A Crossover, Randomized Block Sequence, Double-Blind, Placebo-Controlled Trial for Nicotinamide Riboside in Subjective Cognitive Decline and Mild Cognitive Impairment in Aging

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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ADRD	Alzheimer's Disease Related Dementias
AE	Adverse Event/Adverse Experience
ASL	Arterial Spin Labeling
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BOLD	Blood Oxygen Level Dependent
BRIEF-A	Behavioral Rating Inventory of Executive Function – Adult Version
BVMT-R	Brief Visuospatial Memory Test-Revised
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CRF	Case Report Form
CNS	Central Nervous System
CNY	Charlestown Navy Yard
Co-I	Co-Investigator
CPT	Continuous Performance Task
CSF	Cerebrospinal Fluid
DKEFS	Delis-Kaplan Executive Function System
DNA	Deoxyribonucleic acid
DOB	Date of Birth
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FABP	Fatty Acid Binding Protein
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GRAS	Generally Recognized as Safe
HIPAA	Health Insurance Portability and Accountability Act
HRC	Human Research Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
LC	Longitudinal Cohort
LP	Lumbar Puncture
MADRC	Massachusetts Alzheimer's Disease Research Center
MCI	Mild Cognitive Impairment
MFQ	Memory Function Questionnaire
MDU	Memory Disorders Unit
MGH	Massachusetts General Hospital
MRI	Magnetic Resonance Imaging
MRN	Medical Record Number
T-MoCA	Telephone Montreal Cognitive Assessment
MoCA	Montreal Cognitive Assessment
N	Number (typically refers to subjects)
NAD^+	Nicotinamide Adenine Dinucleotide

NFL	Neurofilament light-chain protein
NMN	Nicotinamide mononucleotide
NR	Nicotinamide riboside
PBO	Placebo
pCASL	Pulsed Continuous Arterial Spin Labeling
PHRC	Partners Human Research Committee
PI	Principal Investigator
PT/INR	Prothrombin Time and International Normalized Ratio
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
rsBOLD	Resting State Blood Oxygen Level Dependent
rsfMRI	Resting State Functional Magnetic Resonance Imaging
SAE	Serious Adverse Event
SCD	Subjective Cognitive Decline
TMT	Trail-Making Test
TOPF	Test of Premorbid Functioning
TSH	Thyroid Stimulating Hormone
UCH-L1	Ubiquitin Carboxy-Hydrolase-L1

1. ETHICS/PROTECTION OF HUMAN SUBJECTS

1.1 Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

1.2 Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonisation (ICH) and the ethical principles of the Declaration of Helsinki.

1.3 Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subjects will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

If the subject is not fully competent and able to provide consent, he or she will not be eligible to participate in this study. Our study population of scientific interest is Mild Cognitive Impairment (MCI); subjects in this category will be expected to have full capacity to consent unless due to another exclusionary factor.

2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Subjective Cognitive Decline and Mild Cognitive Impairment

Advancing age is accompanied by variable declines in neurocognitive functioning. Some crystalized functions such as vocabulary and well-practiced knowledge and skills remain stable or even improve into late-life in normal, non-diseased brain aging. However, fluid functions such as new learning and memory, problem-solving and other executive functioning, attention and concentration, and psychomotor processing speed all show declines. In normally functioning older adults, "Subjective Cognitive Decline" (SCD*) is the most widely accepted term for this decline in fluid cognitive functions with age. It is a state in which patients experience declines from their usual levels of cognitive functioning but score in the normal range on standard cognitive tests relative to peers[1]. Their everyday independent functioning is also "normal", but they are often distressed by their perceived cognitive abilities. SCD may be a very early, prodromal symptom of an Alzheimer's disease (AD) or AD-related dementias (ADRD) for some, or, in the absence of a disease process, it is often attributed to age-related changes in brain metabolism, synaptic plasticity and neurochemistry[1]. The natural history of SCD with or without evidence of an AD/ADRD process is still under investigation, but risk for subsequent decline into dementia is significantly increased and the presence of AD/ADRD biomarkers is higher in this group.[2]

Mild cognitive impairment (MCI) is a term used when there is clinical concern about cognitive decline and evidence of impairment(s) in cognitive testing, more than expected for the patient's age and educational background, but not so severe that the person cannot manage their daily functioning without more than minimal assistance. MCI is frequently, though not always, a transitional, prodromal phase of dementia due to ADRD. "Dementia" is present when independent daily functioning is affected.[3]

AD/ADRDs are associated with synaptic dysfunction and loss, neuronal cell death and gliosis that particularly affect the cerebral association cortices. Abnormal aggregates of amyloid plaques and tau neurofibrillary tangles are the signature histopathological findings of AD, while other mis-folded protein aggregates such as α-synuclein Lewy bodies and TDP-43 cytoplasmic inclusions as well as small- and micro- vessel disease are frequently concurrent and contributory to the overall degenerative processes. Beyond these histopathologies, it is increasingly recognized that a host of pathophysiological processes contribute to neurodegeneration in aging and AD/ADRDs, including bioenergetic/metabolic dysfunction, endoplasmic reticulum stress, inflammation, oxidative damage, neurovascular injury, apoptosis and more. These processes may interact with AD/ADRD

*Also referred to as age-associated memory impairment, subjective cognitive concerns, impairments or complaints, subjective memory concerns, impairments or complaints, "senior moments" and cognitive aging.

signature histopathologies in vicious cycles, presenting considerable challenges, but also novel entry points for potential disease modification and neuroprotection across pathologies[3].

2.1.2 Nicotinamide Riboside Rationale

Nicotinamide riboside (NR) is a form of vitamin B3, naturally found in low levels in milk, that is a precursor to nicotinamide adenine dinucleotide (NAD+). NAD+ is a central metabolic cofactor that functions as the primary electron donor in the mitochondrial respiratory chain, regulates the activity of various metabolic pathway enzymes in glycolysis, Kreb's cycle, and fatty acid oxidation and enables signaling for major cellular metabolism processes involving sirtuins. These processes are critical for maintenance of synaptic plasticity and protection against neurodegeneration. Neuronal mitochondrial dysfunction occurs with advancing age, but this is especially pronounced in pathological brain aging, such as with AD or vascular cognitive impairment. NR is a safe, well-tolerated, potent NAD+ precursor available as a "generally recognized as safe" (GRAS) dietary supplement. It has promising pharmacokinetic and systemic pharmacodynamics properties and has been shown to ameliorate bioenergetic, behavioral and pathological abnormalities in AD mouse models.

2.1.3 Preclinical Use of Nicotinamide Riboside

Mice expressing mutant forms of Alzheimer's Precursor Protein, presenilin-1, and tau with a polymerase β null allele were used to model age-dependent neuronal death and DNA damage. Exposure to 12mM NR for 6 months led to significant reversals in cognitive behavioral deficits, enhanced long-term potentiation and neurogenesis, and decreased oxidative and inflammatory damage. NAD^+/NADH ratio was elevated in mice treated with NR compared to vehicle groups. NR-treated mice also exhibited improved cognitive performance, such as improved observation times in the novel-object tests and improved spatial learning in water maze tests. NR treatment in this model was also implicated in upregulation of neurogenesis-related genes, leading to a 9.6% change in hippocampal gene expression, and downregulation of pro-apoptotic *p53* expression. NR treatment was further linked to decreased astrocytosis and microgliosis (equivalent to wild-type controls), and significantly reduced levels of phospho-tau-231. NR treatment applied to human AD fibroblasts demonstrated reduced expression of the oxidative 8-oxo-dG DNA and reduced levels of acetylated substrate, suggestive of the effect of NR on upregulating NAD^+-dependent sirtuin deacetylases.[4]

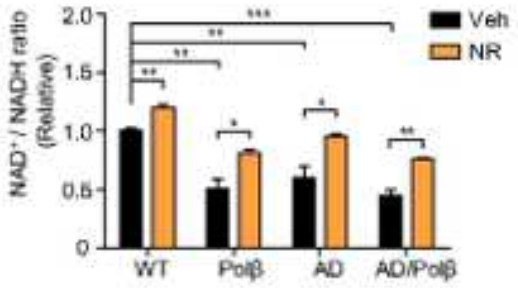


Figure 1. NAD+/NADH ratio following 6 months of NR treatment (12 mM) among wild type, transgenic AD model mice, mice with polymerase β mutation, and AD/polymerase β hybrids simulating age-dependent neuroinflammation and oxidative damage. NAD^+/NADH is a measure of the available pool of NAD^+ and shows significant elevation in NR-treated mice compared to vehicle controls among all models.[4]

NR has also been implicated in modifying the toxicity of amyloid plaques associated with AD. Administration of mg/kg/day of NR to Tg2576 AD mice led to marked changes in hippocampal long-term potentiation and increased expression of PGC-1α, which in turn upregulated degradation of β-secretase, a key enzyme implicated in amyloid plaque formation. [5]

2.1.4 Pharmacokinetics of Nicotinamide Riboside in Humans

An open-label, non-randomized study of the pharmacokinetics of NR carried out at the University of Washington demonstrated both the impact of NR on NAD^+ levels in humans and its tolerability. Eight subjects underwent dose escalation from 250 mg of NR twice daily to 1000 mg of NR twice daily over the course of nine days, with NR and NAD^+ levels determined at baseline Day 1 and over the course of a 24-hour pharmacokinetic study on Day 9. No adverse events were reported in clinical laboratory values, including potassium, which had been noted to be elevated in previous NR studies. Slight, but significant decreases in hematocrit, hemoglobin, and platelet count were reported. All but one subject showed an increase in blood NR levels from baseline to Day 9, ranging from 10% to 127% increases. Blood NR showed exponential decline from 3 to 12 hours with a half-life of 2.7 hours. Despite relatively fast clearance of NR, blood NAD^+ levels were significantly increased to steady state (see Figure 3) by 35 to 168% from baseline. NR treatment did not produce side effects commonly seen in niacin treatment, such as flushing, pruritus, hyperglycemia, hyperuricemia, or elevation of liver or muscle enzymes. However, a variability in NR bioavailability was observed, suggesting that NR relies on active transport across intestinal mucosa, which can vary highly from person to person. [6]

