.
- **COVID-19 Questionnaire.** We will ask subjects about their history with COVID-19, if they were ever hospitalized, experienced symptoms, and details regarding vaccination.
- **Blood pressure and resting heart rate.** Blood pressure will be measured from the non-dominant arm in the seated position after at least 10 minutes of quiet rest using a semi-automated blood pressure device. Repeat measurements will be made (with 2 minutes of quiet rest between recordings) until 3 blood pressure values obtained are within 5 mmHg of one another. These values will be averaged to determine resting systolic and diastolic blood pressure and pulse pressure.

VISIT 1.1: Screening Blood Draw (0.5 hours)

- **Baseline Blood Sampling.** A total of 74 mL (5.0 tablespoons) of blood will be drawn by the research nurse or certified research team member. Some of the blood will be sent to LabCorp for analysis of clinical parameters and the rest will be processed in our own laboratory for determination of APOE4 genotype and isolation of peripheral blood mononuclear cells (PBMC's) (see below). All clinical parameters from LabCorp will be reviewed by a Nurse Practitioner at the NMPCC along with the subject's medical history form. Alternatively, if participants choose to complete all screening remotely, the option is available to the participant to have baseline blood work and urine collected at local LabCorp patient service centers using our research requisition form. This will be billed directly to the lab and will not go through the subject's insurance or cost anything out of pocket. In the event of a clinically significant abnormality, as determined by the Nurse Practitioner, the NMPCC will refer the subject to their PCP for follow-up and that participant will be excluded from the research study. Participants will sign a HIPAA authorization in the informed consent form that includes permission for sharing of patient protected health information with the research team. Blood samples will be

obtained to assess safety to the intervention and as control measures for primary outcomes. If enough blood cannot be obtained during a blood draw visit, the participant may be asked to repeat the blood draw on a separate day (e.g., due to missed stick, insufficient volume, or laboratory error). If a subject is accepted into the study, these clinical labs will also serve as their baseline sample. Screening markers will consist of:

- **APOE Genotype (Visit 1 only).** Individuals with the E4 variant of the Apolipoprotein E (APOE) gene are at increased risk of developing late-onset Alzheimer's disease (AD)[8]. Because the APOE gene is also involved in the regulation of lipid metabolism, specific variants of this gene have been associated with increased risk for cardiovascular diseases. To ensure that APOE genotype does not confound any of our primary cardiovascular outcomes, and to ensure that subjects carrying the E4 variant are evenly distributed between the active and placebo arm of the study, we will isolate each subject's DNA and will determine their APOE genotype using PCR, prior to randomization. The results of this test will be made available to subjects, upon request, with a disclaimer that the results were generated in a non CLIA-certified laboratory for research purposes only.
- **General blood chemistry.** Includes a comprehensive metabolic profile, lipid profile, complete blood count (CBC) with differential, prothrombin time and partial thromboplastin time.
- **Kidney and liver function.** Because kidney and liver dysfunction could influence the metabolism and bioavailability of NR, markers of liver function (ALB, ALT, AST, ALP, total bilirubin (TBILI) and kidney function (blood urea nitrogen (BUN), creatinine (CRE), sodium, and potassium) will be measured prior to subject randomization.
- **Vitamin B6, B12 and Folate.** Known to influence memory.
- **Peripheral Blood Mononuclear Cells.** Required for assessment of NAD^+ related metabolites and mitochondrial respiration.
- **COVID Antibody.** Because recent COVID-19 infection has been associated with increased incidence of stroke and other cardiovascular complications, we will test for the presence of COVID-19 antibodies at baseline and at the end of the study and will perform a sensitivity analysis at the end of the study to determine the potential confounding effect of previous COVID-19 infection on all study outcomes. This test will only be performed in individuals not vaccinated against the SARS-CoV2 virus.

- **Baseline Urine Sampling.** A midstream urine specimen will be collected into a clear container during the baseline laboratory visit, or at their local LabCorp patient service center and a complete urinalysis will be conducted. Urine samples will be collected after the early morning void has been discarded. Urinalysis will be repeated after the 12-week intervention period.
- **Middle Cerebral Artery Screening:** A research team member will use a transcranial doppler ultrasound probe to examine the temporal window for screening of a visible middle cerebral artery. This is done during the screening visit to simplify the preparation of Visits 2 & 7. The results of this screening test will not impact eligibility for this study.

RANDOMIZATION ***(will occur after VISIT 1.1)***

- **Randomization Procedures.** Subjects who qualify for the study based on screening assessments will be randomly assigned by the study coordinator to receive either treatment A or treatment B. The identity of each treatment (i.e., NR or placebo) will be blinded by the manufacturer (ChromaDex, Inc.) and the blinding code will be sent to a member of the PI's department (Megan Wenner) who is unaffiliated with this study. Dr. Wenner will enter the blinding code in a secure REDCap form that will remain hidden from the rest of the study team. The PI will be given a separate login ID and password to access this REDCap form and determine the assigned treatment condition of a subject in the event of an emergency (described below under serious adverse events). A blocked randomization scheme will be uploaded into REDCap by the biostatistician with block size (*b)* resulting in an imbalance of no more than 2 subjects (*b*/2) between group assignments. To address potential imbalance between key biological variables including sex (male vs. female) and APOEε4 status (carriers vs. non-carriers),

the blocked randomization scheme will be stratified such that participants within each strata will be block randomized to either placebo or NR.

Study Restrictions. All experimental visits will be conducted under overnight fasted conditions (no meal within ~12 hours of testing) with the exception of cognitive function and MRI sessions. Participants will be asked to discontinue use of any vitamin B_3-containing supplements (i.e., nicotinamide, niacin, nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide) 2 weeks prior to and throughout participation in the study. Subjects will be asked to refrain from non-prescription medications for 48 hours; alcohol and exercise for 24 hours, caffeine for 12 hours prior to all study visits as these are factors known to modulate vascular function as well as circulating biomarkers of interest. If subjects receive the COVID-19 vaccine, they will not perform any experimental visit until at least one week following vaccination.

PRIMARY & SECONDARY OUTCOMES

All experimental visits will be performed in the PI's Laboratory unless otherwise indicated. An optional snack will be offered following all experimental visits in which fasting is required.

VISIT 2 & 7: VASCULAR FUNCTION (NOVA Lab; 3 hours each)

Subjects will be instructed to refrain from taking over the counter medications for 48 hours, vigorous aerobic exercise and alcohol for 24 hours, and to fast (no food, supplements or caffeine) for ≥12 hours before testing.

