**STUDY TITLE:** Food supplement approach for prevention of age-associated cognitive decline in older adults with obesity: the nutrition for Brain and Body HEALTH (BB-HEALTH) feasibility trial

**STUDY SPONSOR:** National Institutes of Health/National Institute on Aging (NIH/NIA)

**PRINCIPAL INVESTIGATOR**

Name: Susan B Roberts, PhD

Department: Energy Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University

Telephone Number: 617-556-3238

Email Address: susan.roberts@tufts.edu

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ABREVIATIONS

AD: Alzheimer’s disease

ADLs: Activities of daily living

AE: Adverse event

AEL: Accessible Emission Limit

ANSI: American National Standards Institute

APOE: Apolipoprotein E (APOE)

AVLT: Auditory Verbal Learning Test

BMI: Body Mass Index

CBF: Cerebral Blood Flow

CBFi: Microvascular CBF

CBFV: Macrovascular cerebral blood flow velocity

CLIA: Clinical Laboratory Improvement Amendments COWAT: Controlled Oral Word Association Test

CRF: Case report forms

DHA: Docosahexaenoic acid

DPP: Diabetes Prevention Program

DSMB: Data and safety monitoring board

DSMP: Data and safety monitoring plan

SDMT: Symbol Digit Modalities Test

EEG: Electroencephalgram

EPA: Eicosapentaenoic acid

HbA1c: Hemoglobin A1c

HNRCA: Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University

MCA: Middle Cerebral Artery

MCI: Mild Cognitive Impairment

MCNS: Multicomponent Nutrition Supplement

MIND: Mediterranean-DASH Intervention for Neurodegenerative Delay **diet**

MPE: Maximum permissible exposure

MRU: Metabolic Research Unit (MRU)

NEL: Nutrition Evaluation Laboratory at the HNRCA

NIH-TB: NIH-Toolbox

NIRS/DCS: Near-infrared Spectroscopy/Diffuse Correlation Spectroscopy

NOHD: Nominal ocular hazard distance

RCT: Randomized controlled trial

SAE: Serious adverse event

Stroop: Stroop Interference Test (Stroop)

TCD: Transcranial Doppler Ultrasound

TICS-M: Modified Telephone Interview for Cognitive Status

TMTA, TMTB: Trails Making Test A and B

WL: Weight loss

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**A. STUDY SCHEMA**

Graphical user interface, text, application

Description automatically generated

**B. INTRODUCTION**

This protocol is titled Novel food-based approach for prevention of age-associated cognitive decline in older adults with obesity: the nutrition for Brain and Body HEALTH (BB-HEALTH) feasibility trial. The goal of the trial is to determine the feasibility of implementing a specific nutrition regimen used alone or in combination with a behavioral weight loss (WL) intervention on cognition and cerebral blood flow in older adults at risk of cognitive decline, and collect data on intervention adherence for power calculations to be used in an NIH proposal for a powered randomized trial.

**B.1 Background and Rationale**

1. Gaps in knowledge, and relevance and usefulness of the objectives

Declines in age-sensitive cognitive functions including working memory, processing speed and conceptual reasoning occur in most adults as they grow older (1), but individuals with overweight or obesity are especially vulnerable to major cognitive decline, dementia and Alzheimer’s disease (AD) (2-8). This is a crisis at the public health level, because the prevalence of overweight and obesity in middle-aged and older adults is increasing (currently >70% of adults over 60 years in all population subgroups (9-11)) and several ethnic and racial minority populations are particularly affected (12). Identifying nutritional interventions that support optimal cognitive functioning in older adults and slow or prevent cognitive decline and neurodegeneration would have enormous societal value. However, research on nutritional interventions has been fragmented and largely restricted to testing individual nutrition factors or general healthy dietary patterns.

2. Relevant preliminary data and background for the proposal

Our conceptual model for nutrition to support brain health in older adults with overweight and obesity focuses on nutrition support of cognition via multi-component nutrition supplementation (MCNS) and implements the MCNS approach alone and in combination with potentially synergistic effects of a behavioral WL intervention. Our model postulates that any one limiting nutritional factor has the potential to impede the collective work of supporting brain health, which may explain why the prior randomized trials of individual nutrients or chemical agents have had relatively limited success. By supplying more comprehensive nutritional support for cerebral nutrition needs, this project aims to create a favorable brain milieu in which there are no limiting nutritional factors hampering the brain’s neuroregenerative potential. By combining this MCNS approach with a behavioral WL intervention, the beneficial effects for cerebral health are potentially additive. The current proposal to be implemented in 48 enrolling adults focuses on the feasibility of the study design, participant adherence to the interventions, and gathers data to be used to refine power calculations for a powered trial.

Specific components of our MCNS are drawn from existing foods and supplements available commercially and used widely by Americans. These include cocoa (the food with the strongest evidence for beneficial effects on brain health via reducing inflammation) (13-18), as well as omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which could theoretically improve brain health including suppression of inflammation via effects on fatty acid composition of cell membranes (19, 20), and increased cerebral blood flow (CBF) (18, 21-23). In addition, our MCNS includes essential micronutrients, which are consumed in generally inadequate amounts by U.S. older adults (24). We have recently had exceptional success in applying the MCNS approach to undernourished children (25, 26), and found significant benefits for both cognitive function and CBF. While this work is not in older adults, the same principles of comprehensive nutrition theoretically apply to both groups, especially in view of the fact that both groups have low micronutrient intakes (27, 28) and share a vulnerability to cerebral inflammation and oxidative damage (29-34) that amplify risk.

3. and 4. Provide the scientific rational for and significance of the work and usefulness of the work

There are currently no effective treatments for cognitive decline, dementia and AD in old age. Under such circumstances — when ‘only prevention makes sense’ (35) — developing effective preventative strategies is of crucial importance. The expected outcomes of this initial phase of work is pilot demonstration of a successful feasibility of implementing a randomized controlled trial (RCT) on this topic, with collected data being used to apply for funding to complete a powered trial. The anticipated future powered randomized trial is expected to identify one or more feasible interventions for improving cognition in older adults with overweight and obesity, and will be a strong foundation to inform future studies on MCNSs. These results will be of vital importance in helping confront the crisis of growing numbers of older adults at risk of cognitive impairment.

5. Is this the first time the proposed supplement has been used in humans?

No, we previously used a similar supplement in children, see above. All the ingredients are available for commercial purchase.

6. Is there an active control group?

☒ Yes ☐ No

If Yes, respond to all of the following:

☒The active control is an established effective intervention. Control participants will receive similar tasting foods that are comparably healthy to other foods in the participants’ regular diet. Because the food is calorie controlled, it will beneficially support weight management as a portion-controlled item.

☒ N/A *There is no potential bias in the selection of the active control such that there will be an unfair advantage for the investigational intervention*

☒ Check to confirm that the sample size and the randomization ratio for this active control study is ethically justified with regard to the number of participants who will be exposed to the risks of the study.

**B.2 Risks to Subjects**

1. List the reasonably foreseeable risks, discomforts, hazards, and/or inconveniences to the subjects related to their participation in the research, including risk of unintentional loss of confidentiality. Include a description of the probability, magnitude, duration, reversibility, and potential consequences of the risks

Overall this is a low risk study. Risks of participation include the following.

* Physical risks
  + Possible hunger for participants randomized to the WL intervention, as well as during the fasting for screening. Risks include non-adherence and overeating as a result of hunger. These risks will be minimized with appropriate food recommendations for satiety. If hunger occurs it will be reversed on cessation of intervention.
  + Possible effects of WL, as in any WL intervention. For example, precipitation of a weight-loss related co-morbidity such as cholecystitis. Also, light-headedness or postural hypotension from WL if it results in a reduced need for medications for hypertension. This risk will be mitigated by informing participants they might have to adjust these medications if they lose weight, and recommending seeing their physician if any symptoms are reported. Participants may also report a range of symptoms when losing weight including headache, stress symptoms, sleep disorders, insomnia, back pain, diarrhea, constipation, nausea, nasopharyngitis, sinus congestion, irregular heart rate, arthralgia, asthenia, irritability, dizziness, depressed mood, anemia, fatigue, pain in extremity, multiple allergies.
  + Possible bodily injury from the recommended exercise. Common risks of exercise include injuries to the muscles, ligaments, tendons, and joints of the body and rarely abnormal blood pressure, fainting, dizziness, disorders of heart rhythm. To minimize this risk, walking and other low-risk excercises will be the only recommended exercises, and anyone reporting problems with walking during the study will be advised to stop and seek medical advice.
  + Possible symptoms due to change in diet, including transient or more persistent gastrointestinal symptoms, unanticipated allergic reactions to ingredients in study-provided foods, or boredom with the taste of particular foods over time. However, it is important to note that there are no known risks associated with consumption of the foods, supplements, or placebos included in this study, and all participants will be screened for food allergies prior to enrollment to reduce the risk of allergic reactions. All study-provided foods will be palatable and within the range of healthfulness of the participants’ usual diet. These foods will be prepared in isocaloric quantities in our metabolic kitchen by ServSafe®-certified staff, together with the commercially available supplements that are included in the MCNS.
  + Transient pain during needle insertion and a little temporary bruising, infection, or bleeding at the site of the blood draw.
  + Near-infrared Spectroscopy/Diffuse Correlation Spectroscopy (NIRS/DCS) devices (used to measure cerebral blood flow) are investigational tools and, though no adverse effects have been reported in the 10-20 years they have been used, it is possible that effects not yet reported may occur. For NIRS/DCS devices, the exposure to the skin is within than the maximum permissible exposure (MPE) to a laser beam at 670-850 nm, as indicated by American National Standards Institute (ANSI) standards Z136.1-1993. The use of American National Standards Institute (ANSI) approved light levels do not involve any known risk. The laser light used to conduct the measurements has very low power and is considered to present minimal risk–that is, no more risk than the subject would encounter in everyday life.   
     The lasers inside the NIRS/DCS devices are class 3B, but at the sensor, because the laser beams are very divergent, during standard operation their Accessible Emission Limit (AEL) is between class I and low-end class 3R. Hence, they do not pose any known risk to the eye for accidental viewing. Nevertheless, we will take all precautions to ensure that the sensors are not pointed towards anyone’s eyes. The light is turned on only after the sensor is attached to the head and turned off before the sensor is removed.

The NIRS/DCS systems for distances lower than the nominal ocular hazard distance (NOHD) may exceed MPE for eye exposure as 3R laser products. While the risk of injury in most cases is relatively low, the risk increases with exposure duration. To avoid any risk to the subjects, extended direct exposure to the eye should be avoided. We rely on the oversight of study staff members, trained as laser operators, during measurements to promptly turn off the laser if the optical sensor is accidentally detached.

NIRS/DCS devices require coupling optical sensors to the skin on the scalp. This is achieved by fastening the optical sensors to the head with either tape and/or bandages. The subject's hair may need to be parted in the location of the probes to provide better coupling to the skin. This procedure does not cause pain or distress. It may cause some minor skin irritation, but this effect is no greater than that encountered from clinical electroencephalogram (EEG) monitoring sensors. There is a slight potential for allergic reactions to the adhesives used, however to mitigate this participants will be screened for history of allergies including adhesives before measurement.

* + Subjects will be asked to keep their head relatively still for the monitoring periods of up to approximately 10 minutes during the NIRS/DCS and TCD measurements, but strict subject compliance is not essential. Fatigue and/or boredom from performing the breath-holding task are the most likely risks in this study. Time periods between data collection will be subject-controlled in an attempt to reduce both.
* Psychological and emotional risks
  + Unintentional loss of confidentiality is always a risk in research studies, and will be minimized here with consistent application of Clinical Good Practices. The unintended consequence would be sharing of participant data.
  + While unlikely, possible psychological or emotional risk from partaking in cognitive testing.

2. State which study interventions may have unknown risks.  
☒N/A

3. State which study interventions may have risks to an embryo or fetus (if a subject is or becomes pregnant) or to a nursing infant of a study subject

☒N/A

4. Describe risks to people other than the participating subject, e.g., risks to family members, friends, others or risks to the community

☒ N/A

5. Are there any risks to study investigators or staff performing the study procedures due to research with high risk populations (e.g. prisoners, intravenous drug users, patients with major psychiatric issues, etc.)?

☐ Yes ☒ No

**B.3 Potential Benefits to Subjects**

1. Describe potential benefits to individual subjects

This study has potential for very positive weight-related health benefits resulting from weight loss. The CDC recognized [DPP program](https://www.cdc.gov/diabetes/prevention/research-behind-ndpp.htm) can reduce weight typicallly by 5-7% on average for participants. WL is reliably able to support such health benefits as reduced cardiometabolic risk factors, and is helpful in mitigating symptoms of diseases such as sleep apnea, osteoarthritis and urinary incontinence. These benefits are likely to be sustained during and possibly beyond the study period. On completion of the protocol participants who were not randomized to a WL arm will be eligible to receive a 12-week WL intervention, allowing for all participants to receive this trial component. At this time, there is no recognized benefits of the supplement being tested.

2. Check if there is no direct benefit

N/A

3. Describe any benefit to the population from which the subject is drawn

☒ N/A

4. Describe any benefit to science, society, and humanity in general

U.S. older adults are at high risk of cognitive decline and debilitating noncommunicable diseases resulting from overweight and obesity. Because this trial is testing a MCNS and WL intervention (separately and in combination) that address these issues, society would benefit enormously from having demonstrated effective interventions targeting the older population.

**B.4 Alternatives**

1. Describe alternatives to participating

The alternative to participation is to not participate in a weight loss program or too participate in your own weight loss program or one recommended by your physician or other health professional.

2. Describe the standard clinical care that may be an alternative: ☒ N/A

3. Describe how the subject can receive the research procedures/drug/device used in this study in a non-research setting: ☒ N/A

**C. OBJECTIVES**

The overarching goal of this study is to conduct a feasibility trial preparing for a powered randomized trial to identify effective nutrition-based interventions to improve cognitive and brain functioning in older adults with overweight and obesity, a population that is particularly vulnerable to cognitive impairment, dementia and AD. The prevalence of obesity continues to increase in older adults; therefore, this protocol is related to our NIH/NIA grant proposal in response to PA-18-877 (Early Stage Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline [R01 Clinical Trial Optional]) and has been designed to directly address a growing public health crisis.

Our central hypothesis is that age-sensitive cognitive functions and CBF, an important biomarker of brain health (1, 36), can be significantly increased in older adults with overweight or obesity by consumption of a MCNS used alone or in combination with a behavioral WL intervention. Our scientific premise, supported by our first-of-its-kind demonstration of improved cognition and cerebral blood flow with a MCNS in young children (25, 26) and calls for testing multiple-component interventions (37, 38), is based on the following:

1) Older adults with obesity have pathophysiological changes in the brain secondary to obesity that may lead to brain structure and function damages, including cerebral inflammation, oxidative stress, glucose-insulin dysregulation and other metabolic disturbances; (31, 32, 39-75)

2) To be effective, nutritional interventions should include a comprehensive panel of essential nutrients and bioactive constituents to support structural and functional brain remodeling and reduce inflammation and oxidative damage. These include not only essential micronutrients but also flavanols such as epicatechin and catechin (present in cocoa) and the omega-3 fatty acids DHA and EPA. However no comprehensive MCNS has been tested in older adults, and since older adults typically consume unhealthy diets (27) comprehensive MCNSs should be powerful tools to support brain health;

3) Behavioral WL interventions can also support nutrition status and reduce oxidative stress, with potential for beneficial effects on cognition (76) and cardiometabolic health; therefore, the combination of a MCNS and a WL intervention holds particular promise.