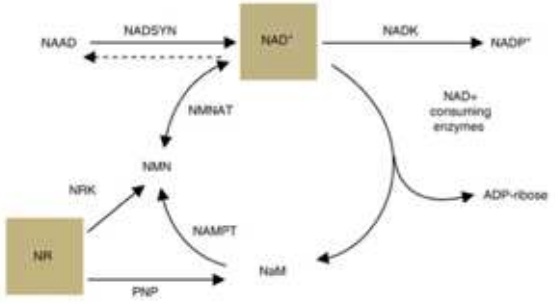
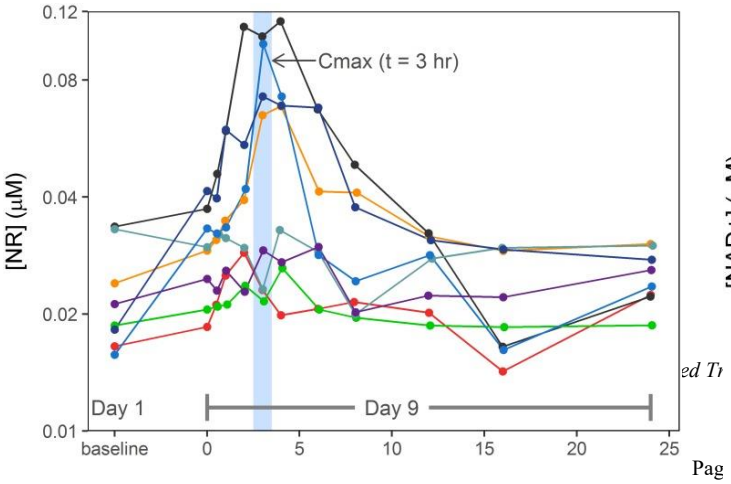
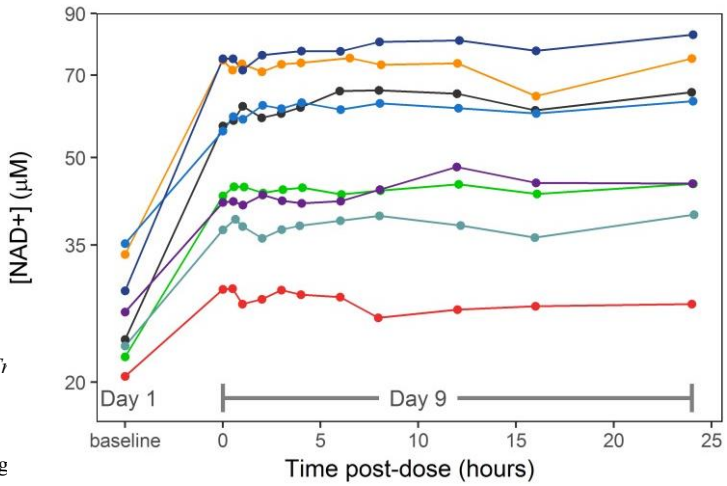


Figure 2. Nicotinamide riboside (NR) enters the NAD^+ salvage pathway through conversion to nicotinamide mononucleotide (NMN) via nicotinamide riboside kinase (NRK) or conversion to nicotinamide (NaM) via purine nucleoside phosphorylase (PNP). From there, NMN (or NaM after modification by NAMPT) is converted into active NAD^+ and shunted into a variety of metabolic processes. NAD^+ consuming enzymes, such as sirtuins, drain the NAD^+ pool, which must be replenished. Declining activity of nicotinamide phosphoribosyltransferase (NAMPT) with age is implicated in declining levels of NAD^+ with age. NR supplementation bypasses this rate-limiting step by introducing NAD^+ precursors directly into the salvage pathway.[6]





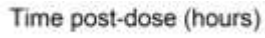


Figure 3. Blood NR and NAD^+ levels in $n = 8$ subjects following NR dose escalation from 250 to 1000 mg twice daily.[6]

2.1.5 Prior Clinical Use of Nicotinamide Riboside

While there are no reports yet of NR being used in patients with SCD, MCI or ADRDs, NR has been characterized to be both tolerable and effective in healthy middle-aged and older adults in improving markers of cardiovascular health in healthy middle-aged and older adults. A 12-week randomized, double-blind, single-crossover trial provided 30 healthy subjects with twice daily 600 mg doses of NR for 6 weeks and 6 weeks of PBO. Over the course of the study, 14 treatment-emergent adverse events (AEs) were reported in 7 subjects, all of mild severity. During the NR treatment period, AEs included nausea, flushing, leg cramps, and increased bruising. During the PBO period, AEs included headache, skin rash, flushing, fainting, and drowsiness. Only 2 subjects (less than 10%) dropped out of the study because of these AEs, both of whom were in the PBO period. Two out of the three instances of flushing reported also occurred during the PBO period, suggesting the AE is likely unrelated to treatment. No significant changes in clinical labs were observed, including renal function markers and blood lipid profiles. Blood NAD^+ levels increased by 60% from baseline following NR treatment. A five-fold change in nicotinic acid adenine dinucleotide (NAAD) and a 1.5-fold change in a nicotinamide mononucleotide (NMN) were also detected. Adenosine and adenosine triphosphate (ATP) levels also increased following NR treatment, demonstrating its robust impact on metabolism. When all subjects were grouped, mean systolic and diastolic blood pressure was significantly lowered following NR treatment, and carotid-femoral pulse wave velocity, a gold-standard marker of aortic stiffness, was also reduced, although not significantly. NR treatment did not correspond to any significant change in total energy expenditure, oxidative fuel source, physical activity patterns, body mass index, glucose and insulin regulation, and exercise capacity in any subject.[7]

3. SPECIFIC AIMS

3.1 Overall Study Design and Plan

This is a single-site single crossover, double-blind placebo (PBO)-controlled randomized sequence block trial of nicotinamide riboside (NR, TruNiagen®, Chromadex) vs. PBO in 40 volunteers with SCD or MCI. All volunteers will receive a four-week PBO-lead in and acclimatization followed by two 8-week "blocks". Each block consists of either NR or PBO for an 8-week period in random assignment order. Outcomes will consist of standardized cognitive, neuropsychiatric, functional and biomarker assessments as well as daily cognitive, mood, sleep, and activity monitoring with computer-based brain games and wearable actigraphy. Due to the COVID-19 pandemic, some of the study visits will be completed fully or partially via Zoom videoconference to minimize the amount of contact between study staff and research subjects.

3.2 Study Objectives

The primary objective of the study will be:

1. To measure the effect of NR vs PBO on RBANS.

The secondary clinical objectives of the study will be:

1. To measure the effect of NR vs PBO on other, standard assessments of cognition, functional activities, and neuropsychiatric symptoms.
2. To measure the effect of NR vs PBO on daily assessments of cognition, functional activities, and neuropsychiatric symptoms.
3. To monitor the safety and tolerability of NR in volunteers with SCD or MCI.

The exploratory biomarker objectives of the study will be:

1. To measure the effect of NR on plasma biomarkers of metabolic target engagement and central nervous system (CNS) neural and glial integrity measurable in blood.
2. To measure the effects of NR on cerebral neurophysiology (in an optional sub-study) using resting state functional magnetic resonance imaging (rs-fMRI). Connectivity will be measured with rsBOLD and regional cerebral blood flow will be measured with pseudo-continuous arterial spin labeling (pCASL).
3. To measure the effect of NR on cerebrospinal fluid (CSF) biomarkers (in an optional sub-study) of metabolic target engagement and emerging biomarkers of relevance to AD/ADRD.

3.2.1 Standardized Cognitive, Functional, and Neuropsychiatric Outcome Measures

The primary outcome of this study will be objective measures of cognitive performance measured with the Repeatable Battery for the Assessment of Neuropsychological Status[15] (RBANS) at Baseline (Day 28), Crossover (Day 84), and End of Study (Day 140). Other secondary outcome measures of cognition include the Trail Making Test (TMT)[16], Delis-Kaplan Executive Function System (DKEFS)[17] Verbal Fluency subtest, a computerized Continuous Performance Test (CPT), and the Brief Visual Memory Task-Revised (BVMT-R)[18]. Subjective ratings of everyday cognitive functioning and independent/instrumental/basic activities of daily living will be measured using items from the Behavior Rating Inventory of Executive Function (BRIEF-A)[19] and the Memory Functioning Questionnaire[20] (MFQ). Ratings of mood and anxiety will be measured using the Beck Anxiety Inventory[21] (BAI) and Beck Depression Inventory[22] (BDI). These standard measures will be administered at Baseline (Day 28), Crossover Visit (Day 84), and End of Study (Day 140).

The secondary clinical and exploratory biomarker outcomes of this study will be:

- Performance on daily measures of cognition, functional activity, and neuropsychiatric symptoms as assessed by:
    - A "daily" battery of approximately 15 minutes of Lumosity brain games (Lumos Labs, Inc.). Subjects will be asked to complete Lumosity games at least 6 times per week.
    - Objective daily physical activity levels and sleep data collected using wrist actigraphy (Fitbit Charge 3).
    - Subjective mood and sleep ratings will be collected using questionnaires 6 days per week.
- Safety and tolerability will be assessed with adverse event (AE) reporting conducted at every visit.
- Plasma for biomarkers will be collected at baseline, crossover and end-of-study. Plasma biomarkers assays will consist of ultra-sensitive immunochemical measures of proteins reflecting neurodegeneration/neural injury (NfL, amyloid $\beta_{42/40}$, and GFAP) as well as NAD+/NADH.

- Cerebral neurophysiology will be measured with functional MRI (optional sub-study).
    - Optional MRI will be conducted at three time points: Baseline (Day 28), Crossover (Day 84), and End of Study (Day 140. Each MRI scan session will include functional MRI with resting state BOLD (rs-fMRI) and pseudo-continuous arterial spin labeling (pCASL) sequences. rs-fMRI measures spontaneous yet synchronized fluctuations in the BOLD signal to determine the strength of functional connectivity within various intrinsic brain networks (i.e., default mode network). pCASL quantifies regional cerebral blood flow using magnetically labeled blood water content and will serve as a marker of resting state cerebral perfusion that has also been shown to correlate with regional brain metabolism and levels of neuronal activity.
- Cerebrospinal Fluid Biomarkers (optional sub-study): All subjects will have the opportunity to participate in a lumbar puncture (LP) sub-study in which they can receive 1-3 optional LPs at Baseline (Day 28), Crossover (Day 84), and End of Study (Day 140). A panel of CSF biomarkers will be measured to determine the effects of NR on pathophysiological targets relevant to aging and neurodegeneration, including 1) neuronal degeneration markers neurofilament light chain (NfL) and neurogranin, 2) classic AD biomarkers including amyloid-$\beta_{42/40}$, total tau and phospho-tau, 3) markers of mitochondrial function and redox dysregulation such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) and pyruvate/lactate, and 4) selected other biomarkers of inflammation and immune response and vascular injury of relevance to the aging brain and cognition.