- **Height and body mass**: Subject height and body mass will be measured using a physician's scale.
- **Arterial Stiffness Assessments**
    - **Pulse Wave Velocity (PWV).** Carotid-to-femoral PWV (a measure of aortic stiffness) will be assessed non-invasively using sequential transcutaneous tonometry of the carotid and femoral arteries (SphygmoCor, AtCor Medical). The time delay (transit time) between the foot of pressure waves obtained at each location will be determined using the R-wave of an ECG recording taken during the tonometry procedure as a timing reference. PWV will be calculated as the distance between measurement sites divided by the transit time of the arterial pulse wave.
    - **Pulse Wave Analysis (PWA).** Central hemodynamic properties, including the central pulse pressure (PP), augmentation pressure (AP) and augmentation index (AI) will be derived from the radial artery pulse wave using applanation tonometry.
    - **Carotid Artery Compliance.** Carotid compliance (a measure of large artery distensibility) will be measured non-invasively using high-resolution ultrasonography of one carotid artery and applanation tonometry of the opposite carotid artery (SphygmoCor, AtCor Medical). Compliance will be calculated as the change in carotid diameter for a given change in pressure as described previously by our laboratory.

- **Cerebrovascular Function Assessments**
    - **Cerebrovascular Reactivity** will be measured by assessing the change (from baseline) in mean blood flow velocity (MFV) of the middle cerebral artery (ΔMFV_{MCA}) in response to increases or decreases in the end-tidal (end-expiratory) partial pressure of carbon dioxide ($P_{ET}CO_2$), which evokes a vasodilatory or vasoconstriction response in the cerebral arteries, respectively. MFV will be measured using a 2-MHz Transcranial Doppler (TCD) ultrasound probe positioned over the temporal window as previously described in the literature[9,10]. The control of $P_{ET}CO_2$ will be achieved using computer assisted gas blender (RespirAct, Thornhill Medical) that automatically adjusts the composition and flow rate of inspired O_2 and CO_2 in order to manipulate $P_{ET}CO_2$ independent of a subject's ventilation and breathing pattern. Following a baseline recording at normocapnia (simulated room air) at the subjects preferred breathing rate, a second baseline will be recorded at a controlled breathing rate of 20 breaths/minute (to improve $P_{ET}CO_2$ targeting). The vasodilatory stimulus will consist of 3-minutes of hypercapnia in which $P_{ET}CO_2$ is abruptly increased to +9mmHg above the

subject's baseline. Subjects will then return to their baseline $P_{ET}CO_2$ for 2 minutes, followed by a -9mmHg decrease in $P_{ET}CO_2$ to initiate vasoconstriction. Subjects will maintain the paced breathing pattern throughout the protocol. End-tidal partial pressure of oxygen will be held constant throughout the protocol. Breath-by-breath end-tidal CO_2 and O_2 ($ETCO_2$) will be continuously monitored to confirm successful end-tidal targeting and beat-by-beat arterial blood pressure will be continuously recorded using a Finapres (Finapres Medical Systems), to control for changes in mean arterial pressure (MAP), which may influence cerebral perfusion. This test will only be performed on individuals with a suitable temporal window, as determined during screening.

- **Pulsatility Index (PI).** Pulsatile blood flow velocity within the cerebral arteries is positively associated with large elastic artery stiffness and implies a reduction in the ability of the cerebral arteries to buffer the pressure delivered to the brain through the carotid arteries. The Gosling Pulsatility Index (PI), will be calculated from the baseline cerebral blood flow velocity (obtained during the above-described protocol) as follows: $PI = (MCAv_{(systolic)} - MCAv_{(diastolic)})/MFV_{MCA}$.
- **Total Cerebral Blood Flow** (CBF) will be assessed using extracranial high-resolution duplex ultrasound to capture both diameter and time-averaged MFV of the bilateral internal carotid arteries (ICA) and vertebral (VA) arteries (i.e., the two primary arteries that supply the brain)[11–13]. CBF will be calculated for each artery as follows: $CBF = MFV \times \pi(diameter/2)^2$ and summed in order to estimate total cerebral blood flow[12].
- **Neurovascular Coupling** will be assessed by measuring the ΔMFV_{MCA} and ΔMFV_{PCA} from baseline in response to the progressively difficult N-back mental task of executive function and a repetitive visual stimulus[14–16]. MFV_{MCA} and ΔMFV_{PCA} will be collected using TCD ultrasonography as described above. The N-back Cognitive Test measures working memory and executive function and activates areas of the brain supplied by the MCA. A computer program will display a series of numbers and the subject will be required to respond by clicking a button when the current number is identical to the same number N-trials previously. A control (0-back) level and two N-back levels will be performed. Numbers will be displayed with an inter-stimulus duration of one second with a 10-second pause between each level. Number sequence will vary between N-back conditions. The number of correct responses will be recorded to control for performance on the cognitive task itself. The repetitive visual stimulus activates the areas of the brain supplied by the PCA. A computer program will display a visual stimulus, as previously described. Briefly, the subject will be asked to focus their eyes on a screen displaying an alternating black and white checkerboard stimulus for varied amounts of time (~40s each stimulus). The subject will be required to open and close their eyes when prompted by the research team.
- **Post-testing blood sampling and urinalysis (visit 7 only).** A urine sample and 4.0 Tablespoons of blood will be collected to repeat baseline blood measures (except for APOE genotype and B vitamins, which only need to be performed once).

VISITS 3 & 8: COGNITIVE FUNCTION (Virtual; 1.5 hour each)

Subjects will be instructed to refrain from taking over the counter medications for 48 hours, vigorous aerobic exercise and alcohol for 24 hours before testing. Subjects will be encouraged to eat a light meal or snack before coming to the lab.

Cognitive function assessments can be performed in-person during visit 3 and visit 8, or alternatively can be completed virtually through REDCap with research staff. If participants choose to complete cognitive testing through virtual means, visit 3 and visit 8 will no longer be needed.

Patient-Reported Outcomes (PROMIS) Measures (Visit 12). Subjects will answer a brief battery of self-guided questionnaires (administered using an iPad, or online via REDCap) to determine their own perception of their health and well-being. Outcome measures will include: fatigue, pain, physical function, sleep disturbance, anxiety, depression, alcohol use, and social abilities.

Memory function will be assessed by performance on the **California Verbal Learning Test III (CVLT III)** and the **Wechsler Logical Memory and Visual Reproduction Tests.** Additional domains of cognitive function will be surveyed using the **NIH Toolbox** computerized tests to evaluate 1) attention, 2) episodic memory, 3) working memory, 4) language, 5) executive function, and 6) processing speed.

- **CVLT III** - The subject will hear a list of 16 nouns in a fixed order over 5 learning trials. After each trial, the subject will be asked to recall as many words as they can in any order. Following the learning trials, the subject must recall as many words on the list as they can immediately (short-delay) and following a 20-minute wait (delayed recall). A second task involving recognition of target words from a list of 44-words read by the investigator is completed at the end of the test. This test can be performed in-person or virtually through REDCap and virtual screen sharing.

- **Wechsler Logical Memory Test (I and II).** This test assesses logical memory function. The subject will listen to the examiner read two stories and will be asked to repeat as many details of the stories as they can remember. An immediate recall and delayed (20 minute) recall component will be used. Scores will be based on accuracy in recalling elements of the story. This test can be performed in-person or virtually through REDCap and virtual screen sharing.