Specific Aim 1: Demonstrate feasibility of implementing a MCNS intervention and a behavioral WL intervention alone and in combination, with collection of data on changes in cognitive function and relevant additional variables in at-risk older adults

We will enroll 144 adults in the screening to have 48 adults in the study aged 60-80 years with normal cognition or mild impairment, overweight or obesity (body mass index [BMI] of 27-39.9 kg/m2) and low intakes of target nutrients in a 12-month randomized trial. A 2 × 2 factorial design will assign participants to (a) a comprehensive MCNS or an isocaloric placebo; and (b) a behavioral WL intervention or an attention control. Neurocognitive assessments will be made at baseline, 3, 6 and 12 months.

This N=48 population is intended to be merged with data in a future larger randomized trial of 268 individuals totally. We have R56 funding to start the currently planned 48 individuals and therefore focus on trial feasibility, adherence and collection of preliminary data. If lessons learned from this study indicate that no protocol changes are needed for a powered randomized clinical trial, these data will be merged with the larger dataset and therefore group differences will be evaluated here in a blinded analysis unless unblinding is required for safety considerations.

*Our hypothesis, to be tested in the future powered trial, is that cognitive function will improve in participants randomized to MCNS, WL and MCNS+WL compared to Controls, with greatest mean benefits occurring in MCNS+WL participants.* The primary outcome will be an age-sensitive cognitive composite z-score based on well-established standardized scores on 5 neuropsychological tests: Controlled Oral Word Association Test (COWAT) (77, 78); Symbol Digit Modalities Test (SDMT) (79); Stroop Interference Test (Stroop) (80); Trails Making Test (TMTA, TMTB (81, 82)). Additional tests will show similar improvements: 1) Total Cognitive Performance (Fully Adjusted Score) and Fluid to Crystalized performance ratio from the NIH Toolbox (NIH-TB); 2) The NIH Examiner (83) assessment of overall executive function and components of executive function; and 3) Auditory Verbal Learning Test (AVLT): Total Learning and Delayed Recall (84).

Specific Aim 2: Measure the effects of the MCNS and WL interventions on cerebral hemodynamics and cardiometabolic parameters in the same participants at the same timepoints as Aim 1

We will measure microvascular CBF (CBFi) with NISC/DCS, and macrovascular CBF velocity (CBFV) with TCD, during fasting and postprandial states at baseline, 3, 6 and 12 months.

*Our hypothesis, to be tested in the future powered trial, is that there will be increases in CBFi and CBFV in MCNS, WL, and MCNS+WL participants compared to Controls, with greatest increases occurring in MCNS+WL participants.*

Specific Aim 3: Exploratory analyses

We will measure intervention adherence metrics, cardiometabolic and other health variables (weight, blood pressure, hemoglobin A1c [HbA1c], comorbidities), eating behaviors, physical activity, mood, activities of daily living (ADLs), and variants in the apolipoprotein E (APOE) gene.

We will examine the time course and predictors of changes in cognitive variables, CBFi, and CBFV in different sub-groups. Also assess associations of changes in cognitive functions with changes in CBFi and CBFV.

**D. ENROLLMENT AND WITHDRAWAL**

Screening and enrollment procedures as well as documents related to MRU standard policies and procedures, approved under **IRB #6701**, will be used for this protocol. Additionally, a prescreening questionnaire will be administered over video conference or phone to determine preliminary eligibility. This will be followed by an in-person screening visit, during which a fasting blood and urine sample, as well as vital signs will be obtained, and the participant’s diet and medical history will be reviewed by a nutrition professional. Participants will be given samples to taste of the supplement-containing foods to be used in the study, and asked to rate the acceptability of each. They will also receive instructions on completing 3 separate diet recalls, to be done by phone after the screening visit and before the baseline visit.

**D.1 Inclusion Criteria**

1. Adult women and men aged 60-80 years.

2. BMI of 27.0-39.9 kg/m2 at screening (note higher BMIs not used at this time to facilitate program adherence);

3. Scores at 34 or greater on the Modified Telephone Interview for Cognitive Status (TICS-M) at screening.(85) The purpose of this screen is to ensure that participants can give consent and implement the study requirements including the outcomes testing.

4. Willing to be randomized and participate in all study components including consuming the foods and supplements, participating in the WL intervention or control meetings, being available for outcome assessments, using the provided home Wi-Fi scale and activity monitor daily, and completing questionnaires; also providing the login information for scales and activity monitor so investigators can download the data.

5. Satisfactory screening review of health history questionnaire by nursing staff for relevant factors including no evidence of exclusions listed below. This will include verbal confirmation of completing a full course of vaccination against COVID-19 consistent with the current Centers for Disease Control recommendations. The reason for this study component is that live group meetings are planned with other participants, and this will reduce participant risk.

6. Has access to computer or smartphone with Wi-Fi and possesses a freezer at home with space for supplement storage.

7. Rates representative food with characteristics of the MCNS and control foods at least 4 on a 5-point scale of liking at screening, and says is willing to eat daily.

8. Average energy intake in 3 24-h dietary recalls to be within physiological (i.e. plausible) range.

None of the following are eligible to participate:

a) Adults unable to consent

b) Pregnant women

c) Pregnant minors

d) Minors

e) Wards of state

f) Non-Viable neonates

g) Neonates of uncertain viability

h) Prisoners

**D.2 Exclusion Criteria**

Describe criteria for exclusion

1. >25% percentile in most recent data for US adult population intake for reported DHA/EPA in screening questionnaire for rich food sources, which is equivalent to 1 or fewer servings of fatty fish/month (86, 87).

2. Regularly taking a multivitamin or DHA/EPA supplement (>1/week). Washout period of 2 months will be accepted.

3. Does not like, or alternatively reports eating >1 serving per week of >60% chocolate or cocoa.

4. Severe cardiovascular disease including stroke, heart failure, coronary bypass and valve replacement, coronary bypass or any surgical procedures or signs and symptoms of current severe cardiovascular disease.

5. History of neurological brain disease, including stroke, traumatic brain injury (moderate to severe); including prior diagnosis of a neurodegenerative disease including AD, Parkinson’s disease, frontotemporal dementia.

6. Major psychiatric disorder history (schizophrenia, bipolar affective disorder, intractable depression).

7. Hypertension not controlled to within acceptable ranges (at the discretion of the study physician); also

diagnosis of type 1 or type 2 diabetes or HbA1c at screening of >6.5, or screening labs (complete blood count with auto-differential and clinical chemistry profile) outside recommended ranges.

8. History of gastric bypass surgery, or any other surgery, medical complication or medication use that would prevent full participation in a program for healthy eating for WL and physical activity. This includes GI diseases, conditions or meds known to influence GI absorption including active peptic ulcer disease or inflammatory bowel disease(such as ulcerative colitis, Crohn’s disease), Celiac disease, Cystic Fibrosis, malabsorption disorders.

Note that use of statins and or hypertensive medications are not exclusions provided individual has not had a change in dosing level within 3 months).

9. History of chronic kidney diseases or kidney stones.

10. Currently undergoing treatment for cancer.

12. Currently have known thyroid disease or uncontrolled hypercholesterolemia.

13. History of acute or chronic pancreatitis or gall bladder disease.

14. Active WL, or weight change > 4 kg in past 6 months.

15. Consuming a restrictive diet (e.g. gluten-free, vegan, Paleolithic diet).

16. Self-reported allergy to any ingredient in the provided intervention foods.

17. Self-reported severe allergy to adhesives.

18. Unwilling to use a mobile phone or computer with videoconference software to participate in intervention group meetings. Unwilling to eat on camera.

19. Reports of consumption of > 2 alcoholic drinks per day on average or >14/week.

20. Reports of use of recreational and/or illegal drugs.

21. Current smoker (tobacco or marijuana).

22. Non English speakers.

23. No social security number (required for stipend payment).

2. Describe how the eligibility criteria will be assessed

Interested individuals will first be pre-screened by telephone using a script, after providing informed verbal consent. One or more screening telephone interviews will ensure that the volunteers are fully aware of all study activities and the full participant study burden as well as exclusion criteria. A health history will be conducted as part of this screening phase, as well as a cognitive screen, body mass index (BMI) screen and preliminary diet screen.

Potentially eligible subjects will then be invited to screen in-person for the study in order to further determine eligibility. During the screening visit, written informed consent for the screening portion only will be obtained by authorized Metabolic Research Unit (MRU) personnel using the screening informed consent form. The consent interview will take place in a private area in the MRU at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts (HNRCA). Subjects will then undergo routine blood tests, weight and height will be measured, the inclusion and exclusion criteria will be reviewed again, and during the next 1-2 weeks participants will have three 24h recalls on random days to confirm their ability to give a plausible dietary record and that their intake fits the inclusion and exclusion criteria.

The research team will meet to review the results of the screening, and if the subject meets study criteria and the team agrees that the participant is likely to be compliant with all aspects of the protocol, they will be invited to participate in the main study. These stepwise recruitment criteria allow for identification of individuals who are likely to be highly committed and therefore are at lower risk of dropping out.

Prior to study enrollment, subjects will read the main study consent form. Once all of their questions and concerns are addressed, and they understand all procedures in the study, a designated staff member will obtain their informed consent in person using the main study informed consent form. Subjects will be provided with a copy of the signed consent form for their records.

3. State who will determine eligibility

After screening is completed the research team will meet to review the screening data. Only individuals who are eligible based on the predefined screening criteria will be considered. A consensus on participants who are invited to enroll will be reached. In the case of any individuals for whom consensus is not reached, Dr Roberts will make the decision.

4. Can study subjects participate in another research study while participating in this research study?

☒Yes ☐ No They could enroll in a survey, but not in another intervention trial.

**D.3 Withdrawal of Subjects**

1. Describe circumstances under which participants will be withdrawn without their consent

Participants will be withdrawn from the study without their consent for the following:

a). If they are unable or unwilling to complete all baseline testing.

b). If reported adherence to supplement/placebo consumption is <75% or meeting attendance for the WL intervention/attentional control is <75% during Month 1.

c). Participants must have at least 1 post-intervention cognitive assessment at month 3 or month 6 to continue to be eligible to continue after 6 months.

d). Weight loss of >10% over 8 consecutive weeks or BMI < 23 kg/m2. It is unlikely that any participant will achieve these levels.

e). Any significant medical event or SAE that would indicate risk to the subject of remaining in the trial.

2. Describe the procedures that will be followed when subjects are withdrawn

Information on the grounds for withdrawal are listed in the consent form. As appropriate (e.g. for low supplement adherence) participants will be notified if they are at risk of being withdrawn without their consent. Anyone who is withdrawn will be called by the PI or Study Physician, and will receive a follow-up email confirming the withdrawal. Subjects who withdraw during pre-screening will have all information destroyed. Subjects who withdraw after the screening visit is completed will have information retained, but no further information will be collected from them. We will tell them about any new information that may impact their health or welfare.

3. Describe any necessary safety precautions to be applied to subjects who withdraw or are withdrawn ☒N/A

**D.4 RECRUITMENT AND RETENTION**

**D.4.1 Local Recruitment Methods**

1. When where and how will potential subjects be recruited?

As with previous studies conducted by our laboratory, in addition to recruitment by the research team this study will utilize Volunteer Services, which is an experienced core recruiting facility in the MRU of the HNRCA that specializes in targeting individuals for participation in aging research. Recruitment will start after IRB approval is obtained and will continue until N=48 subjects are enrolled. We will be following the procedure previously approved in IRB protocol #6701, with deviations stated in this Protocol.

Participants will be recruited using several strategies by the research team and Volunteer Services unit. These will include local print and electronic media (e.g., advertisements in newspapers, craigslist, newsletters, bulletin boards, websites and blogs by influencers) as well as posting flyers in public places such as local YMCAs, senior centers, health centers, supermarkets, libraries, laundromats and local community organizations. The research team also will give informational presentations at local organizations and host information tables at these organizations and public meetings. Importantly, Volunteer Services has access to a roster of >40,000 potentially qualifying men and women, who can be contacted through the use of direct mailings if their age and BMI fall within the study’s inclusion criteria. Only subjects in the Volunteer Services database who have agreed to be contacted for future studies will be contacted for this study.

*1a) If potential subjects will be recruited by telephone, describe how many times the research team will attempt to call / leave a voice message*: 3-5 times depending on whether contact is made with the potential subject. If no contact is ever made, the attempts will be lower end but if contact is made and calling back at another time to complete the call is requested, the attempts will be at the higher end.

☒Check to confirm that a script for both the telephone conversation and the voice message is included with the submission.

*1b) When subjects respond to recruitment material, describe the information that will be provided to them about the study and the information that will be collected from the subject*. Information provided to prospective subjects will include a summary sheet titled “Study Overview” that describes briefly what the study is about and what is required from the participant. This will be attached to a PDF to an email for virtual communications. Information collected from prospective subjects will include name, age, telephone number(s) (up to 2), email address(es) (up to 2) and home address. In addition, inclusion/exclusion criteria (as listed above) will be reviewed to determine initial eligibility.

Describe also, how many times you will attempt to respond to call the subject back / leave a voice message: 3-5 times depending on whether contact is made with the potential subject. If no contact is ever made, the attempts will be lower end but if contact is made and calling back at another time to complete the call is requested, the attempts will be at the higher end.

☒Check to confirm that a script for both the telephone conversation and the voice message is included with the submission.

*1c) If potential subjects will be recruited at institutions that are not owned and operated by Tufts Medical Center or Tufts University, check one of the following*. ☒Activities at non-Tufts institutions are limited to one or more of the following:

Informing prospective subjects about the availability of the research,

Providing prospective subjects with information about the research (NOT obtaining consent or acting as representatives of the investigators),

Providing prospective subjects with information about contacting investigators for information or enrollment,

☒Obtaining the prospective subjects’ permission for investigators to contact them.

2. Source of subjects (for example, patient population, local community, etc.)

Participants will be recruited from the general public. In addition, although employees will not be specifically targeted for recruitment and enrollment, employees of Tufts University and/or the HNRCA (employee-subjects) who voluntarily wish to participate in the study will be eligible for screening and enrollment. In order to qualify for the study, prospective employee-subjects must respond to IRB-approved advertisements of their own accord and will not be directly approached for recruitment. Members of the research team, as direct-report subordinates of the PI, and anyone who is direct-report subordinate to any of the research team members in any other capacity, will not be eligible to participate in the study. If prospective employee-subjects qualify to participate in the study, they will not participate as volunteers during hours in which they are being compensated by Tufts University for their regular work; this includes the use of vacation, personal days or sick time to cover time spent on study procedures.