3.3 Protocol Adherence

Each investigator must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by Internal Review Board (IRB). Each investigator will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

4. SUBJECT SELECTION

4.1 Inclusion and Exclusion Criteria

4.1.1 Inclusion Criteria

A subject will be eligible for study participation if they meet the following:

1) Ages 55 and up;
2) Memory and other cognitive complaints consistent with SCD or MCI as defined:
    a. SCD will be defined as:
        i. any *subjective* concern of change in cognitive functioning without objective evidence of cognitive impairment, and
        ii. preservation of functional abilities and independence in instrumental activities of daily living;
    b. MCI will be defined as:
        i. a preexisting diagnosis of MCI given by a trained physician or behavioral health provider, *or*

ii. evidence of *objective* impairment in cognitive functioning in one or more domain with preservation of functional abilities and independence in instrumental activities of daily living as defined by the National Institute on Aging;

3) Minimum score of 16 on the Telephone Montreal Cognitive Assessment (T-MoCA) or minimum score of 24 on the Montreal Cognitive Assessment (MoCA);
4) Ability to provide direct informed consent as assessed by obtaining a score of 70% on questions 1-10, and 100% on questions 11-14 of the Informed Consent Worksheet after two attempts;
5) Education level, English language skills and literacy indicates participant able to complete all assessments;
6) Willing and able to complete all assessment and study procedures;
7) Not pregnant, lactating, or of child-bearing potential;
8) If on cholinesterase inhibitor and/or memantine, doses are stable for 3 months prior to baseline;
9) Basic video conferencing capabilities and a willingness to participate in a partially virtual trial.

4.1.2 Exclusion Criteria

A subject will not be eligible for study participation if they meet any of the following criteria

1) Any specific CNS disease history other than suspected ADRD, such as major clinical stroke, brain tumor, normal pressure hydrocephalus, multiple sclerosis, significant head trauma with persistent neurological of cognitive deficits or complaints;
2) Any impairment in instrumental activities of daily living that would indicate a level of cognitive impairment beyond MCI as assessed by a trained rater;
3) Clinically significant unstable medical condition that could affect safety or compliance with the study and would, in the opinion of the investigator, pose a risk to the participant if they were to participate in the study;
4) History of neuroimaging with evidence of major infarction, injury, infection, or other focal lesions that may be related to cognitive dysfunction;
5) If participating in the optional LP sub-study, any contraindication to undergo lumbar punctures, such as:
    a. Abnormal coagulation PT/INR test result, outside of the normal range of 0.9 to 1.2 platelet counts below 50,000 (interpreted by a licensed study physician or nurse practitioner after reading test results);
    b. Platelet counts below 50,000;
    c. Use of coumadin or other blood thinner medications;
    d. Infection near the puncture site, or spinal column deformities (a licensed physician or nurse practitioner will examine the site visually for infection or spinal deformities before performing the procedure);
    e. Known allergy to Lidocaine.
6) Major active or chronic unstable psychiatric illness (e.g. depression, bipolar disorder, obsessive compulsive disorder, schizophrenia) within the previous year;
7) Current suicidal ideation or history of suicide attempt;
8) History of alcohol or other substance abuse or dependence within the past two years;
9) Any significant systemic illness or medical condition that could affect safety or compliance with study;

10) Laboratory abnormalities at Baseline in vitamin B12, thyroid stimulating hormone (TSH), or other common laboratory parameters that might contribute to cognitive dysfunction or other abnormalities in hematological, hepatic or renal function tests;
11) Current use of medications with psychoactive properties that in the opinion of the principal investigator, may be deleteriously affecting cognition (e.g., anticholinergics, antihistamines, antipsychotics, sedative hypnotics, anxiolytics);
12) Any known hypersensitivity to nicotinamide riboside, or its principal metabolite, nicotinamide mononucleotide;
13) No consumption of dietary supplements containing more than 100mg niacin, nicotinamide riboside (NR), or nicotinamide mononucleotide (NMN) as the primary agents 30 days prior to baseline and for the duration of the trial.
14) Use of other investigational agents or interventions one month prior to entry and for the duration of the trial;
15) If participating in the optional MR sub-study:
    a. Any contraindication to undergo MRI studies, such as history of cardiac pacemaker or pacemaker wires, metallic particles in the body, vascular clips in the head, prosthetic heart valves, and/or severe claustrophobia impeding ability to participate in an imaging study.
    b. Age 60 and older.

4.1.2.1 Women of Childbearing Potential (WOCBP)

For the purposes of this study, women of childbearing potential are defined as all women who are capable of becoming pregnant, unless they meet one of the following criteria:

1. 12-months post-menopausal
2. Post-hysterectomy
3. Surgically sterile

If a female subject does not meet these criteria and is considered of childbearing potential, they will be excluded from the clinical trial.

4.2 Recruitment

Approximately 60 subjects will be screened, and after screening, approximately 40 subjects are expected to complete the study. All subjects will be given the option to participate in the optional repeat LP sub-study and the optional MRI sub-study until 12 subjects have completed each sub-study. Subjects will be given the choice to complete either the repeat LP sub-study, the MRI sub-study, or both.

4.2.1 Recruitment of Subjects through Advertising

Advertisement flyers will be posted on bulletin boards around MGB campuses (both the main campus and Charlestown campus) to advertise for the study as well posting as an advertisement on Partners Rally for Research. A phone number will be provided that will ring directly to the research coordinator, and voice messages can be left for the coordinator on a password-protected voice mailbox. The study coordinator will contact the subject and explain study in further detail and if the subject is interested, potentially complete a telephone prescreening. A listing of the study will also be posted on the MGH ACTRU website as well the websites of our affiliates.

Subjects may also be recruited through the distribution of IRB approved recruitment materials at community outreach initiatives including informational sessions to various community partners such as city-based and non-profit organizations.

4.2.2 Recruitment of Subjects from among the Investigator's own Patients and MGB Providers

Subjects will also be recruited from the outpatient clinical practices of the principal investigator (Dr. Steven Arnold). If the potential subject is the investigator's patient, another member of the study or clinic staff will introduce the study to the patient and determine if they are interested in learning more about the study. An NP investigator will be made available to explain the study to the subject at their request. If the potential subject is not the investigator's patient, the investigators will not directly approach the patient regarding possible participation in the study. Other MDU physicians (not participating directly in the study) may discuss the study directly with their patients, if they choose to do so. The prospective participant will be given informational papers to review and the study coordinator will contact them to discuss interests and research opportunities.

Subjects will also be recruited from physician specialists at the Center for Alzheimer Research and Treatment (CART) at Brigham and Women's Hospital, a database of patients who have indicated a prior interest in participating in studies at the CART, the memory disorders, neurology, psychiatry, and medicine clinics at Brigham and Women's Hospital and Massachusetts General Hospital, and physicians outside of the Partners network.

Other providers across MGB will also be made aware of the study (including key eligibility criteria) via email and provided a link to the Rally ad. These providers (not participating directly in the study) may discuss the study directly with their patients, if they choose to do so. If the prospective patient is interested, their provider will give them a copy of the consent form (unstamped) for review as well as a link to the Rally ad, which contains the research coordinator's contact information.

4.2.3 Recruitment of Subjects from the Massachusetts Alzheimer's Disease Research Center

Subjects will also be recruited from an observational study that follows a longitudinal research cohort (LC) of approximately 400 active research participants in the Massachusetts Alzheimer's Disease Research Center (MADRC) recruited from the MGH's Memory Disorders Unit clinic and other diverse sources. LC subjects are followed-up on an approximately annual basis, either in-person at the MGH or by means of a telephone follow-up 'visit'. Study staff will only contact subjects that have indicated to the MADRC that they are interested in hearing about/participating in other studies. MADRC may also share information regarding our studies with their mailing list.

4.2.4 Recruitment of Subjects from Providers Outside of MGB

Subjects will be recruited from providers outside of MGB through the use of doctor to doctor letters and/or sharing of IRB-approved recruitment materials. Appropriate providers who have been identified by the study team will be mailed IRB approved materials that introduce the study and outline the specific inclusion/exclusion criteria. Providers will be asked to identify eligible participants and share their information with us following all local policies on patient privacy including, but not limited to, obtaining written consent as needed.

4.2.5 Recruitment from the CTRU Registry

A research volunteer registry (P2021000916) was established in 2021 to maintain a list of people interested in participating in clinical research with the MGH Clinical and Translational Research Unit (CTRU). The goal of the CTRU, located in CNY 149, is to serve the clinical and research departments, institutes, centers, programs

and labs that comprise the MGH Neuroscience community and to be a foundational component and research core facility within MGH's emerging translational programs. Eligible participants who have consented to be contacted for future research may be identified using data stored within the volunteer registry.

4.2.6 **Recruitment from the MAINAH Registry**

The Northern Light Health site will be recruiting from their internal research registry, the Maine Initiative for Neurologic Aging and Health (MAINAH). With over 800 enrollees and growing, including people from all over Maine, of all ages, many of whom either have family members with dementia or themselves have some cognitive symptoms. All enrollees have communicated interest in participating in research. MAINAH registry participants will be contacted using an IRB-approved email to let them know about this opportunity.

5. SUBJECT ENROLLMENT

5.1 Informed Consent Process

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations, and ICH Guidance Documents pertaining to informed consent.

Potential subjects will be given general information about the research (e.g., through informational sheets, letters, or discussion with their treating physicians). If they are interested in learning more about the study, they will then contact the research coordinator. The research coordinator will obtain verbal consent in accordance with Partner's Prescreening Guidelines prior to performing a telephone prescreening interview. If the subject meets pre-screening criteria, including scoring a 27 or greater on the Telephone Interview for Cognitive Status or scoring a 3 or greater on the Cognitive Functioning Instrument, and wishes to continue the screening process, a virtual, Zoom videoconference screening visit will be scheduled. If the subject resides outside the state of Massachusetts, the screening visit will take place in person at MGH CNY campus.

At the screening visit, the investigator will meet with the potential subject to review and discuss the details of the study using the informed consent document as a guide. A copy of the informed consent document will be sent to the subject before the screening visit takes place, and they will have at least 24 hours between when the document is received and when the screening visit is scheduled in order for the subject to have adequate time to fully review the Informed Consent Document.

The informed consent process will be conducted using a REDCap-based electronic consent form. The consent form has been developed in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data for other users. If requested by the subject, a traditional hard copy consent form will be provided to the subject along with any materials needed for return shipping.