VISITS 4 & 9: NEURONAL FUNCTION (CBBI; 2 hours each)

Subjects will be instructed to refrain from taking over the counter medications for 48 hours, vigorous aerobic exercise and alcohol for 24 hours before testing. Subjects will be encouraged to eat a light meal or snack before coming to the lab.

Repeat MRI Screening Form. To confirm ability to safely participate in the MR scan.

To gain initial insight into the possible central neural mechanisms mediating improvements in cognitive function with NR supplementation in older adults, we will use exploratory fMRI assessments of global and regional brain structure and task-induced neural activation patterns. As there is no preliminary evidence suggesting a change in activation patterns following NR supplementation in older adults, all fMRI-based outcomes are considered exploratory in nature and are not included as a primary/secondary outcome.

Table 2

Outcome type	Task	Assessment
Structure	--	Grey matter volume
		White matter volume
		White matter hyper-intensities
Resting state	--	Functional brain connectivity
Blood flow		Arterial spin labeling
Cognitive activation	N-Back	Executive function
		Working memory

- The scanning portion of the study will take place at the UD Center for Biomedical Brain Imaging (CBBI). The MRI device for these scans is FDA approved for research with human subjects and has all the safety inherent in a clinical MRI scanner. The radio frequency fields conform to guidelines determined by the FDA and the FDA has designated MRI scanners to be a non-significant risk device. MR techniques non-invasively produce images and measurements from tissues in the intact, living human.

- Prior to going into the MRI scanner, the MRI technologist on duty will ask participants to remove all jewelry and metal objects from their pockets. Participants will be required to change into scrubs to prevent any possible risk from metallic objects or decorations in their clothing.

- In an MRI scan the subject will lay down on a table and will be placed into a long donut-shaped magnet. A specially designed coil will be placed around the head to provide better images (as is done with standard clinical examinations). As the MRI scan is performed, the subject will hear loud rapping and knocking noises that are normal for a MRI scan.

- The protocol will consist of an 8-min high-resolution structural scan to determine brain anatomy, a 6-min scan of resting state activity to examine age-dependent functional brain connectivity, measurement of resting brain blood flow using arterial spin labeling (ASL) and a 10 minute cognitive

task sensitive to working memory and executive function (N-back). This test is sensitive to age-related deficits in cognitive functions and to intervention. Subjects will follow instructions on a screen in front of them in the scanner and use a clicker to indicate their answer. Further, their vitals (heart rate and respiration rate) will be monitored via a respiration band that they will wear around their chest.

- ***fMRI Data Acquisition and Analysis*.** Images will be acquired on a research-dedicated whole-body 3-Tesla Siemens Magnetom Prisma whole-body MRI scanner equipped with 64 channel head coil. Functional imaging will use T2*-weighted gradient-echo, echo-planar imaging (repetition time [TR] = 1900 ms, echo time [TE] = 25 ms, flip angle = 69°, 39 slices parallel to the AC-PC line, thickness = 3 mm, gap = 1 mm, 64 × 64 in-plane resolution, in-plane FOV = 22 cm). High-resolution anatomical images, which take 6 min to obtain, will be acquired with a T1-weighted 3D magnetization prepared rapid gradient multi-echo sequence (MPRAGE; sagittal plane; TR = 2530 ms; TE = 1.64 ms, 3.5 ms, 5.36 ms, 7.22 ms, 9.08 ms; GRAPPA parallel imaging factor 2; FOV = 256mm; flip angle = 7°; 192 interleaved 1mm slices) covering the whole brain. Note that through pilot development, our sequence may change slightly if a different combination of parameters is deemed more optimal. Tools within the FSL framework will be used for data pre-processing and analysis, as well as tools within SPM, where appropriate (e.g., for resting state analyses). At baseline and after treatment: regional grey matter normalized whole brain volume and white matter hyper-intensities will be assessed. Multivariate linear modeling of BOLD fMRI imaging data will be used to identify functional neural network expression during the resting state and working memory and executive function tasks. Whole brain analyses will be performed to assess connectivity and regions of interest specific to each cognitive task. Relations between structures, resting state and functional neural network expression during each task will be assessed. Associations among function, structure and behavioral performance will be determined using a network analysis.

- **Wechsler Visual Reproduction Test (I and II).** This test assesses visuoconstructional and visual memory function. The subject will be presented with a series of complex line drawing and will be asked first to copy the drawing onto a piece of paper, then re-draw the picture from memory following a 20-minute delay. Drawings will be scored for accuracy and correct placement of picture elements. This test must be completed in-person, and will be administered during Neuronal Function visits #4 and #9.

- **NIH Toolbox (**This test must be completed in-person, and will be administered during Neuronal Function visits #4 and #9).

    - **Attention**. Attention will be measured by the NIH Toolbox Inhibitory Control and Attention Test. The Flanker task measures both a participant's attention and inhibitory control. The test requires the participant to focus on a given stimulus while inhibiting attention to stimuli (fish for ages 3-7 or arrows for ages 8-85) flanking it. Sometimes the middle stimulus is pointing in the same direction as the "flankers" (congruent) and sometimes in the opposite direction (incongruent). Scoring is based on a combination of accuracy and reaction time.

    - **Episodic Memory**. Episodic Memory refers to cognitive processes involved in the acquisition, storage and retrieval of new information and will be measured with the NIH Toolbox Picture Sequence Memory Test. The Picture Sequence Memory Test is a measure developed for the assessment of episodic memory. It involves recalling increasingly lengthy series of illustrated objects and activities that are presented in a particular order on the computer screen. The participants are asked to recall the sequence of pictures that is demonstrated over two learning trials; sequence length varies from 6-18 pictures, depending on age. Participants are given credit for each adjacent pair of pictures (i.e., if pictures in locations 7 and 8 and placed in that order and adjacent to each other anywhere – such as slots 1 and 2 – one point is awarded) they correctly place, up to the maximum value for the sequence, which is one less than the sequence length (if there are 18 pictures in the sequence, the maximum score is 17, because that is the number of adjacent pairs of pictures that exist).

    - **Working Memory**. Working Memory refers to a limited-capacity storage buffer that becomes

overloaded when the amount of information exceeds capacity and is measured by the NIH Toolbox List Sorting Working Memory Test. This test requires immediate recall and sequencing of different visually and orally presented stimuli. Pictures of different foods and animals are displayed with accompanying audio recording and written text (e.g., “elephant”), and the participant is asked to say the items back in size order from smallest to largest, first within a single dimension (either animals or foods, called 1-List) and then on 2 dimensions (foods, then animals, called 2-List). The score is equal to the number of items recalled and sequenced correctly.

