Official Title: MIND Food and Speed of Processing Training in Older Adults with Low Education, The MINDSpeed Alzheimer's Disease Prevention Pilot Trial

NCT03419052

Version date: 8/1/23

SPECIFIC AIMS

Low education, i.e., fewer than 12 years,(1) is the top population-attributable Alzheimer's disease (AD) risk factor worldwide.(2) The National Academy of Medicine has recommended that education be routinely recorded in electronic health records (3) and the IOM 2015 report Cognitive Aging recommended evaluation of multi-component cognitive interventions for vulnerable populations.(4) In the proposed study, we seek to test an intervention that could reach millions of older adults with low education.(5) Our intervention involves two positive behaviors that may have additive effects on cognition—consumption of "MIND" foods and speed of processing training. Consistent with the objectives of PA-16-365, we aim to establish feasibility, mechanisms of action, and initial efficacy of these non-pharmacological interventions in older adults with low education receiving care in federally qualified health centers (FQHC). FQHCs provide care to over 40 million lower SES adults in the U.S.(6)

Low cognitive reserve is a leading hypothesis for low education's effects on early cognitive decline and AD onset.(7) Adult hippocampal neurogenesis (AHN) is fundamental to cognition and learning(8) and diet is critical to maintaining an environment conducive to AHN.(9) Specific dietary components (e.g., polyphenols and polyunsaturated fatty acids) have emerged as key interventions for cognitive health and function.(9, 10) Unfortunately, low education is associated with unhealthy dietary habits(11) that are very difficult to completely eliminate.(12) Fortunately, supplementing unhealthy dietary habits with healthy foods has real potential to impact cognitive health and function.(12) For example, moderate adherence to the "MIND" diet developed by the Rush Memory and Aging group cut AD risk by one-third over 4.5 years.(13) The diet was built from epidemiological (14, 15) and randomized controlled trial evidence that consumption of certain foods can improve short-term cognition including speed of processing.(16-18)

While dietary components support the creation of new neurons, cognitive training promotes integration of neurons into neural networks.(19-21) Speed of processing training, in particular, results in very large performance gains(22-24) that are maintained for years.(25, 26) "Speed training" targets fluid mental processing speed and reduces the length of time needed to process more and more complex information.(27) In a secondary analysis of data from the largest cognitive training trial ever conducted, we showed speed training had a large effect among participants with fewer than 12 years of education; the effect size was 50% larger in those with fewer than 12 years of education compared to the subsample with 16 or more years.(28) The training advantage in the less educated carried out to 5 years.

At this time, there are no published randomized trials of speed of processing training combined with MIND foods. Nor are there published randomized trials of MIND foods or speed training properly designed and powered to assess effects in older adults with low education. Using a 2x2 factorial design, we propose to test in a randomized controlled trial the effects of speed training and MIND foods in older adults with low education. The ultimate goal of this line of research is to prevent or delay AD in this vulnerable group.

This study will enroll older adults receiving care in Eskenazi Health who have 12 years of education or less. Interested and eligible individuals who consent to the study will receive a study-provided tablet running our professionally designed MINDSpeed app. The app will provide each participant with 12wks of access, support, and incentives for all 4 study arms: 1) MIND food + Speed training, 2) MIND food + Speed training control, 3) MIND food control + Speed training, or 4) MIND food control + Speed training control. We refer to the combinatorial MIND food and Speed training intervention as MINDSpeed, while the combination of food control and speed training control we refer to as Double Control.

The primary specific aim of this pilot clinical trial is to determine MINDSpeed effects on an executive cognitive composite (ECC) score relative to Double Control. The primary hypotheses are that MINDSpeed is more effective than MIND food or Speed training alone and all are more effective than Double Control in improving the objective ECC measure at immediate post-training and 6-month follow-up.

Exploratory aims are to determine: 1) feasibility (i.e., adherence, adverse events, costs), 2) treatment effect modifiers (e.g., education, age, baseline cognitive status, *APOE* ε4 carrier status), and 3) mechanisms including the roles of inflammation, oxidative stress, hippocampal volume, and functional connectivity.