During the screening visit, the informed consent document will be reviewed with the participant by an experienced clinician investigator. The potential subject may ask to speak with the physician investigator should a non-physician investigator be involved in obtaining informed consent. The informed consent discussion will include all of the required elements of informed consent, including the purpose of the research, the procedures

to be followed, the risks and discomforts, as well as potential benefits associated with participation, and alternative procedures to study participation. Their questions will be answered to their satisfaction. The subject will be provided with adequate time to reflect on the potential benefits and risks and possible discomforts of participation, and to make an informed decision.

After reviewing the consent form in full, the informed consent worksheet will be administered verbally to the participant by an experienced clinician investigator. The subject must get 70% of questions 1-10 correct, and 100% on questions 11-14. If the subject does not get 70% of questions 1-10 correct and 100% on questions 11-14 on the first attempt, the investigator will review the details of the study again with the subject. The subject will then be given another opportunity to answer the consent worksheet questions. If the subject does not answer 70% of questions 1-10 correct and 100% on questions 11-14 on the second try, they will be considered unable to consent and therefore ineligible for the study.

If the participant is deemed capable of consenting, a link will be sent to the REDCap e-consent form or the subject will sign the provided hard-copy informed consent form. For REDCap e-consent, participant signatures will be obtained using electronic signature via mouse followed by electronic signature by the experienced clinician investigator. Signed hard-copy consent forms will be mailed back or given to the study team and signed by the consenting investigator upon receipt. Upon completion of the informed consent process, participants will be provided with an electronic copy of the signed consent form and hard copies will be stored in each subject's binder. If a participant prefers a hard copy of the consent form, it will be given to them at their Lead-In in person visit.

While some subjects will have MCI, they must be deemed capable of providing informed consent by the Investigators. Subjects who are not capable of providing informed consent will be excluded from this study.

5.2 Remuneration

Subjects will receive $25 for their participation in each Visit. Subjects will receive $100 for the first optional LP they undergo, and $75 for each additional optional LP they elect to receive. If subjects elect to undergo all three LPs, they will receive a $50 LP sub-study completion bonus. If subjects elect to participate in MRIs, they will receive $75 for the first MRI, $100 for the second MRI, and $150 for the third MRI. Subjects will also receive $100 upon completion of the overall study as long as they were at least 80% compliant with all at-home study activities, including medication dosing, brain games, and Fitbit activity tracking. Therefore, if a subject elects to undergo all additional procedures and are at least 80% compliant with at-home study tasks, the maximum amount a subject would be eligible to receive is $925 at study completion. If a subject does not elect to participate in any optional procedures but is compliant with study tasks, they would be eligible to receive $300. We anticipate some subjects may come from outside the region and if so, appropriate travel expenses may be reimbursed including transportation up to $150. To minimize the inconvenience of frequent visits, meal and parking vouchers for use at the MGH Charlestown Navy Yard (CNY) facilities will also be offered at each visit.

5.3 Randomization

Subjects will be randomized into one of two counter-balanced block sequences (see Figure 4 below). Randomization will be done by MGH Clinical Trials Pharmacy.

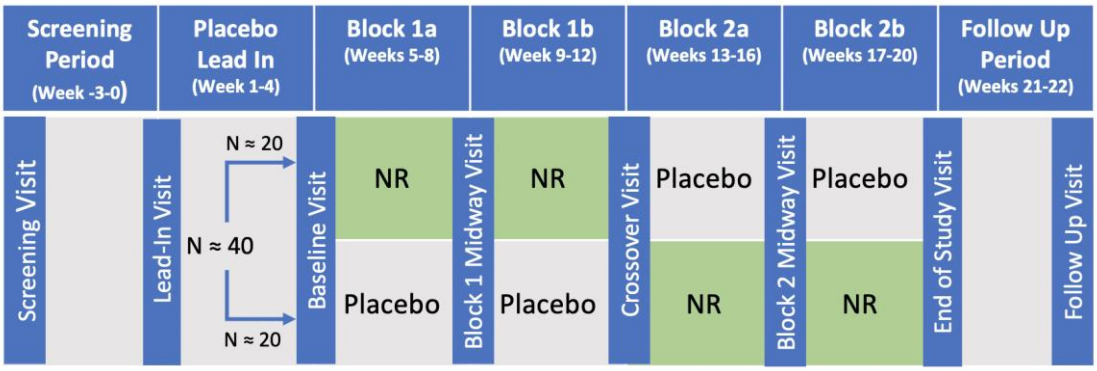


Figure 4. Study Schema

5.4 Reasons for Withdrawal

A study subject will be discontinued from participation in the study if:

- Any clinical adverse event (AE), concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets any exclusion criteria (either newly developed or not previously recognized).
- The subject is less than 80% or greater than 120% compliant with NR dosing.

Subjects are free to withdraw from participation in the study at any time upon request.

5.4.1 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. An Early Termination visit will occur when a subject withdraws consent, i.e. withdrawing his or her participation in future study procedures

5.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the investigator, there is sufficient reasonable cause.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Unsatisfactory enrollment.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.

If the study is prematurely terminated or suspended, the investigators will promptly inform the institution and regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the investigator, as specified by applicable regulatory requirement(s).

6. STUDY PROCEURES

6.1 Schedule of Assessments

	Days -28 to -1	Day 0	Day 28 ± 4 days	Day 56[2] ± 4 days	Day 84 ± 4 days	Day 112[2] ± 4 days	Day 140 ± 4 days	Day 154 ± 4 days	Early Termination
	Screening Visit[1]	Lead-in	Baseline	Block 1 Midway	Crossover (PBO/NR)	Block 2 Midway	End of Study	Follow Up[1]	
Informed Consent[1,3]	X								
Informed Consent Worksheet[1]	X		X		X		X		
Demographics[1]	X								
Medical History[1,4]	X	X	X	X	X	X	X	X	X
Height & Weight		X							
Vital Signs		X	X		X		X		X
Inclusion/ Exclusion Review[1]	X	X							
Safety Labs[5]		X					X	X[6]	X
Concomitant Medications & Supplements[1]	X	X	X	X	X	X	X	X	X
Physical & Neurological Examination		X	X		X		X		
Cognitive Screening Measures[1,7]	X								
Test of Premorbid Functioning		X							
Randomization		X							
Dispense Supplement/Placebo		X	X		X				
Accountability and Compliance			X	X	X	X	X		X

	Days -28 to -1	Day 0	Day 28 ± 4 days	Day 56[2] ± 4 days	Day 84 ± 4 days	Day 112[2] ± 4 days	Day 140 ± 4 days	Day 154 ± 4 days	Early Termination
	Screening Visit[1]	Lead-in	Baseline	Block 1 Midway	Crossover (PBO/NR)	Block 2 Midway	End of Study	Follow Up[1]	
Virtual Cognitive Assessment[1,8]			X		X		X		X
Virtual Neuropsychiatric Surveys[1,9]			X		X		X		X
Virtual Functional Survey[1,10]			X		X		X		X
Blood Collection for Biomarker Analysis		X	X		X		X		X
Technology Training/Check-in		X	X	X	X	X	X		
Daily Cognitive Assessment[11]		→	→	→	→	→	→		
Daily Mood and Sleep Assessment		→	→	→	→	→	→		
Daily Activity Assessment[12]		→	→	→	→	→	→		
Lumbar Puncture[13]			optional		optional		optional		
fMRI Scan			optional		optional		optional		
Adverse Events[1,14]	X	X	X	X	X	X	X	X	X

[1]These visits and procedures will take place virtually via Zoom videoconference and will take place up to 4 business days prior to the office visit date. If the subject resides out of state, their screening visit will take place in person at MGH CNY campus.
[2]Block Midway visits will be conducted over the telephone. The participant will not be required to come in MGH for these visits.
[3]No study procedures apart from the telephone pre-screening visit will be performed prior to the signing of the informed consent form (ICF). All subjects will sign an ICF prior to undergoing any study tests or procedures.
[4]After initial assessment of medical history during the screening period, updated medical history will be collected in subsequent visits.
[5] Safety labs include Hematology (CBC with differential), Complete Chemistry Panel, Liver Function Tests, B12 and TSH**.**
[6]Safety labs will only be drawn if any labs from the End of Study (Day 140) Visit were abnormal and clinically significant.
[7]Including T-MoCA **(**or MoCA**)**, BDI and IADL Scale.
[8]Including RBANS, CPT, BVMT, DKEFS Verbal Fluency Subtest, and Trail Making Test.
[9]Including BDI/BAI.
[10]Including MFQ and BRIEF-A.
[11]Lumosity Brain Games (Lumos, Labs, Inc.)
[12]Fitbit Charge 3 Wearable Device
[13]CBC, PT/INR point of care @ Days 28, 84 and 140 if undergoing optional LP

[14]All adverse events that occur AFTER signing consent form until the end of the follow up visit will be included.

6.1.1 Screening Visit (Day -28)

Subjects located outside the state of Massachusetts will complete their screening visit in person. Subjects located in Massachusetts will complete a virtual screening visit via Zoom videoconference. The screening procedures will determine the subject's eligibility for the study and will take approximately 2 hours.

The following procedures will take place at the Screening visit:

- Obtain written informed consent from the subject after assessment of capacity to consent via the Informed Consent Worksheet.
- Obtain demographics and medical history
- Administer T-MoCA (or MoCA), BDI and IADL Scale
- Review and document concomitant medications, supplements, and therapies
- Assess inclusion and exclusion criteria to determine subject eligibility
- Assess and document adverse events (AEs) after subject signs informed consent form (ICF)

6.1.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum,* the following information should be captured and entered into the Electronic Data System (EDC).

- Inclusion/Exclusion Criteria
- Demographics
- Reason for screen failures

6.1.2 Lead-In Visit (Day 0)

This visit will take place within 28 days of the Screening Visit. The following procedures will be performed and will take approximately 1.5 hours.

The following procedures will take place at the in-person office visit:

- Measure Vital Signs
- Physical and Neurological examination
- Blood draw for safety labs and biomarker analysis
- Dispense PBO
- Explain and practice how to use technological devices and software that will be used during study-computer, daily Lumosity brain games, Fitbit device, Fitbit software.
- Administer TOPF
- Assess inclusion and exclusion criteria to determine subject eligibility

6.1.2.1 Technology Check-In Phone Calls- Days 1, 7, and 21

At Days 1, 7, and 21 (± 1 day) during the initial Lead-in Period, a study coordinator will call the subject to ensure they understand how to use all technology and discuss compliance if necessary. The subjects will also be provided with the study coordinator's phone number and email and encouraged to contact the study coordinator with questions or concerns at any time.