- **Processing Speed**. Processing Speed is either the amount of time it takes to process a set amount of information, or, the amount of information that can be processed within a certain unit of time. It is a measure that reflects mental efficiency, and will be measured with the NIH Toolbox Pattern Comparison Processing Speed Test. This test measures speed of processing by asking participants to discern whether two side-by-side pictures are the same or not. Participants’ raw score is the number of items correct in a 90-second period. The items are designed to be simple to most purely measure processing speed.
- **Executive Function.** Executive Function is the capacity to plan, organize, and monitor the execution of behaviors that are strategically directed in a goal-oriented manner and is measured by the NIH Toolbox Dimensional Change Card Sort Test (DCCS). DCCS is a measure of cognitive flexibility. Two target pictures are presented that vary along two dimensions (e.g., shape and color). Participants are asked to match a series of bivalent test pictures (e.g., yellow balls and blue trucks) to the target pictures, first according to one dimension (e.g., color) and then, after a number of trials, according to the other dimension (e.g., shape). “Switch” trials are also employed, in which the participant must change the dimension being matched. For example, after 4 straight trials matching on shape, the participant may be asked to match on color on the next trial and then go back to shape, thus requiring the cognitive flexibility to quickly choose the correct stimulus. Scoring is based on a combination of accuracy and reaction time.
- **Language.** NIH Toolbox focuses on two aspects of language: vocabulary knowledge, which is fundamental to the growth of knowledge and which also has a very high association with overall intelligence, or what has been called the "g factor”, and oral reading skill, which reflects level and quality of prior educational experiences, and provides a fairly robust indication of verbal intelligence that is relatively undisturbed by many medical conditions that affect the brain. Vocabulary language will be measured by the NIH Toolbox Picture Vocabulary Test while oral reading will be measured by the NIH Toolbox Oral Reading Recognition Test.
  - The NIH Toolbox Picture Vocabulary Test measures receptive vocabulary and is administered in a computerized adaptive format. The respondent is presented with an audio recording of a word and four photographic images on the computer screen and is asked to select the picture that most closely matches the meaning of the word.
  - During NIH Toolbox Oral Reading Recognition Test the participant is asked to read and pronounce letters and words as accurately as possible. The test administrator scores them as right or wrong. For the youngest children, the initial items require them to identify letters (as opposed to symbols) and to identify a specific letter in an array of 4 symbols. The test is given in a computerized adaptive format.

Visit 4: START Intervention (30 min; NOVA Lab)

- Receive 12-week supply of study pills (intervention instructions provided by member of research team)

VISITS 5 & 6: Bi-Weekly CHECK-IN Visits (Virtual or NOVA Lab*; 30 minutes)

- **Consult (bi-weekly).** Brief check-in/consult with study coordinator (physical symptom survey);

- **Patient-Reported Outcomes (PROMIS) Measures (monthly).** Subjects will answer a brief battery of self-guided questionnaires (administered using an iPad, or online via REDCap) to determine their own perception of their health and well-being. Outcome measures will include: fatigue, pain, physical function, sleep disturbance, anxiety, depression, alcohol use, and social abilities. Subjects who cannot complete PROMIS measures online will report to the lab on a monthly basis to complete this measure**.**

OVERVIEW OF INTERVENTION (*see attached Investigator's Brochure and Appendix for additional data*)

Nicotinamide riboside (NR) is a safe, naturally occurring vitamin B_3 derivative found in yeast, bacteria, and mammalian tissues. Additionally, NR has been detected in cows' milk[17]. Each NR capsules contain 250 mg of nicotinamide riboside chloride mixed with microcrystalline cellulose. The appearance is a blue powder within a blue vegetarian capsule. NR has been generally recognized as safe by the FDA (GRAS No. 635) and has been developed as a commercially available **dietary ingredient** for human consumption under the trade name NIAGEN® (ChromaDex, Inc. Irvine, CA). No serious adverse events have been reported in humans taking NR. A summary of the currently available safety and toxicity data are presented below.

Summary of NR safety & toxicity in preclinical models and clinical trials to date:

- NR is non-mutagenic in bacteria at doses of 50-5000µg in a reverse mutagenicity (Ames) assay, and non-cytotoxic in chromosomal aberration studies in human peripheral blood lymphocytes treated with 1.25 – 5 mg/ml NR. Results of an *in vivo* micronucleus assay revealed no cytotoxicity or chromosomal damage in bone marrow-derived erythrocytes obtained from Sprague-Dawley rats at 24-48 hours post ingestion of up to 2,000 mg/kg NR. Using the FDA recommended surface area conversion factor, this is equivalent to a dose of ~19,200 mg/day for an average 60kg human and is **~20-fold higher than the dose for the current study (500 mg, 2x/day)**[18]**.**
- No acute toxicity, abnormal pathology, or mortality was observed over 2-weeks following a single dose of 5,000 mg/kg NR in male and female rats. Likewise, no treatment-related mortality was observed during a 90-day oral toxicity study in rats treated with 300, 1000 or 3000 mg/kg/day NR[19]. The lowest observed adverse effect level (LOAEL) reported was 1,000 mg/kg/day[19], which is equivalent to a dose of ~9,600 mg/day for an average 60kg human or **~10-fold higher than the daily dose for this study**[18]**.** Further, a recent study in mice treated with 400mg/kg/day NR for 16 weeks (i.e., ~15% of a mouse's lifespan at a human equivalent dose of ~ 2,000mg/day) reported no adverse increase in mortality rate, end organ damage, or tumor formation[20].
- There is limited evidence in animal models of cancer suggesting treatment with NAD+ boosting compounds could increase cancer cell proliferation and exacerbate cancer progression and metastasis; however, in other studies, NAD+ precursors have been shown to have favorable effects on metabolism that prevent or limit cancer growth[21].
- A recently conducted Phase-1 clinical trial in humans (Trammel et al., Nat Commun. 2016) found that NR was safe for human consumption and reported no serious adverse effects at doses ranging from 100 – 1,000mg[22].
- Data from our own recently published randomized, placebo-controlled crossover pilot study of long-term dosing in 24 healthy older adults[23]. indicate no serious adverse events ***(Table 3)*** associated with the proposed dose (500 mg, 2x/day), and no change in markers of liver or renal function[23].

Table 3. Treatment emergent adverse events from pilot study. Based on N=30 randomized subjects

Adverse Event	Placebo	NR
Headache	4	0
Nausea	0	1
Skin Rash	1	1
Flushing/Hot flashes	2	1
Fainting	1	0
Drowsiness	1	0
Leg cramps	0	1
Increased Bruising	0	1

Data represent number (n) of times AE was reported. Number of subjects reporting AEs (n=7); Number of subjects reporting ≥ 2 AE (n=5).

Rationale for Proposed Treatment Dose, Duration and Frequency

- **Bioavailability.** Because NR is directly taken up by cells and metabolized into NAD^+ and related metabolites, NAD^+ has proven to be undetectable in the plasma.

As such, assessment of the NAD^+ metabolome in peripheral blood mononuclear cells (PBMCs) has emerged as the gold standard method of evaluating NAD^+ bioavailability in response to supplementation with NAD^+ boosting compounds[22,23]. During a recently published Phase I clinical trial with NR in 12 healthy adults (Trammel et al., Nat. Comm. 2016), the concentration of NAD^+ and related metabolites were dose-dependently elevated in PBMCs compared with baseline following a single ingestion of 100, 300, and 1000 mg NR[22].