3. Methods that will be used to identify potential subjects

Recruitment methods are described in detail above. Both in-person and virtual recruiting will be conducted, and via web platforms. Information will be conveyed via print and electronic media (e.g., advertisements in newspapers, craigslist, newsletters, bulletin boards and websites) as well as posting flyers in public places such as local YMCAs, senior centers, health centers, supermarkets, libraries, laundromats and local community organizations. The research team also will give informational presentations at local organizations and host information tables at these organizations and public meetings.

4. If print and media advertisements will be used specify when/where/how long

These recruitment avenues will be continued until all participants are enrolled (estimated 6 months).

☒Check to confirm that any necessary permission will be obtained for posting/airing these (for example, permission to post a flyer on a bulletin board).

5. If recruitment material is being mailed or otherwise distributed, submit the proposed material and describe where/how the distribution list will be obtained.

Materials are being submitted. Distribution lists will include the HNRCA internal email list and mailing lists purchased for study use or ☐ N/A

6. Describe how the recruitment methods described will be effective in attracting the targeted subject population

We anticipate high interest in this study and will use the wide range of recruitment approaches listed above. Informational sessions will be conducted virtually when feasible.

**D.4.2 Study-Wide Recruitment Methods**

Is this a multicenter study where subjects will be recruited by methods not under the control of the local Tufts site (e.g., call centers, national advertisements)?

☐ Yes ☒ No

**D.4.3 Projected Enrollment Numbers**

1. Projected enrollment numbers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ethnic Categories | | | | |
|  | Not Hispanic or Latino | | Hispanic or Latino | | Total |
| Racial Categories | Female | Male | Female | Male |  |
| American Indian/Alaska Native |  |  |  |  |  |
| Asian | 3 | 3 |  |  | 6 |
| Native Hawaiian or Other Pacific Islander |  |  |  |  |  |
| Black or African American | 9 | 3 | 3 |  | 15 |
| White | 72 | 42 | 6 | 3 | 123 |
| More than One Race |  |  |  |  |  |
| Total | 84 | 48 | 9 | 3 | 144 |

\*Individuals reporting more than one race will be further questioned to identify specific categories for inclusion. Overall percentage of non-Hispanic white projected as 85%.

2. Indicate the type of enrollment location

Enrollment will occur at the HNRCA.

3. Inclusion of individuals across the lifespan

This is a study of older adults who are overweight or obese. Therefore, the age group will be restricted to 60-80 years, which qualifies as an older population. Our team works in the HNRCA which specializes in studies of older adults, and our laboratory has conducted many studies on this demographic.

4. Inclusion of women and minorities

Our recruitment targets for percentages of women vs. men will reflect the general population for the 60-80 year age-group (53.5% women/46.5% men based on national census data). We are basing our enrollment projection on national averages for individuals 65 years, which have a slightly high proportion of ethnic and racial minorities within older adults compared to Greater Boston: 77% non-Hispanic White, 9% African-American (not Hispanic); 8% Hispanic origin; 4% Asian; 0.6% Native American or Native Hawaiian; 0.7% more than 1 race), vs. 83% of the population of older adults categorized as non-Hispanic White in Greater Boston. Note that Native American and Native Hawaiian categories are moved to Hispanic or Latino for practicality of recruiting in Boston.

5. Inclusion of Populations affected by the Disease or Condition being studied

Individuals with overweight and obesity will be included in this study because they are at high risk of cognitive decline. In addition, we will include individuals without measurable cognitive decline and those with slight cognitive decline. We will not include people with moderate-severe Mild Cognitive Impairment (MCI) or dementia due to the need to obtain informed consent and the anticipated difficulty of achieving adherence to the study protocol in participants with such conditions. By including senior centers for older adults in our recruitment plan, we will target a population at particular risk of cognitive decline that might particularly benefit from the interventions.

**D.4.4 Payment**

Will subjects receive money, gifts, or any other incentive for participating in this study?

This does not include reimbursement for expenses, which is considered in the next section.

☒ Yes ☐ No

1. Proposed payments. Subjects will receive monetary compensation for time and effort spent on study participation.

2. Payment amount: $1,525.

3. Method of payment: check.

4. To whom payment will be made (subject, parent [which one], legally authorized representative): Payments will be made directly to participant – mailed to them.

5. Payment schedule. Participants will receive compensation for completing the following study activities: screening at the research center ($25 – one month after the screening visit); baseline assessments ($200); 3-month and 6-month assessments ($300 each), 12-month assessment ($400), $10 for each weekly check-in for the first 6-months, and monthly check-in for the last 6-months (total of $300). The weekly payments will be included in the payment after each visit (e.g., weeks 1-12 will be included with the 3-month visit check). The payments will be mailed to the participant as a check after completion of each cycle of the study. The participant should expect to receive the check within 2-3 weeks after mailing. However, in the event of a government shutdown or other emergency situations, payments will be delayed until the shutdown/emergency ends and all systems are restored at the HNRCA. All payment tracking logs are followed by using previously approved IRB protocol #6701.

**D.4.5 Reimbursement**

Will subjects be reimbursed for their expenses, such as travel, parking, meals, or any other study related costs?

☒ Yes ☐ No

1. What qualifies?

Travel to and from on-site assessment visits and (if they occur) monthly visits for supplement collection will be reimbursed (parking and subway tickets). (The study team may alternatively organize and pay for Uber, Lyft or taxi transportation, in which case there is no reimbursement.) Travel for screening visits and for any optional in-person DPP sessions will not be reimbursed.

2. Documentation

Receipts will be required for reimbursement.

3. Frequency of reimbursement

Quarterly.

4. How will reimbursement be made?

By check in the mail.

5. The reimbursement schedule

Participants will be reimbursed within 30 days of the visit.

**E. COSTS TO SUBJECTS**

Does the research involve any costs to subjects?

☐ Yes ☒ No

**F. STUDY DESIGN**

**F.1 Study Timelines**

1. Describe duration of individual’s participation

After enrollment and baseline testing, subjects will be studied for 12-15 months. Completion of the study will be contingent on availability of research funding.

2. Describe anticipated duration to enroll subjects at Tufts

6 months.

3. Describe estimated data for investigators to complete study

12 months for N-48 subjects.

**F.2 Procedures**

1. Summarize the research design and sequentially identify all procedures to be performed to accomplish the specific aims of the project

This is a parallel-group, randomized, placebo-controlled, 2 × 2 factorial trial comparing the MCNS, WL and MCNS+WL with a placebo control for assessing feasibility of the planned approaches, participant adherence to the interventions, and collecting preliminary data on group differences in cognition, cerebral hemodynamics and related health variables. Participants will have overweight or obesity, low dietary intake of target nutrition in the MCNS and no evidence of moderate-severe MCI or dementia (which would hamper assessment of effects). Randomization will occur after baseline testing, and participants will remain in their randomized group throughout the entire study period. The MCNS and placebo will be provided by the study team for daily consumption. Participants will receive instruction and support for adherence and will be discontinued for low predefined adherence metrics. Adherence metrics for supplement consumption and WL intervention implementation will be captured. Neurocognitive and cerebral hemodynamic outcomes will be assessed at baseline, 3, 6 and 12 months. Weight change and other relevant variables will be measured throughout the study. Details of the interventions and procedures are given below.

a) Interventions

An illustration of the 4 groups is given below and a description follows. All participants will be contacted weekly for the first 6 months and monthly thereafter, and a brief Zoom meeting conducted while the participant consumes the supplement, to monitor adherence. These zoom meeting will be one on one with the participants assigned counseler and/or study team member. Participants will be informed of this requirement prior to enrollment. Any adverse events since the last meeting will also be reviewed at each of these meetings.

Text

Description automatically generated

Immediate Weight Loss Program Groups:

* One group is assigned to the immediate weight loss program for one year and receives Nutrition Supplement 1. Another group is assigned to the immediate weight loss program for one year and receives Nutrition Supplement 2.
  + - Either Supplement 1 or Supplement 2 is the placebo, and the investigative team and the participants will be blinded to which supplement this group receives.

No Immediate Weight Loss Program Groups:

* One group is assigned to receive no weight loss intervention during the 12-month intervention period when supplementation occurs. They will receive Nutrition Supplement 1. Another group is assigned to receive no weight loss intervention during the 12-month intervention period when supplementation occurs. They will receive Nutrition Supplement 2
  + - Either Supplement 1 or Supplement 2 is the placebo, and the investigative team and the participants will be blinded to which supplement this group receives.
    - They will also be offered a 12-week weight loss program (no outcome measurements will be made during this time) after completion of the intervention period, to support recruitment and retention into the study.

b) Procedures   
A schema for the outcome assessments in all participants is given below, and each method is described in greater detail below. The questionnaires listed below may be completed online at home during the 2-week period prior to assessments at the research center, if the participant so chooses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Assessments at the research center | | | |
|  | Visit 1  ‘Baseline’ | Visit 2  3-months | Visit 3  6-months | Visit 4  12-months |
| Fasting weight and vital signs (10-20 mins) | X | X | X | X |
| Fasting blood sample (5-10 minutes) | X |  | X | X |
| Urine sample (5 minutes) | X | X | X | X |
| Fasting brain blood flow (8-15 minutes) | X | X | X | X |
| Breakfast meal (15-20 minutes) | X | X | X | X |
| Cognitive assessments (70-100 mins) | X | X | X | X |
| Fed-state brain blood flow (8-15 mins) | X | X | X | X |
| Questionnaires (20-60 minutes) | X | X | X | X |

The MCNS foods and supplements

The MCNS is a nutritional intervention, designed to be consumed to elicit the beneficial effects of improved dietary quality by providing a substantial proportion (>50%) of the daily value of essential nutrients and nutrients associated with improved cognitive function. While the MCNS incorporates, in part, pre-formed nutritional supplements, it is primarily comprised of food-based ingredients and in composite presented as three options of common foods and is therefore classified as a food according to FDA guidance. The two MCNS palatable food items are designed to have high levels of polyphenols, especially flavonoids, and especially those that cross the blood-brain barrier. >50% of essential micronutrient requirements on average will be provided not including calcium (to prevent competitive absorption with other divalent cation nutrients), and including choline to 550 mg, DHA to 1600 mg and EPA up to 400 mg (93).

Examples of food items include: 1) a single-portion brownie or 2) a protein shake (see submitted documents on Drug page of eIRB). All food items will be prepared with commercially available ingredients selected due to their high-flavanol composition such as cocoa (88, 89), green tea, and due to their high polyphenol content, and high-satiety profile, in order to support the primary outcomes of the experiment group (cognitive function and weight loss). Towards the weight management outcome, macronutrient targets are 10g protein and 5g fiber or more per serving, with a maximum calorie target of 300 kcal/serving. All active supplement ingredients are identified in the food and drugs section, along with an overview of the recipes containing the common household ingredients used for the food items. Modification of the common household ingredients (e.g., grams of beans) may occur. Exact recipes, with quantities and calories, will be submitted as modifications when finalized.

Supplement Option:

Once the study has started, if the participants communicate that they are unwilling to consume the food items, they will have the option of receiving supplement pill and powder form options. This will include the omega 3s, multivitamin, polyphenol supplement and protein supplement with choline. Products will be as identified in the food and drugs section.

Placebo foods and Supplements

Placebo foods will be isocaloric and low-flavanol items (<45 mg total flavanols) (88), that are similar in taste and appearance and equivalent in palatability to the active intervention foods, as in our past studies (90, 94). Placebo supplements will be ordered that are similar in appearance to MCNS ones but do not contain essential micronutrients, DHA and EPA. Placebo foods will be the same food forms as active food items. Placebo supplement options will also be available. These are also listed in the food and drugs section (see submitted documents on Drug page of eIRB).

Preparation, delivery and safe handling of the foods and supplements

The Metabolic Kitchen of our core facility will prepare the MCNS/placebo foods and batch the supplements/placebos for distribution to participants via in-person pickup (every 4 weeks) or delivery. Because the food item options will all meet study requirements for composition, they will be interchangeable, and participants will request their desired products. Study participants place orders to state how many of each item they would like, so they can receive a variety and not be expected to consume the same item each day.

Instructions for food and supplement consumption and tracking intervention adherence

The Research Coordinator and/or interventionist will speak with each participant and develop a feasible plan for developing a new habit for regular daily consumption of their study supplement as a substitution for food they would otherwise consume. Participants also will be instructed to keep an online daily log of food and supplement consumption, and adherence will be reviewed in a regular Zoom videoconference with the participant and a study team member in which the participant consumes their daily supplement ration. Security guidelines and Hosting Best Practices defined by TTS will be implemented to ensure the most secure settings are used. This adherence review will be conducted weekly for the first 6-months and monthly thereafter. The study team will work with any participants with food and supplement adherence < 75% to identify barriers and a correction plan. Participants who do not achieve at least 75% adherence during Month 1 will be withdrawn.

Weight loss intervention

For the WL intervention, an interventionist blinded to supplement randomization will implement the CDC recognized Diabetes Prevention Program (DPP), which involves weekly meetings for 16 weeks and monthly group meetings thereafter, based on online materials (95). We will offer both in-person sessions, anticipating that many seniors will prefer this, as well as videoconference sessions. We will also include regular individual check-ins by email, videoconference, or phone or text as in our previous use of this method (96). Because the study will feature rolling enrollment, new participants will have individual or small-group meetings for 1-4 weeks before joining larger group sessions. Participants will be assigned to group meetings based on convenience of the meeting time without consideration of supplement randomization because supplementation is double-blinded, and will be allowed makeup sessions for missed groups.

Meeting content and delivery will be implemented as outlined elsewhere after interventionist training (97). Twenty-four weeks of DPP intervention content have been used by our team in a videoconference-delivered intervention for military families in five US states (98); these materials, with any updates from the developers, will be used in the current study. The program requests that participants provide food logs to the interventionist weekly as a core focus (in the videoconference-based version of this program, these logs will be provided via email). Starting Week 1, goals for exercise (7000 steps) and diet (calorie reduction with healthy foods) are implemented in parallel. Meeting attendance, compliance with self-monitoring activities, and all interventionist contacts with participants will be tracked by the interventionist. DPP materials are generally healthy, low-energy-dense choices. In this study participants will also be provided with information and handouts supporting consumption of foods in the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet, such as berries and green leafy vegetables (99).

Each participant within the immediate WL group will receive a Wi-Fi-enabled scale and a wrist-worn FitBit activity monitor will be provided to each participant to facilitate tracking for weight and activity. Subjects will be permitted to keep their products if they would like after the study completion.

Weight loss control

Participants who are not assigned to the immediate WL group will be contacted by the Study Coordinator or the interventionist on the same schedule as the WL group to collect information on supplement adherence and adverse events (AEs). These contacts will be via telephone, text, email or videoconference as preferred by the participant. In addition, control participants will be wait-listed to receive a 12-week version of the DPP WL intervention after the end of the 12-month trial.