6.1.3 Baseline Visit (Day 28), Crossover Visit (Day 84), and End of Study Visit (Day 140)

These visits will occur 28 days (± 4 days) after the previous visit. These visits will occur in two parts: the first portion will occur virtually via Zoom videoconference no more than 4 days prior to the second portion, which will be an office visit. The following procedures will be performed and will take approximately 2.5-6 hours depending on participation in LP and MRI sub-studies. If the subject has elected to participate in the LP and/or MRI sub-study, the visit may occur over multiple days as long as all tasks are completed during the study visit window.

The following measures will be performed virtually via Zoom videoconferencing no more than 4 days prior to the office visit:

- Assessment of capacity to consent through administration of the Informed Consent Worksheet
- Administer cognitive assessments
- Administer functional and mood assessments
- Assess technology compliance
- Assess and document AEs and changes to medical history
- Review and document concomitant medications, supplements, and therapies

The following procedures will occur in person:

- Measure Vital Signs
- Perform physical and neurological examinations
- Blood draw for biomarker analysis
    - End of Study (Day 140) only- additional tubes for safety labs
    - If undergoing LP, then an additional fingerstick for point of care, same day PT/INR will also be performed
- Assess study supplement compliance
- Dispense study supplement or PBO (except at End of Study Visit)
- Perform optional MRI scan
- Perform optional fasting LP

6.1.4 Block Midway Telephone Visits (Days 56 and 112)

These visits will be conducted over the phone 28 days (± 4 days) after the previous visit. The following procedures will be performed and will take approximately 30 minutes.

- Assess technology and study supplement compliance
- Review and document concomitant medications, supplements, and therapies
- Assess and document AEs and changes to medical history

6.1.5 Follow Up Visit (Day 154)

This visit will take place after 14 days (± 4 days) following the Day 140 visit. The following procedures will be performed. All procedures will be performed virtually via Zoom videoconferencing or by telephone.

- Review and document concomitant medications, supplements, and therapies
- Assess and document adverse events (AEs) and changes to medical history
- Debriefing with Investigator on subject's data during trial and experience in the study

If any labs from Day 140 were abnormal and clinically significant, the subject will also be asked to come in for an office visit blood draw for safety labs within the Follow up visit window.

6.1.6 Early Termination Visit

If the subject withdraws from the study after the Baseline visit and before completion of all study visits, they will be invited to participate in an Early Termination Visit within 7 days of stopping treatment.

The following procedures will be performed virtually via Zoom videoconferencing no more than 4 days before the in-person office visit:

- Administer cognitive assessments
- Administer functional and mood assessments
- Assess technology and study supplement compliance.
- Review and document concomitant medications, supplements, and therapies
- Assess and document AEs and changes to medical history

The following procedures will be performed at the in-person office visit:

- Measure Vital Signs
- Blood draw for safety labs and biomarker analysis
- Assess study supplement compliance

6.1.7 Protocol Deviations

A protocol deviation is any noncompliance with the current clinical trial protocol. The noncompliance may be on the part of the subject, the PI, or the study staff. As a result of deviations, corrective actions will be developed by the PI and implemented promptly. All deviations from the protocol must be addressed in the subject's documents. Protocol deviations will be sent to the IRB per their guidelines and entered in the Protocol Deviations Log in the EDC System.

6.1.8 Missed Visits and Procedures

Missed visits and any procedures not performed (not attempted) for reasons other than illness or injury will be reported as a protocol deviation. Procedures or visits not performed due to illness, injury, or disability, including procedures that were attempted but failed (i.e. weight unable to be obtained due to subject immobility) will not be reported as protocol deviations.

7 CLINICAL ASSESSMENTS AND OUTCOME MEASURES

7.1 Clinical Variables

Assessments will be performed at designated time-points throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, past medical and AD history, family history, and concomitant medication usage.

7.1.1 Safety Measures

7.1.1.1 Vital Signs, Height, Weight

Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, and temperature will be assessed at specified visits. Verbal weight may be documented for those subjects utilizing a wheelchair. Height and weight will be measured and recorded at the Lead-In only.

7.1.1.2 Clinical Laboratory Assessments

Study participants will be asked to provide approximately 13 mL of blood for safety lab analysis at the Lead-In visit (with an additional draw at the Follow-Up visit if any Day 140 labs are abnormal and clinically significant), for a total of 22 ml (about 1.5 tablespoons) throughout the study. The participant will have his or her whole blood collected by either a nurse or phlebotomist from a peripheral vein. Blood will be handled, processed, and analyzed in accordance with regulations set forth by the American Society for Clinical Pathology and the College of American Pathologists.

Tests for Complete Blood Count (CBC) and Prothrombin Time and International Normalized Ratio (PT/INR) which are required prior to performing a lumbar puncture may be performed using CLIA waived point of care testing using the Sysmex XW-100 CBC analyzer and the Roche CoaguChek XS Plus PT/INR analyzer. This will involve an additional fingerstick blood draw.

The investigator may order additional testing, if thought to be necessary, to further assess an adverse event (AE).

7.1.1.3 Physical and Neurological Examination

A physical and neurological examination will be performed at Lead-In, Baseline (Day 28), Crossover (Day 84), and End of Study (Day 140). The following systems will be examined: general appearance, head, eyes, ears, nose, throat, neck, chest, heart, abdomen, extremities, edema, peripheral vascular, skin and appendages, musculoskeletal, central nervous system and back.

7.1.1.4 Adverse Events

Once the informed consent form has been signed by the subject, inquiry about all adverse events (AEs) will be performed at each study visit.

7.1.2 Clinical Assessments

7.1.2.1 Neuropsychiatric and Functional Assessments

All neuropsychiatric questionnaires will be administered via Zoom videoconference; any required materials will be mailed or given to subjects prior to the visit.

7.1.2.1.1 Beck Anxiety Inventory (BAI)

The Beck Anxiety Inventory[21] (BAI) is a 21-item, self-administered questionnaire that measures the presence and severity of anxiety in psychiatric populations. It was constructed to avoid confounds due to comorbidity of depression, and it has been found to be highly valid, reliable, and consistent[23]. The BAI lists 21 common symptoms of anxiety and asks the subject to rate the severity with which they experience each symptom (not at all, mildly, moderately, or severely). Scores of 0-21, 21-35, or 36-63 indicate low, moderate, or severe anxiety, respectively.

7.1.2.1.2 Beck Depression Inventory (BDI)

The Beck Depression Inventory[22] (BDI) is a widely used assessment of depression severity used in clinical and research settings. It is a 21-item, self-administered rating inventory with a mean internal consistency of 0.86[24].The BDI ranks responses to several domains of depression, including mood changes, guilt, self-image, suicidality, affect, and apathy on a scale from 0 to 3 and provides an assessment of the level of depression, spanning minimal to mild to moderate to severe depression.

7.1.2.1.3 Memory Function Questionnaire (MFQ)

The Memory Function Questionnaire[20] (MFQ) is an 8 question, self-administered functional assessment which measures the frequency and severity of self-perceived memory-related capabilities. The questionnaire involves a general rating of frequency of memory problems, a retrospective intraindividual comparison of memory decline, an assessment of forgetting frequency, an assessment of forgetting severity, and an assessment of self-derived mnemonic device usage. The MFQ was determined to show high internal consisteny[20] and moderate concurrent validity with subsequent performance on clinical measures of memory[25].

7.1.2.1.4 Behavior Rating Inventory of Executive Function (BRIEF-A)

The Behavior Rating Inventory of Executive Functioning[19] (BRIEF-A) is a standardized rating scale to assess self-regulation and executive function in everyday behaviors. It is a 75-item scale including a Behavioral Regulation Index consisting of inhibition, shifting, emotional control, and self-monitoring scales and a Metacognition Index consisting of initiation, working memory, planning/organization, task monitoring, and organization of material scales. This study will only use the BRIEF-A self and not the informant report.

7.1.2.1.5 Lawton Instrumental Activities of Daily Living (IADL) Scale

The Lawton Instrumental Activities of Daily Living Scale (IADL) Scale[26] covers 8 domains of functioning and was designed to assess independent living skills in older adults[27]. IADLs are more complex than basic Activities of Daily Living (ADLs) and are therefore more sensitive to disruption due to cognitive impairment. This scale takes about 10 minutes to complete and is administered by a trained rater who will determine whether participants are functionally impaired and therefore not eligible to participate in the study per inclusion criteria 2.

7.1.2.2 Neurocognitive Assessments

All neurocognitive assessments will be administered via Zoom videoconference.

7.1.2.2.1 Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment (MoCA)[28] is a commonly used screening tool in clinical trials and research settings to measure potential presence of cognitive impairment. The MoCA measures five areas of cognitive function: orientation, visuospatial, attention and calculation, recall, and language. This measure will be completed as part of screening procedures carried out by the Northern Light Healthcare site and should take approximately 10 minutes.

7.1.2.2.2 Telephone Montreal Cognitive Assessment (T-MoCA; also referred to as MoCA-Blind)

The Telephone Montreal Cognitive Assessment[28] (T-MoCA) is a commonly utilized abbreviated cognitive screener adapted from the full Montreal Cognitive Assessment for the purposes of measuring levels of cognitive impairment over the telephone. The telephone MoCA measures five areas of cognitive function: orientation, abstraction, attention and calculation, memory, and language. The telephone MoCA will take approximately 10 minutes to complete and will be administered by experienced raters following the administration recommendations specified by the test publishers. The telephone MoCA will be administered only at the Screening visit to determine subject eligibility, and will require participants to achieve a telephone MoCA score of 16-22 inclusive.

7.1.2.2.3 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The Repeatable Battery for the Assessment of Neuropsychological Status[15] (RBANS) is a commonly used 25-minute, standardized neurocognitive battery assessing functioning across multiple cognitive domains. It is designed to be administered repeatably, with several equivalent alternate forms available. The RBANS measures a total of five neurocognitive domains using twelve subtests: immediate memory (List Learning and Story Memory), visuospatial/constructional (Figure Copy and Line Orientation), language (Picture naming and Semantic Fluency), attention (Digit Span and Coding), and delayed memory (List Recall, List Recognition, Story Memory, and Figure Recall). The RBANS has been shown to be effective at both detecting and characterizing forms of dementia. The Delayed Memory domain has been shown to be particularly sensitive to discriminating MCI due to Alzheimer's disease from controls, and also is predictive of cerebral amyloid burden[15]. The RBANS will be administered at Baseline, Crossover, and End of Study using alternate forms. The RBANS will be administered virtually via Zoom videoconference. The coding subtest response form will be mailed or given to subjects before their scheduled visits along with a prepaid envelope for the form to be returned.