- **Rationale for Dose and Duration.** For the present study, we have chosen to study a dose of 500mg, 2x/day (i.e., 1,000 mg/day) for 12 weeks. We have chosen to standardize the dose among subjects, rather than administer NR in relation to body mass in order to increase the translational potential of the proposed work, as formulation of a treatment based on body mass is impractical in a clinical setting. The rationale for this dose and treatment duration is based on:
    - Preclinical studies using NR in mice demonstrating efficacy for improving physiological function (e.g., insulin sensitivity, mitochondrial biogenesis, fatty acid oxidation) with doses ranging from 250-400 mg/kg/day, which translate to an equivalent dose in humans of between 1,200 – 2,000 mg/day for an average 60kg adult;
    - Results of a phase I clinical trial demonstrating an increase in NAD^+ and related metabolites at this dose in healthy humans (***Appendix, Figure 1***);
    - The PI's own pilot study (clinicaltrials.gov registry: NCT2921659) demonstrating efficacy of this dose for improving measures of arterial stiffness and blood pressure in healthy (cognitively normal) middle-aged and older adults with a shorter duration than the proposed study**;**
    - At least 2 other clinical trials in healthy or obese individuals using a similar dose **(**NCT02300740, NCT02303483).
- ***Rationale for Frequency of Doses.*** Subjects will be advised to consume NR or placebo pills twice a day (one dose in the morning and one in the evening) in order to maintain elevated circulating NAD+ levels and to lower the risk of potential side effects caused by a larger single dose. The exception to this will be in the morning of testing, in which subjects will not take their morning dose until completion of the testing session in order to avoid any acute effects of the compound on the proposed outcomes. Subjects will be advised to take pills with food in order to a) maximize absorption; b) increase subject adherence by timing pill intake with meals; and c) decrease risk of nausea or GI discomfort.

Description of Placebo Capsules. The placebo capsules contain microcrystalline cellulose within vegetarian capsules. The appearance is a blue powder within a blue capsule.

Investigational New Drug Application. In addition to the details above related to the safety of NR supplementation in humans, we have also received Investigational New Drug (IND) status from the Food and Drug Administration (CDER Division of Neurological Products) for the use of NR in patients with MCI (IND#:135449).

ADMINISTRATION, ADHERENCE AND SUBJECT MONITORING

Intervention Administration. Placebo or NR capsules will be packaged and distributed by ChromaDex Inc. (under trade name, NIAGEN®) in individual containers. 12 weeks' worth of capsules will be administered to subjects by the research team at the beginning of the trial. Subjects will be provided with explicit instructions for dosing and frequency including instructions for missed doses.

Monitoring Adherence (pill count). Subjects will be instructed to bring their used and unused pill containers to the last post visit and, at which time a member of the research team will count the number of pills remaining in the container to assess adherence to the intervention.

Plan for Monitoring Adverse Events (AE's) and Serious Adverse Events (SAE's)

Adverse Event (AE): any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease,

temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.

Adverse Event Recording

The period for recording AE's will begin at the time of informed consent and will end at the time that the participant completes all testing, or is withdrawn from the trial. At each in-person or phone check-in, a blinded member of the study team will seek information from the participant on adverse events by specific questioning using a standardized physical symptom survey. Information on all AE's reported during the study period will be recorded immediately in the case report form (CRF). Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause, as described in the Data and Safety Monitoring Plan (DSMP).

Serious Adverse Event (SAE): any adverse event that:

- Results in death;
- Is life threatening, or places the participant at immediate risk of death from the event as it occurred;
- Requires or prolongs hospitalization;
- Causes persistent or significant disability or incapacity;
- Results in congenital abnormalities or birth defects;
- Is another condition which investigators judge to represent significant hazards.

If a SAE occurs while the participant is on campus for a scheduled visit, the participant will be sent to the Christiana Care Medical Aid Unit ("Urgent Care") on the STAR Campus. If a participant contacts the laboratory or PI prior to seeking treatment for an ongoing AE or SAE, the participant will be directed to seek immediate medical treatment (e.g., visit their primary healthcare provider or, if requiring immediate attention, the nearest Urgent Care facility or Emergency Department). Any participant who is experiencing a life-threatening **medical emergency** will be instructed to call 911.

Reporting of SAE's

All participants experiencing a SAE will be instructed to notify the principal investigator after seeking immediate medical attention. As detailed in the Data and Safety Monitoring Plan (DSMP), all SAE's will be reported to the IRB, DSMB and other regulatory bodies (e.g., FDA, NIH) within 24 hours of the event being reported to the investigator. An expedited report of the SAE can be submitted by telephone, fax, or email and will be followed by a detailed, written SAE report as soon as possible. In the event that a participant calls the PI or another member of the study team prior to receiving treatment for an ongoing SAE, that participant will be instructed to seek immediate medical attention (e.g., visit urgent care).

Unblinding in Response to a SAE

All participants will be given a "Clinical Trial Participant Card" at the time of informed consent, which will provide the dates of their anticipated involvement in the study and important details regarding the active compound under investigation. The card will also provide a QR code to access the clinicaltrials.gov registry for this trial. Participants will be instructed to keep this card with them at all times (e.g., in their wallet) and to provide it to emergency personnel or other healthcare professionals if seeking treatment for an AE or SAE. In the event that emergency personnel or other healthcare professionals responding to a SAE determine that it is ***medically necessary*** to know the treatment to which a participant is randomized, the card will provide the contact information for the PI's office. PI will program their office line to forward all calls to their personal phone outside of normal business hours. In case of an emergency, the PI will be capable of breaking the blind for that participant.

STUDY TIMELINE

Experimental sessions (baseline & follow-up) will be conducted over a 7 day period. The order of these visits may be switched as needed in order to facilitate scheduling. Subjects will continue their treatment (NR or placebo pills) until experimental sessions for each condition are completed; however, subjects will

be instructed to refrain from taking study pills within 24 hours of an experimental session, to avoid any confounding effects of acute NR supplementation. In the event of a conflict (e.g., due to scheduling or illness), experimental sessions may be scheduled ± 7 days from the ideal testing window. We anticipate that the typical time lapse between completion of screening measures and the beginning of the intervention will be no more than 1 week and that the study will take approximately 15 weeks to complete. The study will require 16 hours of the subject's time in the clinical trial.

7. STUDY POPULATION AND RECRUITMENT

Study Population

58 older adult men and women of all races and ethnic backgrounds between the ages of 60-90 years with cognitive scores indicative of mild cognitive impairment that includes impaired memory function (i.e., "amnestic MCI or "aMCI") will serve as subjects for this study. To account for a 25% subject exclusion rate due to dropout/experimental failure, failed screening or conversion to dementia (15 subjects), **a total of 73 patients with aMCI (~20 subjects/year over 4 years) will be recruited for this study in order to meet our enrollment goal.**

Recruitment and Screening

Participants for this study will be recruited through the *"Screening eligibility of older adults to participate in clinical trials related to memory"* study (UD IRB #1318668), which is being conducted by Dr. Christopher Martens and Dr. Curtis Johnson. Participants will be primarily recruited through posted flyers and direct mailings targeted towards older adults with a self-report of memory complaints. Additional subjects will be recruited through the Swank Memory Center for Memory Care and Geriatric Consultation ("Swank Memory Center") in Wilmington, DE in collaboration with co-investigator, James Ellison, M.D. Specific details regarding subject recruitment are described in the screening protocol. Briefly, subjects will undergo a brief modified telephone pre-screening for cognitive status (TICS-m) and those qualified will undergo a more comprehensive in person cognitive evaluation with a research nurse. Individuals with memory scores indicative of amnestic MCI that express interest in participating in this study will be recruited for enrollment into this clinical trial by the study coordinator. Additionally, this study will be listed on the Alzheimer's Prevention Registry.