With no current treatments for AD, designing and testing interventions to prevent AD in vulnerable populations has become a top priority.(4) The intervention we propose has potential to be highly effective in a vulnerable population. Support for MINDSpeed feasibility and efficacy could lead to a follow-on AD prevention trial through existing and scalable programs and applications.

[During the COVID-19 health crisis, biospecimen collection, imaging, and Viromeaging substudy enrollment will cease.]

Viromeaging sub-study: [Enrollment closed 5/31/22 for this sub-study.]
The purpose of this sub-study is to identify members of the aging population at increased risk for disease and frailty with the detection of biomarker panels applicable to inflammation in aging. Frailty is a significant and independent predictor of poor clinical outcomes, including prolonged hospitalizations and death. It is now understood that aging is the single most important risk factor for chronic diseases and frailty.

A sizable proportion of people over 65 years of age experiences more than one age-associated chronic disease (musculoskeletal, cardiovascular, neurodegenerative, metabolic, cancer) and will live with them for many years and decades, enjoying a reduced quality of life and costing themselves, and society, a disproportional amount of resources. One common thread frequently found in normal aging as well as in age-associated chronic diseases is low-level inflammation, manifested by elevated levels of pro-inflammatory cytokines and acute phase reactants. This inflammation has been associated with development and/or accelerated progression of age-related chronic diseases.

Aims of this sub-study are to 1) measure the blood virome in older adults, 2) assess markers of chronic inflammation in older adults and correlate these with evidence of immune aging and virus-specific immune responses, and 3) correlate the blood virome, evidence of systemic inflammation and the presence of chronic disease and frailty in test subjects and create a potential inflammatory and viral “biomarker” for the detection of the complications of aging.

Investigators hypothesize that older adults will exhibit greater abundance of viruses, leading to increased immune stimulation and chronic inflammation, and this burden will be able to predict propensity for and timing of frailty and age-related diseases. The ultimate goal is to suggest potential interventions that might mitigate the development of chronic disease and frailty in the aging population.

IMPACT
This year’s dependent care costs of one quarter trillion dollars are projected to double to one half trillion by 2030 as the population ages and the prevalence of Alzheimer’s Disease (AD) and other dementias grow.(29) Interventions that improve brain structure and function, and cognitive reserve of vulnerable older adults have the potential to forestall impairment in functional status(25, 30) and possibly delay time-to-onset of AD.(31)(32)

A 2014 Lancet report estimated population-attributable AD risk factors from published meta-analyses and concluded that seven modifiable risk factors are responsible for almost one-third of all AD cases in the U.S.(2) Having completed no more than lower secondary education, equivalent to fewer than 12 years,(1) is one of these risk factors. In addition to the potential direct effects of low early life education on cognition,(33) adults with low educational attainment are significantly more likely to have each of the other six modifiable AD risk factors including obesity, physical inactivity, hypertension, smoking, diabetes, and depression.(34, 35) Adults with low education are also more likely to have a low quality diet,(36, 37) which has emerged as a major AD risk factor as well.(9, 13) According to the U.S. Census, seven million of today’s older adults did not complete upper level secondary education (i.e., no high school degree). This represents almost one-fifth of the current older adult population. Interventions are needed to improve cognitive reserve in this vulnerable population.

Large randomized controlled trials have identified targeted cognitive training, particularly speed of processing training, as having large effect sizes for improved cognitive performance,(22, 24) including unsupervised, home-based training.(23) In fact, speed training has the largest effect size of any known cognitive intervention and this effect is lasting and has far transfer to other outcomes including depression.(38-41) Our analyses suggest that speed training had a dose-response association with 10yr dementia risk in a sample comprised mostly of well-educated seniors.(43) In other secondary analyses, however, we showed that older adults with

low education have the largest performance responses to training.(42) Targeting those most responsive to training—e.g., seniors with less education—and optimizing that response could lower dementia risk.