7.1.2.2.4 Millisecond Continuous Performance Test (CPT)

The Continuous Performance Test is a test of sustained attention and alertness in which subjects are presented with a string of letters[29]. The subject is instructed to press a button every time they see an X and not to press it for any other letter. Letters are presented for five minutes. The subject is then given a brief break before continuing to the next part of the study, the AX task. In this section, the subject is told to press the button when they see an X that follow right after an A but not when there is an X that is not preceded by an A. The subject is then presented with another five-minute block of letters. This section of the task is also a test of inhibition and executive control. Task administration will utilize Millisecond Inquisit software, a computerized stimulus presentation platform optimized for temporally precise stimuli presentation and reaction time recording of psychological assessments. The CPT will be administered at Baseline (Day 28), Crossover (Day 84), and at End

of Study (Day 140). Task administration will utilize Millisecond Inquisit Lab software, a stimulus presentation platform optimized for temporally precise stimuli presentation and reaction time recording of digitized psychological assessments. Inquisit Web requires only a subject code and no personally identifiable information.

7.1.2.2.5 Test of Premorbid Functioning

The Test of Premorbid Functioning[30] (TOPF) provides an estimate of premorbid intellectual functioning that allows for comparisons with an individual's current cognitive status. The test consists of a list of 70 words that have atypical grapheme to morpheme translations. A premorbid estimate of intelligence is calculated based on the raw TOPF score (number of words correctly read) and up to 13 demographic variables. The tests demonstrates excellent reliability across the age groups included in this study (Ages 60-90 = 0.98-0.99), in MCI (0.98), and in AD (0.99)[31].

7.1.2.2.6 Trail Making Test

The Trail-Making Test[16] (TMT) is a graphomotor letter and number-letter sequencing task that will be administered to test processing speed, mental flexibility, and set-switching. The test takes approximately 5 minutes to complete. The standard, paper version of this test cannot be administered virtually, so the oral Trail Making Test (45) will be used at all study visits.

7.1.2.2.7 Brief Visuospatial Memory Test-Revised (BVMT-R)

The Brief Visuospatial Memory Test-Revised[18] (BVMT-R) is a test of visuospatial learning and memory with six equivalent alternate forms.[23] The BVMT-R has three learning trials, a 25-minute delay, and then delayed recall and recognition trials. The visual stimuli for each learning trial will be presented digitally. The recall and recognition portion of this assessment will be given in the traditional, pencil and paper format and scored according to published administration instructions and norms. Permission to adapt BVMT-R stimuli into a digital format was granted by the publisher of the test. The BVMT-R will be administered at Baseline, Crossover visit, and at End of Study.

7.1.2.4 Phlebotomy for AD Biomarkers

Subjects will provide additional blood samples for AD biomarker analysis at Lead-In and each block visit (Day 28-up to and including Day 140). Biomarker analysis will include NfL, Aβ42, Aβ40, tau, GFAP, neurogranin, and other analytes. At each block visit (Days 0-140), 30 ml (2.0 tablespoons) of blood will be collected for research analyses, for a total of approximately 120 ml (8 tablespoons) over the course of the study.

7.1.2.5 Magnetic Resonance Imaging (MRI)

Subjects will be given the opportunity to participate in an optional MRI sub-study at Baseline (Day 28), Crossover (Day 84), and/or End of Study (Day 140). Subjects electing to participate will be asked to complete all 3 MRIs to be part of the sub-study. Data will be collected on a 3-Tesla Siemens scanner with the following scan sequences: a high resolution T1-weighted scan, T2 FLAIR, resting state BOLD MRI, and pCASL at rest.

Resting state BOLD measures low-frequency spontaneous fluctuations in BOLD signal across time, where correlations of this spontaneous activity between brain regions are thought to reflect functional

connectivity. The 10-minute rsfMRI scan will be acquired with whole-brain echo planar imaging (EPI) time series with a voxel size of 2x2x2 mm isotropic, TR/TE = 4000/30 ms, acquisition matrix = 64x64, and 40 axial slices. Subjects will be instructed to keep their eyes open throughout the duration of the scan. rsfMRI data will be processed within the FreeSurfer FS-FAST stream using standard pre-processing steps including motion correction, masking of non-brain tissue, registration to the anatomical high-res image, sampling to the surface, and surface smoothing by 5 mm. Temporal filtering to extract signals in the 0.008-0.08 Hz frequency band will be performed, followed by linear regression to factor out mean global signal, average signal from white matter, and average signal from ventricular CSF regions. To perform functional connectivity analyses, we will use a seed-based approach to examine temporal correlations between the mean time course within anatomically-defined nodes and the rest of the brain.

ASL-MRI is another indicator of brain function, quantifying regional cerebral blood flow, which in turn is linearly coupled to regional brain metabolism and neuronal activity[36,37]. It is especially well suited to drug trials in which change from baseline is of prime interest[38–40]. Pseudo-Continuous ASL (pCASL)[40], which provides excellent sensitivity and temporal stability[41] and accurately differentiates AD from other causes of dementia[42], will be used in the protocol. The single shot pCASL scan will be acquired with the following parameters: 24 cm field of view, 2x2x2 mm isotropic, TR/TE = 4300/3.2 ms, acquisition matrix = 64x64, slice thickness = 6mm, temporal bolus = 1800 ms with a 1800 ms post labeling delay. Corresponding field map scans will also be acquired to correct for blurring and signal dropout during image processing. All data processing and analysis will utilize tools available through FSL's Bayesian Inference for Arterial Spin Labeling MRI (BASIL) toolbox.

7.1.2.6 Lumbar Puncture

Subjects will have the option of participating in three lumbar punctures over the course of the study (at Days 28, 84 and 140). Lumbar punctures will be performed after a minimum of an 8 hour fast. The subject will be positioned seated or lying on his/her side on the examination table. Standard protocols will be used employing palpation to identify the L3-4, L4-5 or L5-S1 vertebral interspaces, sterile conditions, local lidocaine anesthesia, and use of the Sprotte 24-gauge needles. Lumbar punctures will be performed by qualified, experienced practitioners. Approximately 20 cc of CSF will be collected for analysis of various AD/ADRD, redox, and metabolism and other biomarkers, including NfL, 8-OHdG, pyruvate/lactate, tau, phospho-tau, amyloid-$\beta_{42/40}$, and neurogranin. Subjects will also complete a brief REDCap survey after the LP in which they will answer questions about the tolerability of the procedure. After the LP is complete, subjects will be given time to eat breakfast or have a snack before continuing with the visit.

If a subject has difficult back anatomy or is particularly overweight, and the physician or nurse practitioner does not feel comfortable performing the LP because they are not able to locate the proper lumbar landmarks, the subject will be scheduled to undergo the LP under fluoroscopy at MGH Interventional Radiology. Fluoroscopy time will vary person to person. The average radiation dose is 0.06 mSv, or the equivalent of 1 week of natural background radiation, per LP for a total of 0.2 mSv (3 weeks of natural background radiation) over the duration of the study.

7.1.3 Daily Outcomes

7.1.3.1 Mood and sleep ratings

Before beginning each Lumosity session, the subject will be asked to rate their mood and sleep. The survey will consist of two brief questions and require no more than two minutes of the subject's time.

7.1.3.2 Cognitive Exercises

Each subject will be provided with a Lumosity (Lumos, Labs, Inc.) account. The subjects will be required to complete 6 different cognitive exercises (about 10-15 minutes) at least 6 days per week for the duration of the study (from Days 0 to 140). The assigned cognitive exercises will sample the domains of attention, processing speed, memory, cognitive flexibility, and problem solving. Compliance can be assessed by the study coordinator by logging on to each subject's Lumosity account. If the subject is not adhering to the cognitive exercise requirements, the study coordinator will contact the subject to remind them of proper exercise procedures. If a subject requires more than three such reminders, they will be considered non-compliant, and PI will determine if the subject should continue in the study or if their study participation should be discontinued.

7.1.3.3 Daily activity and sleep tracking

Each subject will be provided with a Fitbit Charge 4 that they will wear throughout the study. The device will track the participant's heart rate, activity, and sleep. The subject will be instructed to wear their device at all times, except for when it is being charged and when they are bathing.

7.1.3.3.1 Charging and syncing procedures

Subjects will be instructed to ensure that the device is charged at all times. The subject will be instructed to charge their device when their battery is low (roughly once every 4 days) during a period of inactivity, such as when watching TV. The subject will be explicitly instructed not to charge their device during times of sleep. The subject will be instructed to sync their device at least 3 times per week. Charging and syncing compliance will be routinely monitored by a study coordinator. The coordinator will conduct periodic reviews of the subject's charging and syncing compliance by logging onto the subject's Fitbit dashboard. If the device has not been appropriately charged or synced, the study coordinator will contact the subject to remind them to sync or charge their device as needed. If a subject requires more than three such reminders, they will be considered non-compliant, and the PI will determine if the subject should continue in the study or if their study participation should be discontinued.

7.2 Training and Validation

All evaluators must be certified by the study PI to perform cognitive and psychiatric outcome assessments. It is strongly preferred that a single evaluator performs all measures with a given instrument throughout the study, if possible.

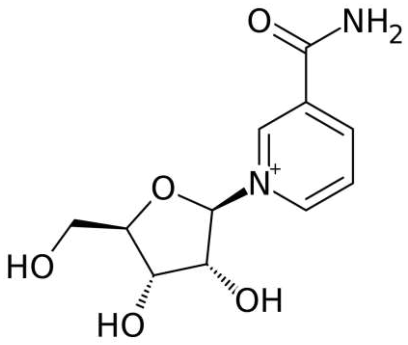
8. INTERVENTIONS ADMINISTERED

8.1 INTERVENTION

8.1.1 Study Product Description

Niagen® is a commercially available form of nicotinamide riboside (NR) manufactured by ChromaDex, Inc. Niagen® is generally recognized as safe (FDA GRAS Notice No. 635) for use in a variety of media, including vitamin waters, protein shakes, and powdered beverages. Niagen® has five issued process and use patents in the United States and several others internationally.

Chemical Structure of nicotinamide riboside:



8.1.2 Drug and Placebo

Chromadex Inc. will provide 250 mg capsules. Identical appearing capsules with only filler will be prepared as placebo.