*1a) How individuals will be screened for eligibility.* Screening and enrollment procedures as well as documents related to MRU standard policies and procedures, approved under **IRB #6701**, will be used for this protocol. Prior to proceeding with pre-screening, a member of Volunteer Services or the research team will obtain verbal consent from prospective participants via telephone and or videoconference. Pre-screening interviews will be conducted by Volunteer Services, other staff within the Metabolic Research Unit or the research team to ensure that prospective participants are aware of inclusion/exclusion criteria, study activities, and expectations (e.g., time commitment) of full study participation. If the pre-screening questionnaire can not be completed in one phone call, it can be completed in multiple phone sessions (up to 5).This thorough pre-screening process will facilitate triage of individuals who are not only interested in the study but also confident that they will be able to complete all study activities, and appear to be eligible based on information that can be obtained remotely. This will be achieved via the Prescreening Questionnaire and the Telephone Interview for Cognitive Status-modified (TICS-m).

Individuals who pass pre-screening will be invited to attend an in-person screening at the HNRCA to further determine eligibility. Subjects will be asked to come in fasted (8-12 hours) before their visit. Prior to completing screening activities, written informed consent (Screening ICF) will be obtained by a member of the study team using the screening informed consent form. Information on study requirements may be given to individuals one at a time or in small groups if multiple people screen on the same day. Written informed consent only will be obtain after the individual’s questions and concerns have been addressed in full and the consent interview will take place in a private area in the HNRCA. After consent is obtained, participants will have their vitals (pulse, respiration rate, temperature, blood pressure), height and weight measured, and will complete a detailed medical history with a Metabolic Research Nurse (MRU), which will include a verbal review of their covid vaccination status. A nurse will then draw 12 ml (less than 1 tablespoon) of blood from a vein in their arm for laboratory tests, including a Complete Blood Count with differential (CBC) and a comprehensive chemistry profile. They will be provided with a standard breakfast (muffin or bagel,coffee or tea, and orange juice). They will complete a questionnaire containing questions about their demographics, health habits, prescription and over-the-counter medications and dietary supplements that they take and foods they eat. They will also talk with a nutrition professional to review their diet history and will taste some foods similar to the foods planned for this study to see if they would like to eat them regularly. At the end of the screening visit, if they meet the study requirements, a member of the Dietary Assessment Unit will provide instructions and materials for completing diet recalls by phone. In the 1-2 weeks following screening, subjects will complete 3 24-hour recalls on random days to confirm (a) their ability to provide a plausible dietary record and (b) that their intake aligns with inclusion and exclusion criteria. Throughout the screening process, hardcopy and electronic case report forms (CRF) will be used to document screening eligibility and inclusion and exclusion factors.

The research team will review the results of each screening. If a prospective participant meets study criteria, and the team agrees that the participant is likely to be compliant with all aspects of the protocol, they will be invited to participate in the trial. Using the main informed consent form, designated study staff will obtain written informed consent from each participant prior to enrollment. Informed consent will either be obtained at the start of visit 1 or a separate visit after all screening criteria is met depending on work-flow and subjects preference. The prospective participant can stop the consent process at any time if they wish to not continue. The consent interview for the main trial will take place in a private area in the research center.

The study’s stepwise recruitment criteria will support efficient recruiting while allowing potential participants to self-exclude who are less committed.

*1b) Procedures being performed to monitor subjects for safety or to minimize risks.* A weekly call for 6-months followed by a monthly call to review AEs. In addition, AEs are additionally reviewed when participants come into the research center for assessments.

*1c) All drugs and devices used in the research, their regulatory approval status, and the purpose of their use*. No drugs are used in this study. All food items/biologics are available in stores/over the counter. TCD is approved by the Food and Drug Administration and materials on this method are appended. The NIRS/DCS research device is IDE exempt on the basis that the testing is noninvasive, does not require invasive sampling procedure(s), is not used as a diagnostic procedure without diagnosis by another, medically established diagnostic product or procedure, and is not being used to determine safety or effectiveness for commercial distribution. Although the device introduces energy into the subject in the form of non-ionizing light, the device is compliant with the ANSI permissible light standards and is considered a non-significant risk device. We will comply with the common rule and 21 CFR 50/56. Materials are appended on NIRS/DCS for review as part of this IRB application. See 4c below for other device details

*1d) The source records that will be used to collect data about subjects.* All CRFs will be provided.

2. Is there a placebo control arm?

☒ Yes ☐ No

If Yes, respond to all of the following:

*2a) Describe the scientific, methodological, and medical reasons to use a placebo.* The placebo is an isocaloric food and supplement regimen without the specific ingredients intended to support cognitive health in the MCNS group. Without a placebo it would not be possible to tell whether changes in cognition were due to provided food or to the specific properties of the food. All placebo food items are within the composition of normal diets of US adults, and the placebo supplements are inactive. The placebo foods and supplements will not be unhealthy relative to foods typically consumed by participants. The foods also will be portion controlled, which is a well-known method of facilitating weight management; therefore they will be beneficial as replacements for home-prepared foods. Placebo foods will be prepared with the same considerations for food safety preparation and storage as the active intervention foods.

*2b) Describe the care that will be given to the placebo group*. The placebo group for the supplement will receive identical care to the active supplement group.The placebo group for the WL intervention will be offered a shortned version of the DPP program after the study is completed.

*2c) Describe all potential risks to the placebo group and how the risks will be minimized.* The potential risks to the placebo group are no different than those to the active intervention group. Please see **B.2 Risks to Subjects** for a list of all potential risks.

3. Describe the following concerning pregnancy testing and birth control

N/A as all women will be over 60 years of age.

4. Describe the data that will be collected during the study and how that data will be obtained.

The following data will be collected at baseline, and months 3, 6 and 12 after randomization to the interventions, unless specified otherwise.

*4a) Assessments of cognitive function (primary outcome).* Study hypotheses will be tested with dependent measures derived from performance on well-established neuropsychological tests, and also the NIH-Toolbox cognitive module (NIH-TB) and the NIH Examiner. Performance outcomes will include accuracy and speed (reaction times) measures. All cognitive tests will be completed by participants at baseline, 3, 6 and 12 months within a postprandial window (between fasting and 3-hour postprandial NIRS/DCS and TCD measures), to standardize testing between and within participants. These tests will occur once at the beginning of the visit (fasted) and once after the participant has eaten the breakfast meal.

Five standardized scores from 5 tests will be obtained and summed to yield a composite executive function z-score, which will be the primary outcome measure: COWAT,(77, 78), SDMT(79), Stroop (80), and TMT-A/TMT-B (81, 82). This battery of well-established tests has been used in the evaluation of older adult subjects with overweight/obesity in the Look AHEAD trial (100) and also in Dr Cohen’s bariatric surgery study (40).

In addition, the AVLT will provide two dependent measures of verbal learning and memory (AVLT-Total Learning, AVLT-Delayed Recall) (84). Secondary outcome measures of overall cognitive function and executive function respectively will also be derived from the NIH-TB (77, 101-106) and the NIH Examiner (83). NIH-Toolbox (NIH-TB) and NIH examiner have shown to be clinically reliable and valid (77, 102, 103). Dr Cohen and his colleagues are using both batteries in ongoing NIH-supported studies and clinical trials (50) and have published previous studies showing the sensitivity of NIH-TB to cognitive function in older adults (107, 108). The cognitive battery is summarized in the table below.

**Table:** Cognitive Tests

|  |  |  |
| --- | --- | --- |
| TEST | DOMAIN(S) ASSESSED | DESCRIPTION |
| Primary Outcomes | | | |
| COWAT | Controlled response generation | Examines phonemic verbal fluency and response generation. Participants verbalize during 1-minute periods as many words as possible that begin with particular letters (e.g., H, O). The restriction of response set requires executive control and executive function for controlled response production. | |
| SDMT | Working memory, focused attention, processing speed | Widely used and well standardized test speed and accuracy in the coding symbols associated with specific numbers is a highly sensitive tests of brain dysfunction. The final score is the total number of correctly entered symbols in 90 seconds. | |
| Stroop | Executive inhibitory control, focused attention | Assesses the extent of slowing created by attentional interference created by the demand of naming colors that are discordant with the printed word of a different color. The score on the Color-Word interference trial is compared with scores on color word reading and color naming in the absence of interference. For each of the 3 tasks, scores are based on the number of colors correctly named in 45 seconds, with these scores computed to yield a Stroop Interference z-score. | |
| TMT-A | Attention, speed of processing | Involves the use of a pencil to connect a series of numbers in ascending order that are distributed over a sheet of paper as quickly as possible. Completion time provides the performance measure that is converted to a z-score. | |
| TMT-B | Attention and executive control – inhibition (set switching, processing speed) | Similar to Trail A, except that alternation of an ascending sequence between numbers and letters distributed over the page is required (e.g., 1-A-2-B…). The number-letter sequence is connected by pencil. Completion time provides the performance measure that is converted to a z-score. | |
| Additional Outcomes | | | |
| AVLT | Learning and memory | The AVLT is a verbal learning and memory test. A list of 15 words is presented to the participant over five trials with recall assessed immediately following each trial. Recall for these words is assessed after presentation of an interference list B after the five trials. Delayed recall of the first 15 word list is then assessed after a 15-minute interval. | |
| NIH-TB | Fluid and crystalized cognitive function | The cognitive module of the NIH-TB was developed to assess fluid and crystalized cognitive functions via computerized (IPAD) administration. It takes 30-45 minutes to complete and contains seven primary tasks (Flanker Inhibitory Control and Attention Test, Dimensional Change Card Sort Test, List Sorting Test, Pattern Comparison Processing Speed Test, Picture Sequence Memory Test, Picture Vocabulary Test, and Oral Reading Test). | |
| NIH Examiner | Executive functions | Assesses executive and related functions. It consists of tasks to assess fluency, inhibition, insight, planning, set shifting, working memory, and social cognition and other behaviors requiring executive control. It is conceptually well grounded in neuropsychological theory and empirical evidence, provides a more comprehensive assessment of executive functions, has strong reliability and validity (83), and is sensitive to executive function impairments in clinical populations (102, 103, 109-112). | |

Training and quality control for cognitive measures: During the study, the research assistant will record 10% of the assessments with only their identity shown in the video (i.e., the camera will be directed away from the participants face), which will be reviewed with feedback given to optimize performance.

*4b) Safety measures and reporting of adverse events.* Participants will be called weekly for capture of adverse events and food/supplement adherence for 6 months and then monthly thereafter.

*4c. Measurement of cerebral blood flow and cerebral blood flow velocity.* For these measurements, we will use an 8 channel NIRS/DCS system and a TCD device.

The NIRS/DCS device includes 1 laser source at 850 nm and 8 detector channels arranged in an optical probe similar to the one we used for our recent study of young children (25, 26). The device also includes 8 low power laser diodes at wavelengths ranging from 670 to 830 nm, and 4 detectors for simultaneous near-infrared spectroscopy (NIRS) measurement of hemoglobin oxygenation. One DCS detector will be located at 5 mm from the source to measure scalp blood flow, and the remaining detectors will be collocated at 20-25 and 30 mm from the source, to increase depth sensitivity in adults and average their signal to increase signal to noise ratio. The NIRS sources will be co-localized with the DCS laser and the detectors will be localized at 2, 2.5 and 3 cm. An NIRS additional detector will be placed 3.5 cm from the source. The DCS and NIRS data are acquired simultaneously without optical interference. We will also use additional devices to monitor and continuously acquire data on the subject’s physiology. These devices are FDA approved or IDE Exempt, completely safe, and commonly used in hospitals. Specifically:

* A pulse oximeter will be attached to one finger to measure arterial oxygenation.
* Three single-use electrodes will be attached to the upper chest and lower belly and will be connected to an electrocardiogram (ECG) device to continuously monitor the heart.
* One fabric belt will be placed around the chest to measure breathing rate. The belt will go over the subject’s clothes and will not be tight.
* A small accelerometer will be attached over the NIRS-DCS sensor to detect and isolate motion artifacts. This accelerometer is smaller than a 10-cent coin and will not be in direct contact with the skin.

DCS (and NIRS) data will be acquired for 30 seconds in a forehead location, with the optical probe gently touching the skin of the subject. DCS measures will be repeated in the left and right frontal cortex close to the hairline up to 6 times. Then we will attach the sensor to the location in the forehead which gave us the best signal with tape and bandages, and we will record data while the subject performs a breath holding task. For this task, the subject will be asked to hold his/her breath for 20 seconds every 30-60 s for 6 times. We will then conduct a posture changing procedure, where the subject will be lying face up in a bed that can be tilted up and down from the horizontal position. This type of bed is known as a tilt table. The subject will be secured to the bed and the changes in posture will be gradual. We will start with the subject lying in the flat position for 1 minute, then we will tilt the head down at around a -20-degree angle for 1 minute. Next, we will tilt the head up at around a 20-degree angle for 1 minute and ask the subject to sit for 1 minute. Finally, we will ask the subject to lay back flat for 1 more minute. We will take detailed notes of the forehead location to make sure that at the following visits the measurements are done in the same locations.

We will ensure the irradiance transmitted to the subject remains within American National Standards Institute limits for safe skin exposure. In addition, our sources will maintain a Nominal Ocular Hazard Distance of less than 10 cm, so no eye protective measures are required.

The TCD device includes a handheld 2 MHz transducer probe. We will scan left and right middle cerebral artery (MCA) through the temporal bone window. If the subject temporal bone window is too small we will not perform the TCD measurements. The blood flow velocity at rest will be recorded for 1-2 minutes on each side. For the TCD we will strictly adhere to the standard protocols for safety and good clinical practice, and will perform all tests as indicated by the Food and Drug Administration.

Data processing of raw data for both NIRS/DCS and TCD will be performed blinded under Dr. Franceschini’s guidance, by the Massachusetts General Hospital collaborators using her MATLAB codes, and analyses of processed data will be performed by the study statistician and graduate students who will also be blinded to treatment code. A data sharing agreement will be implemented before data is shared. All data shared will be in a de-identified format.

We will standardize the NIRS/DCS and TCD measurements across time using similar principles to those that are well established in our lab for measures of resting metabolic rate. For the day before each testing visit (baseline, 3, 6 and 12 months), subjects will be instructed to eat as they usually do and avoid strenuous physical activity for 24 hours. On the morning of testing they will arrive at the research center after an overnight fast or at least 3 hours since the last meal and will rest, meet the researchers and have vital signs taken. Each measurement day will involve multiple measurements by NIRS/DCS and TCD in the fasting state (no food, or caffeine containing items overnight or for at least 4 hours) and in a standardized postprandial state after consuming the randomized food and supplements in the provided daily amount together with additional foods to make up a complete meal of approximately 300 kcal. A standard meal of approximately 300 kcal/serving will be given at baseline that does not contain either the MCNS or Control foods. Photographs will be taken at each measurement to provide quality control data and to ensure repositioning of the optical and TCD sensor in the same location during the multiple visits (to within ±2 mm of baseline positioning).