Importantly, dietary components have been shown to be critical in optimizing the brain's ability to change in response to stimuli such as speed training. In a comprehensive review, the Institute of Psychiatry & Neuroscience, King's College London, concluded that "animal and some human studies now demonstrate that diet-induced brain plasticity offers tremendous potential in promoting emotional and cognitive well-being" and recommended translational research with a focus on polyphenols to both reduce risk and increase reserves.(9) In a complex process, food-derived components serve as precursors of neurotransmitters and AHN, both keys to learning. Thus, polyphenol-rich diet with speed training is a potentially high impact multi-component intervention for older adults with low education.

Testing multi-component interventions in vulnerable populations is a research recommendation of the IOM April 2015 Cognitive Aging report(4) and a prevention recommendation of the May 2015 NIH AD summit.(44) Evaluation of treatment mechanisms was a further recommendation of the IOM report. Successful outcomes in the proposed study would support a properly powered AD prevention trial, contribute to the National Plan to find effective therapies to prevent AD by 2025, and establish a multi-component prevention intervention that could reach millions of vulnerable adults.

SIGNIFICANCE

Low education is associated with a significantly higher risk of dementia.(2) In analyses of six independent samples from four different countries, Piccinin et al showed education was associated with cognitive decline up to 11 years of education.(45) Another recent study found a direct effect of years of education on cognitive decline up to 10 years of education.(46) In these studies, years of education beyond 10 or 11 years were either indirectly associated with cognitive decline(46) or had very little association.(45) The International Standard Classification of Education classifies 10 to 11 years of education as lower secondary.(1) Recent estimates of population-attributable risks for AD identify low education as responsible for 19% of AD cases worldwide; the largest of seven modifiable risk factors.(2) Low education was found to be a top five modifiable risk factor in the United States(2) where lower secondary education has been compulsory since 1918.(47)

The leading hypothesis for education's effect on cognitive decline and AD is cognitive reserve,(48) which can be defined as the brain pathology level at which clinical deficits appear.(49) A recent study with imaging showed that in the case of both amyloid and vascular effects on cognitive decline, more years of education and higher self-reported cognitive activity were associated with later onset of cognitive deficits but not rate of cognitive decline. In fact, the data predicted that a participant with a high education/occupation score who had both amyloid and vascular pathology would have a 5-year advantage in cognitive function compared to a participant who had similar pathology but a low education/occupation score.(7) Evidence that low educational attainment adversely affects cognitive reserve and dementia risk raises the question of how to protect the brains of millions of adults who did not complete upper level secondary education.

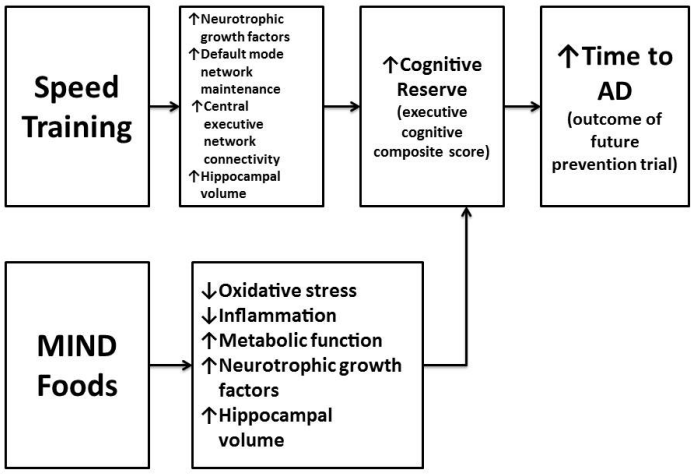
Fortunately, promising interventions exist. For example, the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) randomized controlled trial,(25) and others,(23, 24) showed that short-term targeted cognitive training improves cognitive function in older adults. All three major cognitive training trials to date have enrolled well-educated older adults with 80% to 95% having completed upper level secondary education(23-25) and five smaller trials have had samples with the same restricted range of education.(27) Across these trials, speed of processing training has had an effect size ranging from 0.61 to 1.67 on Useful Field of View (UFOV), a measure of visual attention and psychomotor integration.(27) The ACTIVE study was a large multi-site randomized trial and had an effect size of 1.46 at immediate post-test and 1.21 at one year after training. ACTIVE's sample size was large enough to allow secondary analyses of training effects by educational attainment. As we describe in the Preliminary Studies section, we found evidence that speed of processing training had an effect size in older adults with no upper secondary education around 2.0 on the UFOV measure at immediate post-test and at one year after training.(42)