8.1.3 Acquisition

When the study intervention shipment arrives at the site, the person accepting the shipment must perform an inventory and fill out a supplement receipt log. The contents of the shipment must be counted, verified, and inspected for damage. Any damaged shipments will be documented. The study supplement will be picked up from MGH Clinical Trials Pharmacy on the same day it will be dispensed. Detailed logs will be kept recording quantity of supplement dispensed and study staff in charge of supplement transport and dispensing.

8.2. Product Storage and Stability

Niagen NR is found to be stable under ambient conditions of 25 °C and 60% relative humidity for 11 months in a solid, powdered form.

8.3 Dosage, Preparation and Administration of Study Intervention

Niagen NR 500 mg (two capsules) or PBO (two capsules) should be taken orally twice daily with food. The capsules should be swallowed whole.

8.4 Modification of Study Intervention for a Subject

Dosing may be reduced or suspended at any time by the PI or designated licensed clinician sub-investigator. This will be documented along with the reason(s) and dates of adjustment in the CRF for each subject requiring this manipulation. The PI or designated licensed clinician Sub-Investigator may reduce or suspend the dosage of study dietary supplement or discontinue the study supplement for AEs thought to be related to the study supplement or for other reasons during the trial (the reason and dates of reduction or suspension or dose reduction or suspension must be documented in CRF). If the AE is mild or moderate, the dosage may be re-started after the event improves. If the AE is serious or life threatening and deemed to be definitely related to the dietary supplement, the study dietary supplement will be discontinued immediately. Study subjects must remain off the study dietary supplement permanently. Subjects may not resume study dietary supplement. All AEs will be followed to resolution.

8.4.1 Dosage Discontinuation

Reasons for discontinuation of study dietary supplement may include an AE or PI recommendation, protocol deviation, loss-to-follow-up, patient request, or death. All serious adverse events (SAEs) that occur in a subject who has discontinued early must be recorded and reported within 24-hours of awareness. Study subjects who discontinue study dietary supplement prematurely (early discontinuation from study) and decide not to remain in the study will be encouraged to return for an Early Termination Visit.

8.5 Assessment of Subject Compliance

Subjects will be instructed to return empty and unused study dietary supplement containers at each clinic visit or the Early Discontinuation Visit. Research staff will review returned and unused study dietary supplement and log information into the reconciliation form to determine compliance. Non-compliance will be defined as taking less than 80% or more than 120% of study agent as determined by unused capsule counts. If a study subject is non-compliant with study dietary supplement, research staff should re-educate and train the subject in administration of study agent. If the subject's non-compliance persists, it will be left up to the PI to determine whether the subject should be discontinued from the study.

9. STATISTICAL ANALYSIS

9.1 Biostatistical analysis

This is a crossover randomized controlled trial with the goal of determining the effects of a potential cognition-enhancing dietary supplement for older adults with SCD and MCI. The primary analysis will be based on an intention to treat approach and will include all subjects randomized at the onset of the trial to either NR then placebo or placebo then NR as described below.

In this study, the participants will pass through intervention blocks with administration of both NR and PBO for eight weeks administered in random order. The primary cognitive outcome measure will be the Total Scale score of the RBANS which will be administered at Baseline (Day 28), Crossover (Day 84), and End of Study (Day 140). The RBANS is a robust 25-minute, standardized neurocognitive battery with multiple alternate versions whose 12 subtests yield five Index scores, one for each cognitive domain, and the Total Scale score.

Normative data have been published for cognitively normal older adults, as well as for MCI and AD populations. For example, in a study of MCI, the mean Total Score Index was reported to be 92.4 with a standard deviation of 9.1[43]. Based on test-retest reliability, a minimal detectable change in community-dwelling older adults (including normal, MCI and mild dementia) for the Total Score is 5[43]. A "minimum clinically important difference" in MCI was reported to be 8 points[44].

The basic data of a crossover trial are the measurements obtained in 2 or more different intervention conditions occurring over time. The principal goal is to compare outcomes for each condition within a randomized block structure while accommodating repetition *within* subjects and possible effects of carryover or disease progression.

Assessment of normality of data distribution will be completed prior to inclusion in any analysis. If the distribution of the outcome variables (e.g., RBANS Total Score, etc) are not normal, appropriate transformations (e.g., logarithm, square-root, Box-Cox transformation, etc) will be applied. Data analysis at the individual and group levels for the primary outcome will begin with visual inspection of RBANS Total Scores at each assessment for differences from baseline for each measurement and differences between intervention conditions. Ignoring potential for carryover, a simple matched pair t-test will be performed using data from each condition. Should carryover effects or other time trends be suspected, we can use a regression model adjusting for sequence.

Mixed-effects models will be used to compare the scores at the end of each study period (Days 28, 84, and 140) between NR and PBO incorporating variance within and between participants**.** This within-patient level will accommodate variation resulting from the intervention crossovers as well as time effects modeled in various forms (e.g., linear and non-linear trends, carryover effects). Each within-subject regression coefficient will be treated as a random effect that can vary based on these between participant factors. The primary analysis will calculate the average intervention effect (i.e., will model the mean of the individual intervention effects as a constant). Secondary analyses will model the mean as a function of factors that vary among patients such as age and sex. These terms describe the main effects of these factors, their interactions with the intervention, and their interaction with time. The analysis will be completed using SAS PROC MIXED through a restricted maximum likelihood analysis with a mixed model with fixed effects for sequence and treatment. Appropriate covariance structure will be investigated using Akaike's information criterion (AIC).

The full multilevel model will estimate both average effects across the population of participants, as well as effects for individuals informed by the results on other participants. The predicted effects for an individual participant are weighted averages of his or her data and the averages from others. This model incorporates correlations among the measurements within an individual and enables comparison of the individual's predicted intervention effect using the multilevel model with that from using only that individual's data.

When conducting study analyses we can also calculate 95% confidence intervals for the difference in response for active treatment versus placebo treatment for descriptive purposes. Similar analyses will be applied to all

outcomes. All outcomes in this pilot study are considered exploratory and no claims are attached to them, thus multiple comparison correction is not applied. Therefore P value <0.05 will be considered significant.

Carryover is always a potential confound in crossover studies. While the pharmacokinetic half-life of NR is short (2.7 hours), its pharmacodynamic effects may be long-lived and we cannot exclude carryover and practice effects affecting measures in successive assessments if NR precedes PBO. We do not expect carryover pharmacological effects from PBO, but assessment practice effects might differentially affect measurements made in an NR intervention period that follows PBO. This may be difficult to gauge with our primary RBANS outcome measure taken only once at the beginning/end of each intervention period. However, we will have other resources with which to evaluate this using our exploratory outcomes, especially the daily cognition measures. We will investigate various adjustments for carryover, such as discarding the first measurements in an intervention period on outcomes measured daily, to determine whether results are sensitive to carryover. These adjustments can allow for potential differential carryover by intervention regimen.

10. SAFETY AND ADVERSE EVENTS

10.1 Potential Risks and Benefits

At screening, we will collect standardized historical, symptomatic, physical exam, and laboratory parameters to identify health conditions that represent risks for participation in the study's procedures. Chief among these would be unstable coagulopathies or anti-coagulant therapies that increase the risk of phlebotomy and lumbar puncture and unstable medical conditions.

10.1.1 Potential Risks

Nicotinamide Riboside: Nicotinamide riboside is well-tolerated in patients, with few adverse events reported in a minority (3.3%) of patients[6]:

- Mild nausea
- Mild skin rash
- Mild flushing/hot flashes
- Mild leg cramps
- Increased bruising

None of the reported adverse events led to discontinuation of NR during its intervention period.

Phlebotomy: The risks associated with having blood drawn include bruising and local discomfort. Rarely an infection may occur at this site, and if an infection does occur it will be treated by the study physician.

Magnetic Resonance Imaging: MRI is a safe procedure for subjects who do not have metal implants or other contraindications. Individuals who have electrically, magnetically, or mechanically-activated implants (such as heart pacemakers) or those who have clips on blood vessels in their brain will not be allowed to participate in the study due to risks associated with MRI scanning. The MRI will be operated in a manner accepted by the FDA. The protocol requires subjects to remain still within a relatively confined space during the scanning

session, and the scanner makes loud knocking and beeping sounds as it takes images. While subjects with severe claustrophobia are excluded from the study, some subjects may find the physical confinement or noise uncomfortable. Every effort will be made to enhance each subject's comfort level, and subjects will be given earplugs to reduce discomfort due to noise. If a subject notices any discomfort while in the MRI scanner, (s)he should notify the administrator immediately. If the discomfort cannot be stopped, the scanning session will be stopped. The MRI has the potential, during normal routine use, to cause localized warming of the skin and underlying tissues. Subjects should immediately inform the study staff if they experience discomfort due to warming of the skin and the procedure will be stopped.

Neurocognitive testing: The neurocognitive tests that will be administered to assess mental performance may be stressful and potentially cause anxiety, fatigue, and frustration. In our prior experience with similar protocols, risks have occurred infrequently and very few subjects have terminated testing. However, testing will be discontinued immediately upon any request by the subject to do so. With the evaluation of individuals with cognitive complaints, there is always the potential for a subject to enter the study with SCD and subsequently demonstrate a decline in cognitive performance throughout the course of the study to a level consistent with a diagnosis of MCI. If this occurs, a licensed clinician will meet with the subject and suggest that they see their primary care provider for further evaluation.

Daily Cognitive Exercises: The daily Lumosity (Lumos Labs, Inc.) brain exercises may be stressful, tedious, and potentially cause fatigue, and frustration. However, the program was designed to resemble games and the duration is short, so the likelihood of these potential risks is low.

Questionnaires: Questionnaires administered during the protocol may cause subjects to feel sad or upset about how their neurocognitive functioning affects their quality of life. Study staff is experienced with such evaluations and sensitive to these issues. Any question can be omitted per the subject's request.

Wrist Actigraphy: The wrist actigraph, the Fitbit Charge 3, may cause some minor discomfort due to prolonged wearing, but the risk of such discomfort will be minimized by ensuring the wristband fits the subject properly (e.g. is not too tight or does not cause irritation). If the subject does experience discomfort, another wristband will be ordered to try to eliminate the discomfort.