*4d). Health measures.* Measurements of weight will be taken to ±0.1 kg at baseline, 3, 6 and 12 months in light indoor clothing when participants arrive at the research center for the outcome testing sessions. Our standard protocol (114, 115) requires the use of the same calibrated instrument over time and the removal of shoes, outer clothing and heavy items prior to stepping on the scale. Immediately after weight measurements, brachial systolic and diastolic blood pressure will be measured using standardized procedures (115).

*4e. Adherence metrics.* The adherence metrics planned for the MCNS/placebo are weekly logs of food and supplement consumption. Participants will track these metrics daily and provide their logs to the Research Coordinator during the contacts designed to review adherence and adverse events.

Adherence to the WL intervention has 3 components: WL, activity data and dietary intake. Wi-Fi scales and wrist-worn activity monitors will be provided to all participants for continuous use throughout the trial. This remote data collection will reduce participant burden related to these assessments. Participants will provide their login to the devices so that the interventionist can review data being collected.

For dietary adherence, we will use the multiple pass interviewer-administered 24-h dietary recall method, which is the only method recommended by NIH for individuals with obesity, on account of its acceptable accuracy and ability to provide rich details about consumption (116). Three daily recalls will be performed on random days by telephone or videoconference at screening as an eligibility criterion (to determine if plausible records can be obtained based on our usual criteria (117)) and that data will be used as baseline information for enrolling participants. Three recalls will also be collected on random days after each visit at the 3, 6 and 12 month timepoints. Collected data will include efforts to obtain both general dietary intake information for the period of collection and specific information of particular relevance to this study (e.g. type of chocolate consumed, if any). The records will be analyzed to quantify daily nutrient intakes and also intakes of nutrients of particular interest (cocoa polyphenols, DHA+EPA, % adequacy of micronutrients relative to Dietary Reference Intakes (118)), using Nutrition Data System for Research software (Nutrition Coordinating Center, University of Minnesota, Version 2021 or latest). Information will be used to calculate a Healthy Eating Index score (119) and also a MIND diet score (99) which will be used as metrics for adherence to the dietary recommendations of the WL intervention.

*4f). Questionnaires.* Demographic variables and family history of dementia and AD will be assessed at the start of the study and updated as relevant. In addition, health-related quality of life, functional activities, sleep, depression, eating behaviors, activity and activities of daily living will be assessed, using NIH-recommended instruments when available.

5. Specify which procedures, tests, visits are part of usual standard of care at Tufts.

None. Participants are research subjects, they are not studied as part of usual care at Tufts.

6. Specific which tests are routinely performed for clinical care but are providing data for research.

None. All tests are for research.

7. For Humanitarian Use Device.

***☒*** N/A

**F.3 Evaluations**

Will you perform any laboratory tests for this study?

☒ Yes ☐ No

1. Laboratory tests

A 12-mL blood sample will be taken at screening. Screening blood samples will be analyzed immediately for the exclusion criteria: HbA1c > 6.5%, and routine blood chemistries and urinalysis. Any remaining sample will be discarded.

At baseline, 6 and 12 months a 30-ml blood sample will be taken and processed for storage for batched analyses in the HRNCA’s Nutrition Evaluation Laboratory (NEL).Because of the likely centrality of changes in circulating glucose and insulin sensitivity (120), we will measure HbA1c, glucose and insulin at each time point (HbA1c data will be used from the screening test). *APO4* genotypes also influence risk of AD and therefore will be measured (121, 122) along with brain amyloid content (123). Omega-3 fatty acids in red blood cells will be measured to provide an unbiased assessment of changes in intakes of DHA and EPA (124). For these assays, we will process the blood samples for plasma, serum and red blood cells, to batch analyses. At baseline, 3, 6 and 12 months, a single void urine sample will be collected to test for biomarkers of supplement consumption. Urine tests will be run at the completion of the study. Results of the intra-study blood and urine tests are for research purposes only and will not be shared with participants.

2. Describe laboratory methods for appropriate longitudinal and cross-sectional analyses

Samples will be measured at the time of collection for screening analyses and at convenient times when batched for other analyses. The assays use standard methods that do not require batching for quality control but convenience and economy favor batching. Samples will be saved for repeat analyses as necessary for data quality once measured.

3. Number of laboratories to be used?

One (HNRCA).

4. **☒**Check to confirm that laboratory tests that will be performed are in compliance with [Clinical Laboratory Improvement Amendments (CLIA) of 1988](http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124105.htm).

5. Storage of samples

Samples will be stored at -80C prior to analyses except for HbA1c (which is measured on fresh sample).

**F.4 COLLECTION, STORAGE, AND USE OF HUMAN BIOSPECIMENS FOR UNSPECIFIED FUTURE RESEARCH**

Will bio specimens be collected and stored for future unspecified research.

☒ Yes ☐ No

We will store plasma, serum and red blood cells for future unspecified laboratory analyses. Participants will initial the consent form for approval of this study component, and may opt out by not signing.

Will biospecimens be collected and stored for unspecified future research?

Yes  No

If Yes, respond to all of the following:

1. Describe the role of biospecimen collection and storage in this study (check one):

Biospecimen collection and storage for unspecified future research is the primary purpose of the study

Biospecimen collection and storage for unspecified future research is an optional part of the main study

1. Describe the following:
   1. Where biospecimens will be stored: HNRCA at Tufts University in Boston
   2. How long they will be stored: 10 years
   3. How biospecimens will be accessed for future research: Through written permission from Principal Investigator
   4. Who will have access to the biospecimens for future research: The research team for this study
2. Specify what types of biospecimens (blood, urine, stool, tumor, embryonic germ, or stem cells, etc.) will be stored and how they will be collected (blood draw, bone marrow aspiration, needle biopsy, etc.): Blood, collected during the study visit blood draws at baseline, 6 and 12 months.
3. If fetal biospecimens\* will be collected and stored, explain why they are required to achieve the research goals (cord blood and placenta are NOT considered fetal biospecimens): N/A

\*NOTE: In addition to Federal regulations, Massachusetts state regulations govern the use of fetal specimens for research (M.G.L. Chapter 112, Section 12J). For more information, refer to the [General Laws of Massachusetts](http://www.mass.gov/legis/laws/mgl/112-12j.htm).

1. The biospecimens and associated PHI are being obtained (check all that apply):

Solely for research (would not be obtained as part of standard clinical care)

As part of standard clinical care (leftover/discarded)

In addition to that which would be obtained for standard clinical care. Specify how much more will be collected and stored for unspecified future research:

(When biospecimen collection, storage, and use for future research is an optional component of the main research study) In addition to the amount of biospecimen to be obtained for the main research study, specify how much of this additional amount is for unspecified future research; do this for each type of biospecimen to be collected and stored for future research): The Nutrition Evaluation Laboratory, the analytical core unit at the HNRCA, will handle all specimen processing. Blood will be collected for research measures outlined in the protocol. Any leftover collected blood will be stored in increments of 500 uL and used for potential research in the future.

1. Check/complete one of the following for how biospecimens are being released:

The biospecimens and associated PHI will be de-identified (stripped of all identifiers) and coded; specify where the key to the code will be kept, how it will be secured, and under what circumstances the key to the code could be revealed: The key to the code will be kept in a password-protected file by the Principal Investigator, and will be revealed only with approval from the Principal Investigator

Note: All information related to the code, including the key, is to be retained and secured by the Principal Investigator at Tufts Medical Center/Tufts University and must not be shared outside of the study team or outside of Tufts Medical Center/Tufts University.

The biospecimens and associated PHI will be de-identified (stripped of all [HIPAA](https://viceprovost.tufts.edu/health-insurance-portability-and-accountability-act-hipaa#definitions) identifiers) and not coded.

Note: “Collection date” and/or “Date of surgery” will need to be removed from the biospecimens – in addition to all of the ‘standard’ HIPAA identifiers – for the biospecimens to be considered “de-identified” per HIPAA.

The biospecimens and associated PHI will be labeled with, stored with, or associated with identifiers (for example, date of birth, date of biospecimen, zip code, etc.). Specify the identifiers and the rationale for collecting these. If identifiers will be gathered in the future and associated with the specimen, describe the frequency of gathering such identifiers. Note: If including dates or other HIPAA identifiers with the biospecimens is necessary, a limited data set may be required:

NOTE: For all applicable NIH funded research, all data and biospecimens are to be collected and stored according to the [NIH Genomic Data Sharing Policy.](https://www.govinfo.gov/content/pkg/FR-2014-08-28/pdf/2014-20385.pdf)

6. Describe procedures to release biospecimens and associated PHI, including: the process to request the use of biospecimens for future research, approvals required for use, who can obtain the biospecimens: Blood specimens will be stored in the locked freezers of the Nutrition Evaluation Laboratory at the HNRCA. They will be stored for a maximum of 10 years after the study ends and only this study’s research team members will have access to the specimens. Banked biological materials will not be distributed to any other investigator(s) located at other academic institutions. Use of the biospecimens for future research must be approved by the Principal Investigator of this study.

NOTE: The provision of biospecimens to investigators/entities outside of Tufts Medical Center/Tufts University requires a contract such as a Materials Transfer Agreement (MTA) executed by Tufts Medical Center Grants and Contracts Office ([TuftsMCGrants&Contracts@tuftsmedicalcenter.org](mailto:TuftsMCGrants&Contracts@tuftsmedicalcenter.org)) or Tufts University Office of Technology Transfer and Industry Collaboration ([MTA@tufts.edu](mailto:mta@tufts.edu)).

7. How will the biospecimens and associated PHI be labeled:

* 1. Readily identified with the source individual’s identifiers
  2. Limited data set (stripped of all [HIPAA](https://viceprovost.tufts.edu/health-insurance-portability-and-accountability-act-hipaa#definitions) identifiers except for dates, age, city, state, and/or zip code)
  3. De-identified and coded: specify under what circumstances the key to the code will or could be revealed: At any time after tissue banking permission has been granted, if the study participant wishes to withdraw consent to the research use of biological materials for future studies, he/she must inform the Principal Investigator either by phone or in writing. The code will then be broken, and the specific samples will be retrieved and destroyed.
  4. De-identified and not coded

8. Specify under what circumstances a recipient of the stored biospecimens and associated PHI would seek to contact the source individual (subject):       or  N/A, there are no such circumstances where this could occur.

9. It is anticipated that the biospecimens might be (check all that apply) or  N/A, the following will not occur:

* 1. Subjected to genetic testing (risks of genetic testing must be described in the ICF)
  2. Subjected to genome-wide analysis (must be described in the ICF)
  3. Genotype and phenotype data will be shared for research purposes (this must be described in the ICF, including with whom this data will be shared)
  4. Used to create a perpetual cell lin– - immortalized (must be described in the ICF)

Refer to the NIH’s FAQ on [Human Subjects Researc– - Human Specimens and Cell Lines](https://grants.nih.gov/faqs#/hs-human-specimen-and-cell-lines.htm) for additional information.

10. Describe the mechanism by which the research subject can withdraw permission to use the stored biospecimens and associated PHI for future research. Indicate what will happen to the biospecimens and related research data if permission is withdrawn (must be described in the ICF): At any time after tissue banking permission has been granted, if the study participant wishes to withdraw consent to the research use of biological materials for future studies, he/she must inform the Principal Investigator either by phone or in writing. The code will then be broken, and the specific samples will be retrieved and destroyed.

11. Results of any research performed on the biospecimens and associated PHI will be reported as follows (check all that apply) or  N/A, none of the following will occur:

1. Conveyed to the source individual
2. Conveyed to the source individual’s physician
3. Placed in the source individual’s medical record
4. For each checked item above, specify the rationale for conveying the results:

12.  Testing of research biospecimens is being conducted in a laboratory certified (CLIA-approved) to conduct diagnostic testing. NOTE: If patient-specific research results are reported from the laboratory and those results will or could be used for the diagnosis, prevention, or treatment of any disease or impairment, or the assessment of the health of human beings, the laboratory must be [CLIA certified](https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/Research-Testing-and-CLIA.pdf).

1. If the research tests are experimental or of unknown or unproven clinical significance and the results will be provided to the source individual or physician or placed in the source individual’s medical record, explain the rationale for this:

13.  Check to confirm that a copy of the biorepository’s written policies are uploaded to eIRB, ensuring that items a through d below are addressed. If policies are not available, please complete the following:

1. The types of investigators or entities to whom the biospecimens may be distributed: Banked biospecimens will not be distributed to investigator(s) located at other academic institutions.
2. The types of research for which the biospecimens may be distributed: Banked biospecimens may be distributed to co-investigators for additional genetic and biomedical research studies.
3. The measures taken to guard against disclosure of the source individual’s private and protected health information: There will be no samples transferred to external investigators.Stored samples will be de-identified.
4. Who will oversee the distribution of biospecimens from the biorepository to other investigators or entities: There will be no distribution of biological materials from the tissue bank to other investigator(s).

14. Describe potential risks to subjects and their families associated with the use of the subject’s biospecimens or associated PHI for future research: We do not anticipate any major risks to the subjects or their families associated with tissue banking. Measures in place include:

a. The biological materials will be stored after they have been de-identified and provided with a unique subject code.

b. The master list of the codes will be kept secured electronically (by using a password) and paper form in a locked cabinet.

c. The study coordinator will be solely responsible for all information related to the de-identification and the re-coding of the biological material to be banked. This information will then be transferred to the Principal Investigator and secured electronically (by using a password) and in a locked cabinet.

d. If the Principal Investigator turns the study over to a new Principal Investigator, the key codes will be transferred to the new Principal Investigator.

e. Banked biological material ready to be processed on-site will remain de-identified and coded.

f. When no further use of the banked biological materials has been established the stored samples will be discarded and the key code destroyed.

g. We will not go back to the medical record or study volunteer for additional health information.

a. The results of any research performed on these banked human biological materials will not be conveyed to the study participant or treating physician and will not be placed in the individual’s medical record.

15. Describe risks to groups or populations associated with the use of the subject’s biospecimens or associated PHI for future research use (considering the subject population and the potential future research): We do not anticipate any major risks to groups or populations associated with tissue banking.

Reminders:

* + Refer to the IRB’s [Policy for Human Source Material Banking (Biobanking in a Biorepository)](https://viceprovost.tufts.edu/policies-forms-guides/human-source-material-banking-biobanking-biorepository) to ensure all relevant information is included in the protocol.
  + Refer to the Biospecimen Collection section of the [HRP-351– - WORKSHEET– - Elements of Consent Checklist](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/RMVJ7HTQO134BBFJABPOB5O789/HRP-351%20-%20WORKSHEET%20-%20Elements%20of%20Consent%20Checklist_030321.docx) to ensure all relevant information is included in the ICF.

**G. ETHICS AND PROTECTION OF HUMAN SUBJECTS**

**G.1 Informed Consent Process**

Will subjects be required to provide informed consent?

☒ Yes ☐ No

1. Where the consent process will take place

Consent for pre-screening will take place by verbal consent over the phone or videoconference. The consent process for in-person screening and study enrollment will be in a private room in HNRCA.