Final eligibility will be determined by the PI based on the inclusion/exclusion criteria stated below:

Inclusion Criteria

- Cognitive function scores consistent with amnestic mild cognitive impairment based on pre-screening evaluation;
- age 60-90 years;
- MMSE score >21 at time of initial consent;

Exclusion Criteria

- blood chemistries indicative of abnormal renal, liver, thyroid and adrenal function; estimated glomerular filtration rate using the MDRD prediction equation must be ≥30 ml/min/1.73 m^2;
- any clinically significant abnormal blood chemistry values as determined by the NMPCC nurse practitioner;
- major psychiatric disorder (e.g. schizophrenia, bipolar disorder, major depression within past two years);

- neurological or autoimmune conditions affecting cognition (e.g. Parkinson's disease, epilepsy, multiple sclerosis, mild or severe traumatic brain injury, large vessel infarct);
- concussion within last 2 years and ≥ 3 lifetime concussions;
- current systemic medical illnesses, not including diabetes (e.g. cardiovascular disease, cancer, renal failure);
- prior history of any type of cancer other than basal cell carcinoma;
- substance abuse or dependence (DSM-V criteria);
- current use of medications used to treat dementia (e.g., anticholinesterase drugs) or other drugs likely to affect cognition (e.g., anticholinergic drugs, long-acting benzodiazepines);
- claustrophobia, metal implants, pacemaker or other factors affecting feasibility and/or safety of MRI scanning*;
- current smoking (including marijuana) within the past 3 months;
- hospitalization as a result of COVID-19.
- History of heart palpitations or arrhythmias

* In order to protect the health and safety of the participants, exclusion criteria for this study will include any contraindication to magnetic resonance scanning (e.g., metal in body, claustrophobia, pregnant). These exclusions are specific to MRI and are consistent with most studies involving MRI. Potential participants will be screened for the presence of any of these exclusion criteria prior to participating in this MRI study and before each MRI visit by having subjects complete an MRI screening form.
Describe what (if any) conditions will result in PI termination of subject participation.

The primary exit criteria will include:

- completion of the study;
- failure to comply with the requirements of the study;
- lack of ability to continue to provide consent during the study;
- development of dementia during the study;
- treatment for impaired cognitive function by PCP;
- change in medication status that my affect primary outcomes (as determined by the PI);
- any serious adverse event that results in unblinding, prolonged disruption of the intervention or is deemed unsafe for the participant to continue in the trial.

Other exit criteria will include cardiovascular or metabolic disease-related events (e.g., myocardial ischemia/angina, myocardial infarction, stroke, congestive heart failure, hyperglycemia, etc.), major surgery and other serious changes in health status. Subjects will also have the option of withdrawing from the study at any time for any reason. The number of subjects exiting the study and the reasons for exit will be carefully documented. Subjects who withdraw from the study will be replaced in order to achieve the sample size required for appropriate statistical power.

8. **RISKS AND BENEFITS**

List all potential physical, psychological, social, financial or legal risks to subjects (risks listed here should be included on the consent form).

- Nicotinamide Riboside (NR) and Placebo Capsules - Subjects will either be given nicotinamide riboside (NR) capsules (Niagen®; 500 mg 2x/day) to raise blood levels of NAD+, or placebo capsules that contain inactive ingredients (microcrystalline cellulose within vegetarian capsule). Potential side effects associated with NR may include mild-to-moderate cases of headache, feelings of warmth, hot flushing sensations, gastrointestinal discomfort, and fatigue. There are currently no reports of serious adverse side-effects to NR supplementation; however, all adverse events will be closely monitored. To lessen the risk of side effects, subjects will take capsules twice a day instead of all at once. If they miss a dose, subjects will be instructed not to double the number of capsules that they take and to just take the next dose at the regularly scheduled time. If subjects are allergic to or have had an adverse event after taking one of the above-mentioned drugs or supplements, they will be asked to notify the investigator before participating.

Per Niacin's FDA label, cardiac arrhythmias and palpitations have been reported during Niacin use. Niacin is a closely related compound to nicotinamide riboside. There are ongoing clinical trials using the current compound, nicotinamide riboside, as an intervention for preventing atrial fibrillation in people with heart failure; thus, the actual risks of cardiac arrhythmias with NR are unknown.

- Blood Draw - When the needle goes into a vein, it hurts for a short time and there may be redness or swelling around where the needle goes into the skin. There is a small chance you may feel lightheaded or faint. In about 1 in 10 cases, a small amount of bleeding under the skin will cause a bruise. A risk of a blood clot forming in the vein is about 1 in 100. The risk of infection or significant blood loss is less than 1 in 1,000. The total amount of blood being drawn over the entire study is approximately 9.0 tablespoons (less than 1/3 pint). Subjects should not donate blood 8 weeks before or after taking part in this study.
- Genetic Risk for AD – One of the blood tests will determine whether or not the subject carries a gene that has been associated with risk for Alzheimer's disease (AD). People with this gene have a higher chance of developing AD compared with people who do not carry the gene. Some people who carry the gene may never develop AD. Others may develop AD even though they do not carry the gene. Subjects will be provided with the results of this test upon request only.
- Cerebrovascular Function - There are no risks associated with breathing slightly higher or lower amounts of carbon dioxide. The use of CO_2 to manipulate brain blood flow in humans has been applied clinically and in the research setting for decades without any adverse events. Subjects may feel an increase in heart rate, flushed skin, and minor disorientation or slight metallic taste while breathing the carbon dioxide mixture. A mild headache is also possible. Subjects may experience a dry mouth due to the use of the facemask during the test.
- MRI Scan - MRI is an imaging technique that uses radio waves and magnetic fields to produce images of internal structures in your body. Unlike X-rays, the MRI does not use any ionizing radiation, and it does not use radioactivity, so there is no radiation related risks from having an MRI scan done on you. Below there is a description of MRI related risks and what is being done to reduce any possible risks associated with them:
    - **Metal**: The MRI scanner produces a constant strong magnetic field, which may cause any metal implants, clips, or implanted medical devices within your body to shift position or malfunction. You will not be allowed to participate in this study if you have any implanted metal, clips or devices. You will be screened to make sure that it is safe for you to enter a strong magnetic field. Please provide us with as much information as you can, for example if you had surgery in the past, so that we may decide whether it is safe for you to be a participant. Metallic objects brought into the MRI environment can become hazardous projectiles and can also interfere with the data quality. To minimize this risk, metal earrings, other piercings, necklaces and any other metal in contact with your body must be removed prior to the study. You must also remove all items from your pockets, including coins, electronics (including cell phones and hearing aids) and wallets. You must remove belts with metal buckles, and you may be asked to change into a gown that we will provide if your clothing contains significant metal, including metal underwire bras.
    - **Inner ear damage**: MRI scanning produces loud noises that can cause damage to the inner ear if appropriate hearing protection is not used. Earplugs and/or headphones will be provided to protect your ears.
    - **Claustrophobia**: When inside the MRI scanner, the "bore" of the scanner will surround the part of the body that is being scanned. In the case of fMRI, we are interested in brain activity, and the head will be centered inside a close-fitting scanning coil positioned in the bore of the scanner. If the subject feels anxious in confined, spaces they may not want to participate. If they are unsure, they can try the "mock" scanner at the MRI facility to evaluate their comfort level with the enclosed space of the magnet bore. If they decide to participate and begin to feel claustrophobic, they will be able to communicate with the research team via the intercom or the squeeze ball and the test will be discontinued.