Lumbar puncture (LP): Pain may occur during the procedure. This is usually temporary, confined to the lower back, and minimized with the cutaneous and soft tissue administration of 1% lidocaine as a local anesthetic. Short-lived LP associated headaches occur in <1%-36% of subjects with an incidence that decreases with age[45–47]. Less commonly, a persistent low-pressure headache may develop as a result of a post-LP CSF leakage. Lower rates of post-LP headache have been noted with the atraumatic (Sprotte) 24-gauge needle that will be used in this study[47]. Potentially more serious, but very rare risks, include infection, damage to radicular nerves and bleeding into the lumbar CSF space. The risk of these procedure related complications is much less than 1%.

Videoconferencing: Visits that take place via videoconferencing may allow research staff to learn more about the subject's home and cohabitants than an office visit. The study team will use a secure Zoom

videoconferencing platform and conduct the visit in a private location to ensure personal information about the subject is kept confidential.

Other Risks: Reviewing health-related information might be stressful or make the subject feel uncomfortable. Subjects do not have to answer any questions they do not want to. In addition, there may be incidental medical findings as a result of the physical examination.

10.1.2 Known Potential Benefits

NR has been tested in a number of clinical trials in middle-aged and elderly patients and has shown to be well-tolerated. If successful, this trial will further understanding of the effects of NR on standard and personalized assessment of cognitive, neuropsychiatric, and everyday functioning in brain aging. This trial is also assessing multiple biomarkers in concert with clinical outcomes, which will provide a detailed understanding of NR activity and provide a well-curated data set for the Alzheimer's research community to improve our understanding of cognitive decline in aging and AD/ADRDs.

10.2 Data Security

10.2.1 Laptop computers and iPads

Subjects will be given the choice to use their personal computers or study-provided laptops to use for Lumosity cognitive exercises. Fitbit and Lumosity are web-based applications that require no PHI or personally identifiable information be entered into the system, so there are no additional data safety risks to the subject if they use their personal computers. Older adults may have difficulty adapting to new technologies, resulting in increased cognitive and perceptual demands[48]. Because a major outcome of this trial is performance on computerized cognitive brain games, it is important that subjects' performance is not influenced by the novelty of the study laptop.

If a subject does not wish to use their personal computer, they will be provided with a study laptop. Study laptops will be registered under the Principal Investigator and no identifying information will be required to sign in. The study subject will be provided with a password that adheres to Partners password requirements to log in to the computer. We will also provide an information sheet with detailed instructions on how to use the laptop to complete study tasks in.

Subjects will sync Fitbit activity tracking through the Fitbit app downloaded to their personal mobile device by logging into the application with their provided, deidentified account. If a subject does not have a compatible smart phone, an iPad will be provided. The provided iPad will be a Partners device enrolled under Mobile Iron. The Fitbit app will be downloaded prior to distributing the iPad, and all other applications will be blocked from use.

10.2.2 Lumosity software

For the successful completion of this research study, Lumos Labs will provide de-identified user accounts to the principal investigator. The research team at MGH will be responsible for assigning these de-identified accounts to research participants, as they are enrolled in the trial. These accounts will have a generic date of birth and will not contain any personal health information or personally identifying information. Lumos Labs does not collect or store any personally identifying information of research participants. A copy of Lumos Labs Privacy Policy and Terms of Service will also be reviewed by the subject during the consenting process. By consenting to the study, the subject is also agreeing to these policies and terms.

Any record of cognitive exercises and assessment data provided by Lumos Labs to MGH will be restricted to data created through or generated by study subjects or study subjects access to or use of Lumosity and will not contain personal information of other Lumosity users. Lumos Labs will send data reports via email to the study staff in a CSV file which will be downloaded and stored on Partners compliant workstations.

10.2.3 Fitbit activity tracker

A study coordinator will create a unique, deidentified email and password combination to create an account on Fitbit.com. To create an account, the following information will be required: first and last Name (a de-identified placeholder name], date of birth (January 01, year of birth), gender, height, and weight. The subject will be given the associated account information so that they are able to login, sync their device, and use the Fitbit dashboard. The subject will be notified that the researcher will also have full access to their account and will be able to freely view and download the subject's data for research purposes. A copy of Fitbit's Privacy Policy and Terms of Service will also be reviewed by the subject during the consenting process. By consenting to the study, the subject is also agreeing to these policies and terms.[41]

After the subject has completed or if they are withdrawn from the study, their Fitbit account and all associated data will be deleted. However, prior to deleting the account, the de-identified study data will be downloaded and stored confidentially on Partner's computers for study purposes.

11. MONITORING AND QUALITY ASSURANCE

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonisation (ICH) guidelines. The PI will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It will be important to report all AEs, whether serious or non-serious.

11.1 Independent Monitoring of Source Data

The PI will ultimately be responsible for the validity and integrity of the data collected at the MGH site, and for ensuring that the study is conducted in accordance with the IRB-approved protocol. After data is collected and recorded on forms, the study coordinator may input the data into the Partners approved StudyTrax EDC. Entries will be reviewed for accuracy and completeness by a second study coordinator. Finally, the PI or his designee (Co-I) will conduct monthly reviews to check that data in StudyTrax accurately reflects the data collected on the

original data capture forms. The research team (PI, Co-I, research coordinators) will subsequently meet to discuss the results of this review, as well as case report forms and source documentation.

All electronic documentation will be stored on password-protected devices in locked cabinets located in secured areas. Paper forms will be stored in locked cabinets located in secured areas.

Source documents that are sent to the subject (i.e. informed consent documents, cognitive testing materials) will be returned to the study team. To ensure validity of source documentation, the subject will put the documents into the envelope, seal the envelope, and sign the back of the envelope during the virtual visit.

11.2 Safety Monitoring

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The PI will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It will be important to report all AEs, whether serious or non-serious.

11.3 Definitions of AEs & SAEs

11.3.1 Adverse Event

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device, whether or not considered related to the drug product or device.

Examples of adverse events include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as adverse events in this trial (they are being collected, but analyzed separately). Stable chronic conditions (e.g. arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity are considered as worsened and therefore would be recorded as adverse events. Adverse events are generally detected in two ways:

- Clinical → symptoms reported by the subject or signs detected on examination.
- Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the PI and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the PI to be a component

of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the PI.

Subjects will be monitored for AEs from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, early study termination for other reasons, or following completion of the entire study). An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure.

The study procedures and the well-being of all participants will be monitored closely by the MGH principal investigator, Steven Arnold MD, and the Co-Investigators, Nadine Schwab, PhD and Alison McManus DNP, FNP-BC. Throughout the course of the study, constant feedback with the subject is maintained in order to assess comfort and safety and to minimize risks throughout the procedure. The above investigators will be responsible for determining if a subject should be removed from the study. Criteria for removal include the following: 1) if a subject is unwilling or unable to participate in study procedures 2) if the subject refuses to participate and consent, 3) if the subject acquires a medical condition that prohibits further participation, 4) if in the opinion of the MGH principal investigator, Dr. Steven Arnold, it is decided that it is not in the subject's best interest to continue participation.

Unanticipated problems including adverse events will be reported to the Partners Human Research Committee (PHRC) as described in the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others including adverse events.

The Data Management team will be responsible for the development, execution, and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets. All data will be managed in compliance with applicable regulatory requirements. The study coordinator, under the supervision of the PI, will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track, and record clinical research data. The study coordinator will be instructed to enter this information into the StudyTrax Electronic Data Capture (EDC) System. The StudyTrax platform provides password protection. An edit checking and data clarification process will be put in place to ensure accuracy of the data. Logic and range checks as well as more sophisticated rules may be built into the eCRFs to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing, or not calculated correctly. The platform will have the ability to lock specific visits to prevent any modification of data once the visit is closed. Once this option is activated, every user will have Read-Only access to the data. The PI, Dr. Arnold, will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

11.3.2 Serious Adverse Events

All adverse events will be reviewed by the MGH Principal Investigator, Dr. Steven Arnold, and will be reported to Partners IRB and to the Human Research Committee (HRC) in accordance with HRC Guidelines. A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:

1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurs.
    - This serious criterion applies if the study subject, in the view of the PI, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
    - Hospitalization for an elective procedure or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.
4. Results in persistent or significant disability or incapacity.
    - This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subject's ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject.
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for "seriousness" but is not an adverse experience and will therefore not be considered an SAE. An example of this would include a social admission (subject admitted for reasons other than medical, e.g., lives far from the hospital, has no place to sleep).

The PI is responsible for classifying adverse events as serious or non-serious.

11.4 Assessment and Recording of Adverse Events

The PI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in the EDC system.

11.4.1 Assessment of Adverse Events

At each visit (including telephone visits), the subject will be asked if they had any problems or symptoms since their last visit in order to determine the occurrence of adverse events. If the subject reports an adverse event, the Investigator will probe further to determine:

- Type of event
- Date of onset and resolution (duration)
- Severity (mild, moderate, severe)
- Seriousness (does the event meet the above definition for an SAE)
- Causality, relation to investigational protocol

- Outcome

11.4.2 Relatedness of Adverse Event to Investigational Protocol

1. Not Related:	Concomitant illness, accident, or event with no reasonable association with protocol.
2. Unlikely:	The reaction has little or no temporal sequence from administration of the investigational protocol, and/or a more likely alternative etiology exists.
3. Possibly Related:	The reaction follows a reasonably temporal sequence from administration of the investigational protocol and follows a known response pattern to the suspected investigational protocol; the reaction could have been produced by the investigational protocol or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject.
4. Probably Related:	The reaction follows a reasonably temporal sequence from administration of investigational protocol; is confirmed by discontinuation of the investigational protocol or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state.
5. Definitely Related:	The reaction follows a reasonable temporal sequence from administration of investigational protocol; that follows a known or expected response pattern to the investigational protocol; and that is confirmed by improvement on stopping of the investigational protocol, and reappearance of the reaction on repeated exposure.

11.4.3 Recording of Adverse Events

All clinical adverse events are recorded in the Adverse Event (AE) Log in the subject's study binder. Study staff should fill out the AE Log and enter the AE information into the EDC system within 48 hours of learning of a new AE or receiving an update on an existing AE.

Entries on the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

11.5 Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported via the EDC system within 24 hours of study staff being notified of the event.

- All events that meet the above criteria for Serious Adverse Events

11.6 Safety and Feasibility of Performing Experimental Visits at MGH-East (Charlestown Navy Yard)

The entire study will be performed in MGH Building 149 in examination, testing and procedure rooms of the Neurology Service on the 2nd floor. If an emergency does occur, 911 will be called promptly, with the subject being managed by the study physician until paramedics arrive. This setup has been discussed with and approved by the nursing administration at the CNY Clinical Research Center.

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