2. Anticipated amount of time a potential subject will have to make a decision about participation in the study

A potential subject will be permitted adequate time to make a decision about participation in the study. Pre-screening can take place during one phone call or can be extended over several phone calls if needed (up to 5), and screening will involve 1 visit to the HNRCA. The three dietary recalls done after the in-person visit will each take approximately 30 minutes by phone or videoconference. After these steps, participants will have up to 1 month to decide whether to enroll in the study. During the consent process for pre-screening, screening, and the main study, participants will be allowed to take as long as needed before deciding whether to give informed consent.

3. Processes to ensure ongoing consent throughout the study

Participants will be reminded at regular intervals that, as noted in the consent form, they can drop out at any time and for any reason. This information is included in all informed consent forms.

4. Role of each research team member involved in the informed consent process

This is a low risk study, and the contact PI, MPIs and Co-Investigators, Research Coordinators, and other research team members are all trained in and can each conduct the consent process.

5. ☒ Check to confirm you will follow “[**SOP: Informed Consent Process for Research (HRP-090)**](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/GFRQ9IFG3FGKVB96RUV3Q41F9F/HRP-090%20-%20SOP%20-%20Informed%20Consent%20Process%20for%20Research_2.4.pdf)”.

6. ☐ Check to confirm non English speakers will be enrolled using interpreters.

7. Non-English speakers will not be enrolled. We will enroll participants in whom English is not their first language, provided they can communicate adequately to complete the study. Due to this being a pilot study and having only 1 Spanish speaking team member we do not have the capacity to enroll participants who do not speak enough English to communicate and complete questionnaires in English.

8. ☒Check to confirm you will follow “[**SOP: Written Documentation of Consent (HRP-091)**](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/UL3H8IJ6BNOKLBPKKE9OBC1Q6E/HRP-091%20-%20SOP%20-%20Written%20Documentation%20of%20Consent_2.4.pdf)”.

9. ☒Check to confirm you will follow “[**SOP: Remote Consent Process (HRP-092)**](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/RO2NC13LPRJ43D85S9SK2IEL06/HRP-092%20-%20SOP%20-%20Remote%20Consent%20Process_112320.docx)” if there is ever a situation where consent will not be obtained in person.

**G.2 Waiver of consent**

1. Is a waiver or alteration of the consent process being requested for this study?

Yes  No

If **Yes**, respond to all of the following:

1. *Provide the rationale for the waiver:*

The waiver is being requested to allow the investigators to pre-screen potential participants by telephone. Verbal consent would be obtained from the participant prior to administering a pre-screening questionnaire. If it is determined that the participant is not eligible for the study, then they will not have to travel to the HNRCA for an in-person visit.

1. *How the waiver or consent alteration will* ***NOT*** *adversely affect the rights and welfare of subjects: MRU study team members will be conducting the prescreening for which the waiver is requested; please refer to MRU protocol IRB-6701.*
2. *How the research could* ***NOT*** *practicably be carried out without the waiver or alteration:*

It is anticipated that the majority of participants meeting one or more exclusionary criteria would be identified by this pre-screening telephone questionnaire, which would be far less burdensome to the participant than an in-person visit.

1. *How, subjects will be provided with additional pertinent information after participation. If subjects will not be provided this information after participation, explain why:* *If subjects are eligible based on the results of the prescreening questionnaire (for which this waiver is requested) they will be invited to pariticpate in the in-person screening. Participation in in-person screening will require a signed consent. If the person is not eligible based on the results of the prescreening questionnaire, they will be informed of the reason why.*

**G.3 International research**

N/A

**G.4 Confidentiality**

1. Location of study records

Coded participant ID numbers (HRNCA IDs) will be assigned using Protocol Manager, a volunteer database and protocol management system for management of PHI robust user access restrictions in place (IRB previous approved protocol # 6701). Hard copy data forms of screening logs and informed consents will be stored in locked filing cabinets and rooms within the HNRCA. Access to hard copy data forms is outlined in IRB protocol #6701. Rooms including filing cabinets and computers that house study data will be locked when not in use by the research team. No additional paper copies of data forms will be made. Any PHI will be stored in Protocol Manager, the system the MRU uses (as referenced in IRB protocol #6701). Protocol Manager will generate a unique ID (HNRCA ID) for each participant..The records and data from each study visit will be maintained in a secure, HIPAA compliant REDCap database maintained by the HNRCA Scientific Computing Department with access restricted to essential study personnel only. These electronic records will be study data that includes all demographics, dietary and cognitive screenings, study surveys, anthropometric measures, blood chemistry measures, and cerebral blood flow measures and will be stored using the participant HNRCA ID number. The key linking this participant ID number to the respective personal subject information is kept in Protocol Manager. The video and audio files will be stored on Tufts secure box, in password-protected specific accounts for this study. They will be stored with HNRCA IDs. Record of subjects that have signed a screening consent, including records of ineligible subjects will be stored for a minimum of 10 years post study completion. Only authorized personnel may access the record. The HNRCA Volunteer Services Department is staffed during normal working hours and is locked during non-working hours. If it is necessary for a research record to leave the admissions office, it must be signed out only by an HNRCA employee (who is identified by Tufts ID) in a log of the HNRCA ID, name of employee taking possession of the chart, and the date. The records of this study might also be reviewed to make sure all rules and guidelines were followed. The HNRCA maintains a computerized database of participant information that is backed up nightly. The database is centralized under the Volunteer Services Department (IRB previous approved protocol # 6701). Computer access to the database is provided to investigators through an intranet, and electronic gatekeeper (known as a “firewall”) blocks all terminals outside of the HNRCA from gaining access. The Scientific Computing Department subscribes to Microsoft’s security bulletins and appropriate security updates are applied on an ongoing basis. If a subject withdraws or is terminated from the study, the study data generated so far will be retained as part of the study database. Coded blood sample vials (i.e identified by the participant’s ID number) collected during the study will be stored without personal identifiers (names, addresses, etc.) at the Nutrition Center for a 7-year period. These will be linked to the same key mentioned above. Statistical summaries and reports generated for distribution will not contain personal identifiers (names, addresses, etc.)

2. State where study records will be retained when the study has been closed (long-term storage)

When the study has been closed, study records will be retained in the HNRCA.

3. State who, in addition to the research team, will have access to the study files, data, and/or specimens

No one outside the research team and HNRCA personnel authorized under IRB approval 6701 will have access to the study files, data. The research team includes Maria Angela Franceschini of Massachusetts General Hospital and Ron Cohen of U. Florida, and a data sharing agreement will be signed to allow them to access deidentified data blinded to randomization.

4. In this study, will you be entering research data using a computer software application (on a computer or any other electronic device)?

☒ Yes ☐ No

4a) Tufts Medical Center studies. N/A

*4b) Tufts University studies*. ☒ Check this box if you have confirmed that the software applications used in this study are already on the list of approved software (and will be used in accordance with the details on the list)

5. Will data (or specimens) be sent outside of Tufts Medical Center or Tufts University and/or sent between Tufts Medical Center and Tufts University?

☒ Yes ☐ No

No specimens will be sent out. De-identified data will be sent out. We are working with Tufts OVPR to create the data sharing agreement needed with Massachusetts General Hospital and University of Florida before any data sharing occurs.

6. Explain how data will be transported

De-identified data will be transferred electronically via secure fileshare within RedCap.

7. Explain how data will be coded

Data and specimens will have participant ID and date of collection and type of data. The participant ID will be a unique 5-digit number (e.g. 25312). Participant identifiers will not be used.

8. Explain whether audio/videotapes will be collected

Yes. Photographs will be taken to allow for quality control in positioning the electrodes for brain blood flow assessments (same exact placement at each assessment). Video recordings, including audio, will also be taken during the cognitive testing to ensure accuracy of the measurements. These electronic video files will be used for quality control purposes only and will be kept securely on Tufts Box data storing software. Only authorized research team members will have access and they are required to have two-factor log-in security.

For the participants randomized to the 1-year weight-loss DPP program, some of the group video-conferencing sessions may be videotaped to allow the DPP counselor to review and analyize individual behavior as required by the DPP procedures. These electronic video files will be stored securely on the specific DPP Tufts Box, which will be password protected and only be accessible to the DPP counselor. They will be deleted within 12-months after they are reviewed by the DPP counselor.

9. Explain whether audio/videotapes could potentially identify the subject

Photos and videos taken to ensure accuracy of measurements will be taken so that they do not identify the subject. Videos and photographs will not take full-face photographs and will not include any distinguishing features (tattoos, unique scars), and will only be stored with participant ID and date of collection. Participants may chose to opt out if they want.

Video recordings of the group DPP programs will contain subject identifying information, such as facial and voice features, but will be stored with group session information, not participant information. Participants may opt to turn their video camera off, but pariticpation with audio is required.

10. **☒**Check to confirm that study records will be retained for the timeframe described in the record retention policy

*11.* **☒***Check to confirm that you will follow the “*[*Confidentiality and Data Security Guidelines for Electronic Research Data*](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/78UPM5090RJ4JCGPNBMJ68SE14/Data%20Security%20for%20Research%20Study%20Files_121517.pdf)*” for electronic data.*

12. A Certificate of Confidentiality will be issued (for NIH studies) or obtained  
**☒**Yes **☐**N/A

**G.5 Screening Data Collection Form/Screening Log**

Will a screening data collection form/screening log be used in this research study?

☒ Yes ☐ No

If Yes, respond to all of the following:

1. ☒ Check to confirm you have submitted the Screening Data Collection Form / Screening Log to the IRB.

2. Review the following and provide information about the Screening Data / Screening Log and how it will be used

2a) ☐ De-identified Screening Log will be provided to and/or viewed by the Study Sponsor.

2b) ☒ Identifiable Screening Log that will not be distributed or viewed outside of the institution (although the Screening Log will record [HIPAA identifiers](https://viceprovost.tufts.edu/health-insurance-portability-and-accountability-act-hipaa), the Screening Log will not leave the institution.)

Specify the identifiers that will be collected: Age, name, date of birth, address, email and telephone contacts. Screening Log data with identifiers will only be kept in computer files on password protected Tufts-encrypted computers and or locked filing cabinet. PHI and the numeric subject ID (HNRCA ID) collected at pre-screening are stored in a separate REDCap database and Protocol Manager (PM) system from the study. Study screening, enrollment and intrastudy visit data collection is recorded in a study specific database by the subject HNRCA ID. User access to pre-screening/PM and to the study database are independent and in both cases are limited to study personnel.

2c) ☐ Identifiable Screening Log that will be distributed or viewed outside of the institution.

**G.6 Provisions to Protect the Privacy Interests of Subjects**

1. Describe the steps that will be taken to protect subjects’ privacy interests

The consent process, assessments, discussions of any abnormal test results, and discussions of study exclusion/withdrawal will be conducted by phone, videoconference or in a private room in the HNRCA to protect subjects’ privacy.

2. Describe the steps that will be taken to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed

Participants will be encouraged to ask questions, reminded they can drop out at any time, and provided with phone numbers of the research team to facilitate communication. They also will meet with the PI and be contacted regularly by study team members.

**G.7 Provisions to Monitor the Study to Ensure the Safety of Subjects**

1. Describe the plan to periodically evaluate the data regarding both harms and benefits to assess subject safety

*a) The data that will be reviewed*. Data on all AEs, serious adverse events (SAEs) and unexpected events will be reviewed

*b) Who will review the data*? Data will be reviewed quarterly by the Study Physician. A data and safety monitoring board (DSMB) convened by the NIH will review the SAEs on a timetable determined by the DSMB. During the initial phase of recruitment and study of 48 individuals, efficacy data will not be assessed.

*c) How the safety information will be obtained and documented?* Safety data will be collected via call, email, videoconference, or in-person meeting, as feasible (as well as when they visit the research center for assessments). All information will be recorded by study staff using electronic or hard copy CRFs.

*d) The frequency of safety data collection*. This will be every week for 6-months and then once a month.

*e) The frequency or periodicity of review of cumulative data*. The data safety and monitoring plan (DSMP) will be approved by the DSMB prior to launching the study. Our DSMP proposes that intra-study review will be quarterly, or as requested by DSMB.

f) *The statistical tests for analyzing the safety data to determine whether harm is occurring will be as follows.* During the study, the cumulative incidence of severe AEs will be summarized by intervention group (blinded analysis). Chi-square test will be used to detect significant differences in the incidence among all 4 groups (MCNS+WL, MCNS, WL and the placebo). If a significant difference is detected in the global test, a study statistician will be unblinded to perform ad hoc pairwise comparisons to the placebo group with a Bonferroni correction.

*g) Any conditions that will trigger immediate suspension of the research or other action for the research.* An incidence of SAEs occurring at a significantly greater frequency in one of the active intervention groups compared to the placebo group would be a trigger for immediate suspension of the research. The DSMB would be informed and a detailed review of the data performed to determine whether the study should be stopped.

2. Describe the entity responsible for monitoring the data, and their respective roles

The Data Manager is responsible for collating AE data for review by the Study Physician and the PI. The PI will provide the report quarterly (or at a time frequency requested) to the DSMB and within 2 working days when any analysis indicates significantly more serious adverse events in an active intervention group compared to the placebo control. The DSMB will be requested to respond to data reports within the time period determined by their charter.

A copy of the DSMB Charter if the study is enclosed with the submission: ☒Yes ☐N/A

**G.8 Vulnerable Populations**

1. Can or will pregnant women be enrolled?

☐ Yes ☒ No

2. Can or will the research involve neonates of uncertain viability or non-viable neonates?

☐ Yes ☒ No

3. Can or will subjects who are not yet adults (neonates, children, teenagers) be enrolled? ☐ Yes ☒ No

4. Can or will minors who are: married, widowed, divorced; or the parent of a child; or a member of any of the armed forces; or pregnant or believes herself to be pregnant; or living separate and apart from his/her parent or legal guardian, and is managing his/her own financial affairs be approached for study participation for either themselves or their child?

☐ Yes ☒ No

5. Can or will wards of the state and/or children at risk of becoming wards of the state be enrolled (this includes foster children or any child that is in state custody)? Yes ☒ No

6. Can or will cognitively impaired adults (adults with impaired-decision making capacity) or adults who may lose the capacity to consent be enrolled? ☐ Yes ☒ No

7. Can or will prisoners be enrolled? ☐ Yes ☒ No

8. Can or will students and/or employees be targeted for enrollment in this research?

☐ Yes ☒ No

9. Transgender Subjects: Are you recording sex or gender for your study?

☒ Yes ☐ No. Sex is a biological variable of relevance to the effects of the MSNS and WL. We will collect self-reported identification only.

Is there a scientific and/or safety rationale for collecting information on whether a subject is transgender? ☐ Yes ☒ No

Are transgender individuals eligible for participation in this study? ☒ Yes ☐ No  
☒ Check to confirm that relevant questions for transgender and gender nonconforming individuals have been incorporated into relevant study documents (i.e. protocol eligibility, screening forms, demographic questionnaires, surveys), per the [website guidance](https://viceprovost.tufts.edu/policies-forms-guides/sex-and-gender-research-considerations).