Subjects who do not complete MRI testing will still be allowed to continue with the trial.

- **Burns**: In rare cases, contact with the MRI transmitting and receiving coil, conductive materials such as wires or other metallic objects, or skin-to-skin contact that forms conductive loops may result in excessive heating and burns during the experiment. The operators of the MRI scanner will take steps, such as using foam pads when necessary, to minimize this risk. Tattoos with metallic inks can also potentially cause burns. In addition, please let the MRI operator know immediately if you experience any heating or burning sensations during a scan. The scanning session will be stopped as soon as you tell the operator.
- **Nerve or muscle stimulation**. While the scanner is operating, there is a small chance that the rapidly changing magnetic fields could cause a slight tingling sensation or a muscle twitch, usually felt in the upper arms or torso. While these sensations may be startling, they are not dangerous or a health risk, and they have no lasting consequences. The sensations should stop when the scan ends. Because these sensations may nevertheless be distracting or even possibly uncomfortable, please squeeze the signal bulb to alert the scanner operator if you feel tingling or muscle twitching, and we will immediately stop the scan. You will then have the opportunity to choose to withdraw from the study or to continue.
- **Other Risks**. Besides the risks listed above, there are no other known risks from the magnetic field or radio waves at this time. Although functional MRI scanning has been used for more than 20 years, long-term effects are unknown.

There are no alternative methods that would provide the same type and accuracy of information as the state-of-the-art procedures proposed in this application.

In your opinion, are risks listed above minimal* or more than minimal? If more than minimal, please justify why risks are reasonable in relation to anticipated direct or future benefits.
*(*Minimal risk means the probability and magnitude of harm or discomfort anticipated in the research are not greater than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests)*

The risks associated with this research are minimal and not greater than those encountered during the performance of routine physical or psychological examinations.

What steps will be taken to minimize risks?

Management of **general risks** of the proposed study*:*

- Having only qualified and experienced personnel perform the procedures;
- Using only safe, well-established procedures;
- Constant monitoring of the subjects by the members of the investigative team;
- Instating appropriate clinical supervision, availability of emergency equipment and medications (with well-planned emergency procedures), and the overall safety procedures in place within the STAR Health Sciences Complex;
- Establishing a Data and Safety Monitoring Committee to periodically review and evaluate adverse events related to study procedures and/or treatments.

Management of **specific risks** related to drugs/supplements:

- screening for adverse events and allergies to drugs;
- obtaining Investigational New Drug (IND) approval from the FDA;
- working collaboratively with ChromaDex, Inc. and the FDA to ensure that the NR and placebo pills meet all regulatory and safety guidelines, and that there is continuing effective information sharing;

- using a dose of NR therapy that is based on my pilot study, has been shown to have efficacy, but does not result in adverse side effects in human subjects.

Management of **specific risks** related to the collection of MRI data:

- This protocol will be performed using an MR scanner employing pulse sequences and hardware that have been approved by the FDA for human clinical use. The field strength is 3 Tesla and all relevant operating characteristics (RF power deposition, rate of change of the field gradients, coil design) fall within the limits of FDA guidelines for NMR exposure. Participants will be carefully screened to exclude those who may have metal in or on their bodies that cannot be removed (e g., bullets, metal filings, body piercings, etc.). MR Facility rules strictly forbid staff from entering the magnet room carrying metal objects. The risk of claustrophobia is minimized by screening subjects for self-reported claustrophobia and making sure the subject is lying comfortably with head and neck supported and providing ear protection with headphones, a mirror to see out, a button to signal distress, and an intercom. Scan time will be kept to a minimum. If they are unsure about whether or not they may be pregnant, female participants will be given the opportunity to complete a urine pregnancy test immediately before the scanning period, and those with a positive result will not be scanned. With regard to PNS, participants are given a squeeze ball to use in case of an emergency. They are informed that if they experience PNS related sensations or are otherwise uncomfortable, they can alert the MRI technologist via the squeeze ball and the technologist will stop the scan immediately.
- All information obtained during the study will be held in strict confidence to the fullest extent possible by law. In no case will a participant's personal identifiable information be shared with any other individuals or groups without their expressed written consent. MR Images will be stored on secured computer servers and will be archived indefinitely. Non-identifiable images of MR scans may be used for teaching purposes, be presented at meetings, published, and also shared in databases accessible to other researchers for further research and educational purposes. The name or other identifying information associated with the participant will not be used in any publication or teaching materials without their specific permission.

Describe any potential direct benefits to participants.

There may be no direct benefit to subjects participating in this research study; however, subjects will receive the results of their screening assessments (i.e., blood glucose status, plasma lipids and lipoprotein profile, blood pressure, blood chemistries). These results will not be interpreted but are provided for information. Subjects will be encouraged to discuss their results with their PCP.

Describe any potential future benefits to this class of participants, others, or society.

The findings from the proposed research should provide important new information regarding the influence of NR supplementation upon multiple domains of physiological function including vascular, cognitive and neuronal function in older adults with aMCI. In addition, the proposed research should provide important insight into a potential mechanism involved in the modulation of cerebrovascular function with NR (i.e. oxidative stress). Together, this information will contribute to the knowledge of how to prevent and treat cerebrovascular and cognitive dysfunction in older adults with aMCI and possibly lead to future insight into reducing Alzheimer's disease risk.

If there is a Data Monitoring Committee (DMC) in place for this project, please describe when and how often it meets.

This protocol has been reviewed and approved by a Data and Safety Monitoring Board (DSMB) which will convene every 6 months to monitor patient safety, data quality and evaluate study progress. A copy of the DSMB charter as approved by the DSMB is attached with this protocol.