If no, there is no scientific or safety-related rationale for needing to identify sex or gender for your study, and you will not collect information on transgender and gender nonconforming people’s identification.

**H. ADVERSE EVENT MONITORING**

**H.1 Definitions**

An AE is any untoward medical occurrence in a subject temporally associated with participation. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, etc.) or any combination of these. AEs will be labeled according to severity which is based on their impact on the subject. An AE will be termed ‘mild’ if it does not have a major impact on the subject, ‘moderate’ if it causes the subject some minor inconvenience and ‘severe’ if it causes a substantial disruption to the subject’s well-being. AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either very likely, probably related, possibly related, doubtful or not related to the study intervention. In addition, AEs will be classified as expected or unexpected depending on the event. Unanticipated problems are defined as events that are unexpected, serious and would have implications for conduct of the study.

Serious Adverse Events (SAEs) are any adverse event that results in one or more of the following outcomes: Death, A life-threatening event, Inpatient hospitalization or prolongation of existing hospitalization, A persistent or significant disability/incapacity, A congenital anomaly or birth defect, Important medical event based upon appropriate medical judgment.

**H.2 Reporting Procedures**

1. Describe the protocol-specific reporting procedures

The Data Manager will summarize all SAEs, AEs and unanticipated events reported by subjects in logs (weekly contacts to 6-months and then monthly contacts to end of the study), with additional reports requested from the subjects within 24 hours of occurrence for SAEs and important medical events). The study statistician, PI, and study physician will review quarterly and report a summary organized by group and participant ID to the IRB and NIH on an annual basis and to the DSMB on their defined schedule.

2. Specific reporting

*2a) Deaths, life threatening events:* The standard procedures will be followed (calling 911) in the case of an emergency (e.g. dialing 911). Whichever team member is first aware of the problem, they will immediately inform the PI, who will inform the Study Physician. Reporting to the IRB and the NIH Safety Officer will be within 24 hours of the study team member becoming aware of the problem.

*2b) Other SAEs*. The standard procedures for these events within the HNRCA will be followed (calling 911) in the case of an emergency (e.g. dialing 911). For emergencies occurring off-site, subjects will be instructed to contact his or her personal physician and report the emergency to the investigators as soon as possible. Whichever team member is first aware of the problem, they will immediately inform the PI, who will inform the Study Physician. Reporting to the IRB and the NIH Safety Officer will be within 24 hours of the study team member becoming aware of the problem.

*2c) Other AEs*. In the case of a non-emergency AEs within the HNRCA, these will be recorded and discussed with the PI or Study Physician and summarized monthly for review by the PI and the Study Physician. AEs occurring outside of the research center will be summarized monthly for the PI and study Physician.

*2d) SAEs that are unanticipated, serious, and/or possibly related to the study participation*. These will be reported to the IRB, NIH and DSMB as Unexpected Problems (UPs) in accordance with their respective requirements. Though less urgent in nature, unexpected and serious events that are judged not to be related to the intervention also will be reported to the IRB, NIH and DSMB in accordance with requirements.

**H.3 Reportable New Information**

☒Check to confirm that reportable new information will be reported to the IRB per the Tufts Health Sciences IRB’s [Reportable New Information policy.](https://viceprovost.tufts.edu/reportable-new-information)

**I. STATISTICAL CONSIDERATIONS**

**I.1 Study Endpoints**

1. Primary and secondary endpoints

The primary outcome is age-sensitive cognitive composite z-score based on well-established standardized scores on 5 neuropsychological tests: COWAT (77, 78), SDMT (79), Stroop (80), TMT-A and TMT-B (81, 82). Secondary endpoints are CBFi measured with NIRS/DCS and macrovascular CBFV measured with TCD, during fasting and postprandial states. Additional outcomes are adherence metrics, cardiometabolic and other health-related variables (BMI, % weight change, blood pressure, HbA1c, comorbidities) as well as questionnaire data.

2. Primary and secondary safety endpoints

All SAEs are tracked as the primary safety endpoint. All AEs are tracked as secondary safety endpoints.

**I.2 Sample Size Justification Statistical Analyses**

1. Describe the statistical analysis that will be performed for this study

The focus of analysis for our initial year of the study (N=48 will be summarizing recruitment and enrollment, demonstrating feasibility, and using the data to refine the sample size. Estimated standard deviation of 6 month change will be estimated for each group from blinded study data. Standard deviation estimates will be updated by combining prior information from our previous sample size calculation with collected data using an inverse weighted variance calculation with weights based on the sample size of each estimate. Estimated group differences in treatment effect will also be calculated from the collected data using stage-wise confidence interval estimation, and consistency of newly collected data with previous findings will be assessed. This assessment will be complete with blinded data.

Blinded study data will also be examined, as previously planned, to monitor safety of the study interventions, with a defined process described above for the unlikely event that any concerns arise. Results of all interim assessments will be reported to the DSMB.

*1a). Statistical analysis during the first year of study activity (current proposed study)*

The focus of analysis for our initial year of the study (N=48) will be summarizing recruitment and enrollment, demonstrating feasibility, and using the data to refine the sample size. Estimated standard deviation of 6 month change will be estimated for each group from blinded study data. Standard deviation estimates will be updated by combining prior information from our previous sample size calculation with collected data using an inverse weighted variance calculation with weights based on the sample size of each estimate. Estimated group differences in treatment effect will also be calculated from the collected data using stage-wise confidence interval estimation, and consistency of newly collected data with previous findings will be assessed. This assessment will be completed with blinded data.

Blinded study data will also be examined, as previously planned, to monitor safety of the study interventions, with a defined process described above for the unlikely event that any concerns arise. Results of all interim assessments will be reported to the DSMB.

*1b). Statistical analysis planned for the full, multi-year study with 12 months follow up on all participants*

If funding becomes available to complete this study after the 1-year R56, and if the study protocol remains essentially unchanged, the data collected here will be merged into the larger trial database. The study design is a 12-month, randomized 2×2 factorial placebo-controlled trial in adults aged 60 to 80 years with normal or mild cognitive impairment, BMI between 27-39.9 kg/m2, and low intakes of target nutrients. Control groups are included in the study design in order to make controlled statistical comparisons to test the effect of MCNS without confounding from differences in fiber or macronutrients. Subjects wait-listed for the behavioral WL intervention (CONTROL) will be of a social nature to serve as an attentional control and will receive the WL intervention after the end of the study. We will verify the randomization procedure produced balanced treatment groups by comparing baseline demographic (gender, race/ethnicity) and clinical characteristics (e.g. percent of individuals with normal vs. mild cognitive impairment, BMI) of participants using standard parametric and nonparametric statistical techniques. Should any imbalances be detected, we will include them as covariates in the statistical analyses, as needed. The primary analysis by intention-to-treat will include all randomized participants in their assigned treatment arm. Planned comparisons: Treatment effects of MCNS, WL, and combined MCNS+WL will be tested through contrasts for comparisons between 1) MCNS vs. control, 2) WL vs. control, and 3) MCNS+WL vs. control in the primary analysis. Comparisons of contrasts between MCNS+WL vs. MCNS, and MCNS+WL vs. WL will be performed as exploratory analysis since assessing the interaction between MCNS and WL is not the primary research objective and the study is not powered to test interaction effects. Aim 1: **Cognition** - Cognitive tests will be administered at baseline, 3, 6, and 12 months. Duplicate measures on separate days will be obtained at baseline and averaged. The overall z score will be computed by converting log-transformed scores from the individual tests to standardized scores (all individual tests except for global cognition), summing the individual tests and rescaling to obtain a standardized composite score as the primary outcome. The intervention effect will be tested using linear mixed-effects linear models to partition the fixed effects of treatment group, time, and their interaction. Subject will be included as a grouping term and a random intercept will be included in the model. Statistical significance will be set at a two-sided alpha of 0.05. The primary hypothesis will be tested by assessment of the interaction term between treatment group and time, assuming a linear time trend which tests the overall group effect (omnibus test) between all 4 arms. Planned comparisons will be estimated after the fitting of the model using contrasts with Dunnett’s method to control for experiment-wise type I error rate. Sphericity assumption will be examined using Mauchly’s test and variable transformations will be applied for outcomes that do not satisfy linear modeling assumptions of normality. We will report primary analysis results from minimally adjusted models as recommended in CONSORT guidelines,(125) adjusted for stratifying variables (sex and BMI) and baseline outcome measure only. Additional covariates will be used in sensitivity analyses to increase precision in the estimated treatment effect and address potential confounding including ApoE4 variant, education, race/ethnicity, presence of a known risk factor for accelerated cognitive decline (any of the following diabetes, hypertension, family risk of dementia or AD before 60), physical activity, and any subject characteristics found to be imbalanced at baseline. Treatment effects by sex and ApoE4 gene will be estimated and reported from analyses. Aim 2: **Cerebral hemodynamics -** CBFi and CBFV will be measured in fasting state and fed state on each study visit. Acute effects of the interventions will also be explored using repeated measures ANOVA (within-subject change between fasting and fed states on a single day) in subgroup analysis by intervention. If there are no significant differences between the two states, an average of each will be used for analyses, while if differences are observed the measurements will be analyzed separately. Statistical modeling approach for CBFi and CBFV will be implemented in the same way as described in Aim 1. Statistical significance will be set at a two-sided alpha of 0.05 for each cerebral hemodynamic outcome and no multiple comparison adjustment will be applied as these are secondary outcomes. Descriptive outcomes (changes in cardiometabolic health, self-reported healthy, and activities of daily living) will be summarized by intervention. Aim 3 (Exploratory analyses): **Time-course effects and adherence -** To examine the pattern of intervention effects over time for all cognitive variables, CBFi, and CBFV, nonlinear time trends within the mixed-effects models will be fit using restricted cubic splines or polynomial terms, and their use will be informed by local regression smoothing plots. Exploratory per-protocol analysis will be conducted excluding low-adherent participants. **Predictors of cognitive and cerebral hemodynamic responses -** Predictors of cognitive and cerebral hemodynamic responses will be identified using association analysis. Predictors in consideration include adherence for the intervention, measured dietary intake of constituents of the MCNS (flavanol, vitamins, and omea-3 fatty acids), and cardiometabolic measures (including blood pressure, lipid panel, HbA1C, T2D, obstructive sleep apnea and arthritis, and genetic variants of the ApoE4 gene). Multiple linear regression models will be used with adjustment on intervention group. **Individual cognitive functions –** Assessment of individual cognitive functions will be performed using the same modeling approach as Aim 1.

*Missing Data.* Every effort will be made to minimize missing data. The main source of missing data will be from incomplete study visits. Tufts HNRCA Metabolic Research Unit is experienced in procedures to maximize continued participation for study subjects that minimizes the amount of missing data. Last observation carried forward will be used on outcomes for subjects who do not complete 12 months, but have measured outcomes at 3 or 6 months. As cognitive function and cerebral outcomes are expected to decline over time, this alternative analysis will provide conservative estimates of treatment effect without estimating further treatment benefit after drop out.

2. Provide a sample size justification

2a) The sample size is based on the first year enrollment for a larger trial that will be submitted for competitive funding. Data will be collected in a way that will allow them to be combined with the larger trial upon anticipated receipt of additional funding.

*2b) Sample size calculations for the full study if approved.* The original study plan was to enroll 268 participants in order to have 200 completers (50 per group).The sample size was chosen to have at least 80% statistical power for both the primary outcome, composite z score, and key secondary outcomes, cerebral blood flow index and cerebral blood flow velocity. Statistical power is calculated using simulations based on a mixed-effects linear model with subject-specific random intercepts and fixed effects for group, time, and group by time interaction using outcome-specific correlations among the 4 repeated measures. *Primary Outcome:* ***Cognition -*** In the previous study, high cocoa flavanol supplementation (8 week change=0.7, SE=0.03, n=30) improved cognition z score 0.63 units more than the low flavanol group (8 week change=0.1, SE=0.07, n=30).(126) In a 12 month diet and exercise study, where the diet only group is similar to WL group in the proposed study, the diet only group (12 month change=1.7, SE=0.4, n=26) improved 3MS by 1.5 compared to the control group (12 month change=0.1, SE=0.4, n=27).(76) With N=50 subjects per group, we have 87% statistical power for the omnibus test for group effects (time by group interaction term in the mixed-effects model) for changes in the cognitive z score of 0.31 between control group compared to each of MCNS and WL arms. *Secondary Outcomes:* In a previous 12 month study, mean for overall perfusion increased from 74.4 ml/100g/min (SE=1.7, n=107) at baseline to 87.0 ml/100g/min (SE=2.4, n=107) at 12 month.(127) The sample size yields 80% power to detect 12.6 ml/100g/min change in CBF in MCNS and WL arms. Neurovascular coupling (NVC%) is reported in a previous 30-day cocoa intervention to measure blood flow velocity. NVC had an 8% difference between flavanol-rich and flavanol-poor groups (5.6%, SD=7.2% vs. -2.4%, SD=4.8%; n=17) in those with impaired NVC at baseline.(128) For CBFV, the sample size yields 82% power to detect 5% change in neurovascular coupling in MCNS and WL arms. Power calculations for all outcomes assume 25% additive increases in the MCNS+WL combined treatment group over treatment arms with individual agents. Simulations for power calculations for the mixed-effects linear model with repeated measures was simulated in R v 3.6(129) with a custom algorithm using the lme() function to test the time by group interaction.

**I.3 Number of Subjects**

We expect that up to 144 subjects will be enrolled at screening (all at Tufts) and we anticipate that we will have 48 participants in the study and have at least 36 completers.

**I.4 Data Management**

1. Describe the data analysis plan, including descriptions of the data

The proposed project will generate primary data consisting of recruitment and retention statistics, adherence metrics, and data on the primary and secondary outcomes: cognitive testing (administered electronically via tablet or using hard copy forms), cerebral hemodynamic data, electronic survey questionnaires, clinical measures (weight, vitals, cerebral hemodynamics, cognitive measures), multi-pass 24-hour dietary recalls, and blood measures. The cognitive and cerebral hemodynamic measures will be captured using instrument-specific software. Other data will be collected primarily through use of REDCap-based eCRFs, with diet data recorded separately and stored in NDSR and copied into REDCap. The CRFs will utilize REDCap’s built-in data validation and data range functions to prevent entry of impossible values (e.g., negative body weight or implausible weight changes). All data entry will be blinded to randomization, and raw scores will be entered in duplicate into REDCap by 2 different individuals. Hard copies and/or electronic records of all responses on tests will be stored as backup.

As necessary, secure data transfer of de-identified data between investigators will be performed using shared network drives for internal collaborators (i.e., those at the HNRCA) or the REDCap “send-it” feature for transfers to external collaborators. Brain data will be processed at Harvard Medical School (Cambridge, MA) by investigators blinded to randomization prior to its transfer to the HNRCA Energy Metabolism Laboratory for integration into the REDCap study database. The resulting de-identified data will be maintained on the Energy Metabolism Laboratory’s departmental network drive with all other study documents.

2. Describe the steps that will be taken to secure the data

Members of the HNRCA biostatistics and data management core unit will provide training and guidance to Dr. Roberts and research staff responsible for data entry and management on best practices for data capture, eCRF design and implementation, quality control and data quality checks.

3. Describe any procedures that will be used for quality control of collected data

Monthly data quality reviews will be conducted to minimize missing data. Potential outliers (as identified by the Mahalanobis’ D2 statistic) will be reviewed and verified. If a participant record requires any correction, justification and documentation of the change will be logged in REDCap and linked directly to the record in question. Any missing data or extreme outliers that cannot be confirmed as erroneous will be handled as described in the analysis section. Robust metadata and data dictionaries describing each variable and all information necessary to understand the data will be developed in conjunction with electronic data forms and will be maintained on the Energy Metabolism Laboratory’s departmental network drive for the duration of the study.

4. Describe how data and specimens will be handled study-wide

*4a). What information will be included with the specimens.* All specimens will be labeled with the study ID, the subject ID and date and time. No personal identifiers will be included.

*4b) Where and how data or specimens will be stored*. All specimens will be stored in the HNRCA. All data will be stored in the HNRCA, and raw cognitive data will also be stored in the University of Florida and raw cognitive data will be stored in Massachusetts General Hospital.

*4c) How long the data or specimens will be stored*: Up to 10 years.

*4d) Specify who will have access to the data or specimens*. The PI, research team members at Tufts and members of the NEL who manage the storage facility.

*4e) Specify who is responsible for receipt or transmission of the data or specimens.* The Nutrition Evaluation Labratory staff under the supervision of the laboratory director, Gayle Petty, will be responsible for receipt or transmission of specimens within the study team. Members of the PI’s research team will be responsible for the receipt or transmission of data.

*4f) Specify how data and specimens will be transported*. Data will be integrated in the HNRCA via REDCap. All biological samples will be kept within HNRCA.

*4g) Specify the plan for study data retention and storage.* Specimens will be stored within the HNRCA. We will complete a data sharing agreement for sharing of de-identified data outside the HNRCA with research team members.

**I.5 Randomization**

Will subjects be randomized?

☒ Yes ☐ No

If Yes respond to all of the following:

1. Describe the randomization procedures, including the ratio of subjects randomized to each study arm Enrollees will be randomized using the built-in random number generator included in REDCap, the study’s database and data capture system. The HNRCA bioinformatics team will randomize each enrollee to 1 of the 4 intervention arms: (a) MCNS + WL; (b) MCNS; (c) WL; and (d) control. Randomization will be stratified by age (60-69; 70-80) and BMI (27-32.9; 33-39.9). The bioinformatics team will retain randomization information for each participant.

2. Describe the blinding procedures

For the MCNS supplement/control food, only the Bioinformatics team leader and MRU kitchen staff will have access to the randomization. For the WL intervention, the Research Coordinator, PI, Study Physician and WL interventionist and participants will have access to the randomization schema. The participants, research staff conducting outcome measurements, statistician and team Data Manager will be blinded throughout the study except for above and when safety events require unblinding.

**J. Drugs and Devices**

1. Will the research involve drugs?

☒ Yes ☐ No

If **Yes**, respond to all of the following:

1. If the drug is investigational (has an IND) identify the holder of the IND: N/A
2. Describe your plans to store, handle, and administer study drugs so that they will be used only on subjects and be used only by authorized investigators (if the control of the drugs used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference that SOP in this section): products will be maintained and held by the study team in the HNRCA
3. Who on the research team, in addition to the Principal Investigator, will be accountable for drug(s):study coordinators will be responsible for ordering the products, kitchen staff will be responsible for distributing products to participants.
4. Who will interface with the pharmacy: N/A
5. If pre-printed orders will be created to obtain study drug(s) from the pharmacy, describe the procedures for reviewing and verifying the accuracy of the pre-printed orders prior to their being implemented:       or N/A, there are no pre-printed orders
6. If computerized order sets are created and/or infusion devices need to be programmed to administer an investigational drug, indicate the mechanism to pre-review and verify their accuracy, including who will be involved in this process from the research team, pharmacy, and nursing:       or

N/A, there are no computerized orders sets and/or infusion devices.

1. The study drug or procedure (including beneficial health care procedures) will be available to subjects after participation in the study: Yes N/A
2. There are medications or other substances that should not be taken while participating in the study. A list of these are incorporated into the ICF or submitted to the IRB as a subject handout: Yes N/A
3. Handouts or instructions sheets that will be given to subjects on how to administer study drug(s) have been submitted to the IRB: Yes N/A

2. Will the research involve devices?

☒ Yes ☐ No

If Yes, respond to all of the following:

*2a) If the device has an IDE or a claim of abbreviated IDE (non-significant risk device) identify the holder of the IDE/Abbreviated IDE.* The NIRS/DCS research device is IDE exempt on the basis that the testing is noninvasive, does not require invasive sampling procedure(s), is not used as a diagnostic procedure without diagnosis by another, medically established diagnostic product or procedure, and is used to determine safety or effectiveness for commercial distribution. Although the device introduces energy into the subject in the form of non-ionizing light, the device is compliant with the ANSI permissible light standards and is considered a non-significant risk device. We will comply with the common rule and 21 CFR 50/56.

*2b) Describe your plans to store, handle, and administer study devices so that they will be used only on subjects and be used only by authorized investigators (if the control of the devices used in this protocol will be accomplished by following an established, approved organizational SOP, please reference that SOP in this section).* The devices will be kept in a locked room at all times except when in use and under the direct supervision of the research staff members conducting testing. Each use of the devices will be recorded in a log (individual, date, time) for tracking purposes.

*2c) Specify who will be responsible for the costs of implantation or placement of the device in subjects’ bodies?:* or ☒N/A, the device will not be implanted or placed in the body.

*2d) Specify who will be responsible for the costs of removing the device from subjects’ bodies?* or ☒N/A, the device will not be removed from the body.

*2e) Specify the cost of the device and who will be responsible for the cost.* or ☒N/A

*2f) Who on the research team, in addition to the Principal Investigator, will be accountable for device(s).* The study coordinator and individuals on the research team who have been trained by Dr. Franceschini in its use. Documentation of training will be maintained, and refresher training will be given at 6-month intervals.

*2g) Who will interface with the sponsor.* Dr Roberts.

*2h) The study device or procedure (including beneficial health care procedures) will be available to subjects after participation in the study*: ☐Yes ☒N/A

*2i) Handouts or instructions sheets that will be given to subjects on how to use study device(s) have been submitted to the IRB*. ☐Yes ☒N/A

**K. STUDY ADMINISTRATION**

**K.1 Setting**

1. Describe the sites / locations where your research team will conduct the research, including recruitment activities

*1a) Tufts MC / Tufts University locations (specify which facility/clinic if applicable*). HNRCA

*1b) Other locations*: Local health clinics and health fairs, YMCAs and community centers in Greater Boston (and within Massachusetts by invitation) will be used for recruitment purposes only. Contact information for prospective participants who wish to be contacted will be obtained at these sites.

Please note that while scientists from other institutions are involved in the study, all research collection activities will be conducted at Tufts by Tufts personnel. A data sharing agreement is being organized for our external collaborators to have deidentified data.

1c) Greater Lawrence Family Health Center. N/A

*1d) Other locations.* ☒ A sIRB is not requested. A data sharing agreement will be implemented before participants are enrolled, to allow for transfer and sharing of deidentified data with Massachusetts General Hospital and University of Florida.

2. The research will take place at an international site, and the [International Research Guidance](https://viceprovost.tufts.edu/policies-forms-guides/hs-irb-international-research-guide) and [International Checklist](https://eirb.tuftsmedicalcenter.org/IRB/sd/Doc/0/RNNTKT4QC5JKFD1MH6AP6JM1E2/International%20Research%20Checklist_032921.docx) were utilized: ☐ Yes ☒ N/A

**K.2 Registration**

1. Describe the steps the research team will take to ensure that a subject is appropriately enrolled or registered in the study prior to receiving any study intervention.

Checklists for screening and eligibility and enrollment will be provided for IRB review and approval prior to launch.

**K.3 Resources Available**

1. 2. Describe the roles/tasks of each research team member and their qualifications

PI Susan B. Roberts, PhD, of Tufts University will oversee the project and be responsible for conforming to regulatory requirements, including submitting all required items for IRB review and coordinating participant safety issues and supervising the intervention team. Dr Roberts has designed and led several randomized trials of nutrition interventions including in older adults. Because of her potential conflict of interest (Tufts has been awarded a patent for a related though distinctly different food supplement for children under 4 years of age) Dr Roberts will not have any access to participant supplement randomization, she will not have access to outcome data, will not have contact with participant outcome assessments after randomization, and will not lead data analysis strategies or conduct data analyses.

Co-Investigator Sai Krupa Das, PhD, of Tufts University will be responsible for supervision of outcomes testing and data. Dr. Das is an expert on nutrition and aging, with expertise in outcomes relating to weight change and quality of life. Further, as an on-site investigator, Dr. Das will provide day-to-day oversight of cognitive and brain assessments, working with off-site investigators Dr. Cohen and Dr. Franceschini to ensure that all measures are maintained at the highest quality standards. Dr. Das also will be responsible for safety-related decisions when Dr. Roberts is away. If it becomes necessary for any reason to unblind the supplement randomization of a subject, Dr Das will take responsibility for this event including decisions to withdraw subjects for any adverse events related to supplement consumption.

Co-Investigator Jose Ordovas, PhD of Tufts University will be responsible for laboratory analysis of APOE samples.

Co-Investigator Kathryn Barger, PhD of Tufts University will be the lead statistician responsible for design of statistical plan.

Clinical Trial Consultant (Paul Fuss). Mr Fuss is a highly experienced clinical trials manager. He will oversee coordination of clinical trial activities and supervise Ms Dix for day-to-day trial management.

Research Coordinator (Sadie Dix). Ms. Dix has a background in nutrition and experience in communicating with subjects and study organization and will report to Dr Das. This position is unblinded for WL intervention group status, but — like all other core staff — will be blinded to food supplement randomization. Ms. Dix will coordinate all aspects of the trial including recruitment, enrollment, outcome assessments and adherence in intervention groups. She will also maintain participant contacts and support engagement, and coordinate all activities within the HNRCA, including the MRU and NEL cores. She will also conduct baseline outcome assessments and report to Dr. Roberts for general study coordination activities.

Interventionist and Research Assistant (Suzie Gerber). Ms. Gerber is a PhD student in the Friedman school with prior experience counseling patients and will report to Dr Roberts. She will lead all the WL intervention groups and be responsible for collecting data on participant meeting attendance and intervention adherence metrics. In preparation for this role, she has completed the national DPP online training course. Ms. Gerber, will also work with the recruiting team to enroll participants and conduct baseline outcome measurements. She will not conduct outcome assessments after baseline because post-baseline measurements will be taken by staff blinded to randomization.

Part time Assistant Data Manager and Statistician (Rachel Silver, MPH). Ms Silver will be blinded to randomization. She will work with core services to support the data management system. She also will be responsible for performing and tracking quality control assessments and data management activities. She will contribute to outcome testing measures on an as-needed basis. She will report to Dr. Das.

Metabolic Research Unit (MRU) Staff (see DOA chart below):

[Multiple staff within the MRU will have roles in data collection in this study. The staff have all been trained in their respective roles. IRB permission for all MRU staff is given in IRB protocol 6701.]

MRU Nurses

* Jean McShea
* Janice Klian
* Mary Weiss
* Michelle Hallam-Naing
* Margaret Vilme

MRU Research Coordinators

* Kim Trinh
* Ryan Piccirillo

MRU Volunteer Services

* Kimberly Dupiton, MA (Volunteer Services Supervisor)

MRU Dieticians / Dietary Assessment Unit (DAU)

* Cheryl Gilhooly, RD, PhD (MRU and DAU Manager)
* Andrew Howland, RD (DAU Research Dietitian)

Delegation of Authority (DOA) for MRU Staff Members

|  |  |
| --- | --- |
| Role | Authorized Tasks |
| MRU Nurse | 1,2,3,6,7 |
| MRU Research Coordinator | 1,2,8 |
| MRU Volunteer Services | 9 |
| MRU Dietician / DAU | 10,11 |
|  |  |
| Authorized Tasks Key |  |
| 1. Obtain Informed Consent |  |
| 2. Determine Subject Eligibility |  |
| 3. Evalutate Adverse Event |  |
| 4. Evauate Response/ Clinical Endpoints |  |
| 5. Decision to Administer Investiagational Product/ Dose Modifications |  |
| 6. Control Investigational Products (Storage/ Receipt/ Accountability) |  |
| 7. Medical History/ Vital Signs |  |
| 8. Data Entry, Regulatory Documentation, and other Non-Clinical Activities |  |
| 9. Recruitment and PM logs |  |
| 10. Dietary Recalls  11. Review Diet habits, diet history, food allergies, diet intolerances and supplement intake |  |

Medical or psychological resources that subjects might need, such as for emergencies or medical issues, are available for the study: ☐Yes ☒ N/A

K3. Describe coverage plan while PI is away or unavailable

Dr Das will cover on safety administration and oversight for Dr Roberts when she is away. The MRU has coverage for Dr Ceglia when she is away. The research team will have a backup for every position to ensure that assessments and interventions do not suffer in the case of a team member being unavailable.

**K.4 IRB Review**

1. ☒Check to confirm that an appropriate IRB, registered with the OHRP, will review and approve this study

2. ☒Check to confirm that any amendments to the protocol or informed consent documents will be reviewed and approved by the IRB prior to use, unless required to eliminate an apparent immediate hazard to subjects.

**K.5 Multi-Site Research**

Is this a multi-site study where Tufts IRB is requested to be the Single IRB of record for non-Tufts sites or collaborators, AND/OR where Tufts is the sponsor, primary grant recipient, or coordinating site?

☐ Yes ☒ No

**K.6 Community-Based Participatory Research**

Can or will this study involve community-based participatory research?

☐ Yes ☒ No

**K.7 Sharing Results with Subjects**

Will results be shared with subjects or others (e.g., the subject’s primary care physician or the subject’s treating physician)?

☐ Yes ☒ No

**L. NURSING INVOLVEMENT**

Will your study require the involvement of Nursing? ☒Yes ☐ No

The study requires nursing involvement for routine blood draws, vital signs, and the informed consent process. Blood draws will be obtained on four occasions for each subject: at screening, baseline, month 6, and month 12. Vital signs (weight, blood pressure, pulse, and oral temperature) will be taken on five occasions for each subject: at screening, baseline, month 3, month 6, and month 12. The consent process will be conducted at the screening visit (Screening ICF) and at baseline for those participants who are eligible (Main Study ICF). We expect to enroll 144 subjects at screening, and of these we anticipate that 48 will be eligible to participate in the main study itself. All procedures are routine for the HNRCA nursing staff and do not require any additional training. Enrollment will be staggered over a period of approximately 3 months to mitigate the burden on the nursing staff.

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