9. **COMPENSATION**

Will participants be compensated for participation? Yes

Subjects will receive monetary reimbursements for completion of the main experimental sessions. Subjects will be compensated per visit for their time in the form of a single check that will be mailed upon completion or withdrawal from the study. There is no compensation for the screening session (Visit 1) because subjects will be receiving medical information from all of the screening tests free of charge. This information will be provided upon completion of the study. Subjects will be compensated for any visit that they attempt to complete; however, they will not be compensated if they skip a visit altogether. For example, if they attempt to complete the baseline MRI (Visit 4) but ultimately cannot go through with the procedures (e.g., due to claustrophobia), they will still be compensated for Visit 4; however, since they will not have completed a baseline visit, they will not be scheduled for the follow-up MRI (Visit 9) and will receive no compensation for that visit.

Compensation does not include travel time (we will compensate subjects for their travel if they wish at the current IRS approved mileage rate). If a subject withdraws or is withdrawn from the study, they will receive any health information collected and will be compensated for the study visits that they have already completed up to the time of withdrawal. Compensation is based on completion of study visits, not individual procedures. Subjects will be compensated for completing the majority of procedures in a visit. If a subject does not complete a visit at baseline, that visit will not be scheduled for follow-up and no compensation will be given for the follow-up visit. A table outlining the total compensation for each visit is presented below.

Compensation Schedule

	Visit #	Purpose	Duration	Total
Screening	Visit 1	Informed Consent & Screening Measures	2 hr	No compensation Subjects receive free health information
Baseline Testing	Visit 2	Vascular Function	3 hr	$30
	Visit 3	Cognitive Function	1.5 hr	$22.50
	Visit 4	Neuronal Function/ Start Intervention	2 hr	$30
Intervention Period	No Visit	Check-In	10 min	$9.00
	Visit 5	Check-In	0.5 hr	$9.00
	No Visit	Check-In	10 min	$9.00
	Visit 6	Check-In	0.5 hr	$9.00
	No Visit	Check-In	10 min	$9.00
Follow-Up Testing	Visit 7	Vascular Function	3 hr	$30
	Visit 8	Cognitive Function	1.5 hr	$22.50
	Visits 9	Neuronal Function	2 hr	$30
	Total Compensation			**$210**

10. **DATA**

Will subjects be anonymous to the researcher? No.

If subjects are identifiable, will their identities be kept confidential? (If yes, please specify how)
All subject identities and records will remain strictly confidential. Individual subject data will be coded and will not be associated with subject name. Documents that contain subject identifies (e.g., signed informed consent) will be kept in a separate locked filing cabinet. A list of names and participant numbers will be encrypted and stored on a password protected computer. This list will be kept for 5 years after study completion, after which it will be permanately deleted. All information obtained by the NMPCC, including electronic medical records, will be stored on a HIPAA compliant database.

How will data be stored and kept secure (specify data storage plans for both paper and electronic files. For guidance see http://www.udel.edu/research/preparing/datastorage.html)

Physical data will be stored in a locked file cabinet in the PI's lab space and will only be accessible by members of the research team. Physical data that are transcribed into an electronic database, and electronically captured data will be kept on a password protected laboratory server that is maintained by the University of Delaware. Electronic data will be periodically backed-up onto an external hard drive that will be kept in a locked office space. All of the ultrasound images and files generated by the SphygmoCor and RespirAct devices will be coded with an identification number and will not contain the name of the subject. The names of subjects will not be identified in any publication arising from these studies. Only the PI and research staff will have access to data from this study. With subject consent, biological samples (plasma/serum, peripheral blood mononuclear cells) will be kept for retrospective analyses related to aMCI and physiological function (this will not be conducted without IRB approval).

How long will data be stored? All data will be stored in a locked cabinet or a password protected computer indefinitely.

Will data be destroyed? □ YES **X** NO (if yes, please specify how the data will be destroyed)

Will the data be shared with anyone outside of the research team? □ YES **X**NO (if yes, please list the person(s), organization(s) and/or institution(s) and specify plans for secure data transfer)

How will data be analyzed and reported?

To determine if pre-intervention differences exist between groups, subject characteristics (e.g. total body fat, fasting blood glucose, etc.) will be compared between the two groups using unpaired t-tests. If assumptions of normality are met, separate linear regression models will be fit to compare NR vs. placebo (independent variable) with respect to change (baseline vs. week 12) for each of the outcome variables. Linear regression methods will be used to determine the association between/among changes in the primary or secondary outcomes in response to NR supplementation (e.g., to examine the association between changes in cognitive function and changes in arterial stiffness or cerebrovascular function). If assumptions of normality are not met, alternative strategies will be used, including transformation of variables, generalized linear mixed models, and non-parametric regression. To account for the blocked randomization scheme, indicator variables for block assignment will be included in the model as appropriate. To account for multiple testing of primary outcomes of interest in response to treatment, a Hotelling's T test will be used. If this multivariate test is significant then individual outcomes will be compared between groups at an alpha level of 0.05. Secondary outcomes (brain blood flow and blood pressure/arterial stiffness) will be assessed similarly with an adjusted alpha level of 0.0125. All tertiary outcomes will be considered more exploratory for future hypothesis testing. Given the design, treatment conditions and assessment protocols, we anticipate missing data (due to subject dropout, missed data collections, etc.) to be at least missing at random (MAR), if not missing completely at random (MCAR). Based on my pilot study, we do not expect the assigned treatment condition (NR or placebo) to influence whether a subject opts to continue with the study. Assuming missing data that are MAR or MCAR, a linear model or generalized linear model would yield valid inference.

11. **CONFIDENTIALITY**

Will participants be audiotaped, photographed or videotaped during this study? No

How will subject identity be protected? Information obtained from this study will be kept strictly confidential. Subjects will not be individually identified, except by subject number, known only to the investigators. All data stored as paper files or digitally will be kept indefinitely. The paper files are stored in a locked cabinet. While the results of the research may be published, subjects' names and identities will not be revealed.

Is there a Certificate of Confidentiality in place for this project? (If so, please provide a copy). No.

12. **CONFLICT OF INTEREST**
(For information on disclosure reporting see: http://www.udel.edu/research/preparing/conflict.html)

Do you have a current conflict of interest disclosure form on file through UD Web forms? Yes

Does this project involve a potential conflict of interest*? No.

* As defined in the University of Delaware's Policies and Procedures ,a potential conflict of interest (COI) occurs when there is a divergence between an individual's private interests and his or her professional obligations, such that an independent observer might reasonably question whether the individual's professional judgment, commitment, actions, or decisions could be influenced by considerations of personal gain, financial or otherwise.

13. **CONSENT and ASSENT**

__x__ Consent forms will be used and are attached for review (see Consent Template under Forms and Templates in IRBNet)

14. **Other IRB Approval**

Has this protocol been submitted to any other IRBs? No

If so, please list along with protocol title, number, and expiration date.

15. **Supporting Documentation**
Please list all additional documents uploaded to IRBNet in support of this application.

Informed Consent (subject)
DSMB Charter
CDR Survey
Hopkins Verbal Learning Test
Pre Consent Script
Pre-Screening Questionnaire
Recruitment opt out postcard
Recruitment Letter
MRI screening form
Family history questionnaire
Study Delegation Log
Form 6. Physical Symptom Questionnaire

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