The UFOV gains in the ACTIVE trial have been shown to have meaningfully improved locus of control(50), self-rated health(38), depressive symptoms,(39) health-related quality of life,(40), and driving outcomes including longer time to driving cessation(51), and fewer at fault motor vehicle collisions.(41) Four days per

week for six weeks of computerized speed of processing training was recently shown, relative to leisure mental activities, to maintain two networks susceptible to normal and abnormal aging effects including the default mode network (DMN) and central executive network (CEN).(52) Thus, we include speed of processing training in our intervention for its broad effects and potential to enhance cognitive reserve.(39)

The second component of our multi-component intervention is focused on establishing an environment conducive to training response and learning. Long-term potentiation underlies learning and refers to activity-dependent synaptic changes in response to frequent stimuli.(53) This process depends on a micro-environment that supports the presence of neural stem cells in the dentate gyrus subregion of the hippocampus and their renewal and differentiation into mature neurons.(9) Blood vessels deliver biochemical factors that improve (or worsen) this micro-environment, which is one of the ways in which diet may affect cognition.(9, 10) Recent animal studies have shown multiple subclasses of flavonoids, a class of polyphenols, remain stable after digestion and may cross the blood-brain barrier to reach the hippocampus.(54) Polyphenols play a central role in managing inflammation, oxidative stress, and metabolic function.(54, 55) As reviewed in detail by our consultant Dr. Tangney, plant-based foods like berries, nuts, cocoa, and green leafy vegetables contain a range of polyphenols and improve metabolic function,(13, 54) combat oxidative stress, and are cardio and neuroprotective.(10) Flavonoids in particular have been shown in human trials of 12 weeks duration to improve metabolic function, and to reduce oxidative stress and inflammation.(10, 54) One trial in adults aged 50-69 years showed that participants randomized to high vs low flavonols via cocoa for 3 months had higher dentate gyrus function as evidenced by functional MRI.(56) Importantly, consumption of these foods two or more times per week for 12 weeks has been shown to improve performance on cognitive tests, including speed of processing.(18),(14),(57),(16)

Figure 1. Multi-component intervention, mechanisms, and long-term outcomes.



Diets high in these foods are increasingly studied for their potential to delay AD. Secondary analyses of large randomized trials have shown that the Mediterranean diet improves Mini-Mental State Examination (MMSE) scores relative to low fat diet control(16) and that the DASH diet relative to usual diet improves speed of processing.(17) More recently the MIND diet, which de-emphasizes dairy, potato, and general fruit consumption and specifically emphasizes berries, nuts, olive oil, and green leafy vegetables, has been shown to be associated with lower AD risk in a non-randomized prospective study.(13) Even modest MIND diet adherence was associated with reduced AD risk. As shown in our broad conceptual model (Figure 1), we include MIND foods in our intervention for their potential effects on cognitive reserve.

User centered design and behavioral economic principles guided the proposed interventions to maximize uptake, adherence, and scalability. As we describe under Interventions, our professional design team worked with older less educated adults and consultants to create the proposed intervention. User design preferences were paired with behavioral principles—three in particular: 1) "easy" substantially increases uptake,(58) 2) small and frequent incentives significantly improve adherence,(59, 60) (61) and 3) intrinsic motivation is key to maintaining behavior. Regarding easy, we limit cognitive training to speed of processing to maximize effect per minute of training. Relatively speaking, the MIND diet is easy but we make it really easy; tablet based food selection with home delivery. Intrinsic motivation is designed into speed of processing trainings by continuously adjusting to an individual's competency, while foods are self-selected.(62, 63)

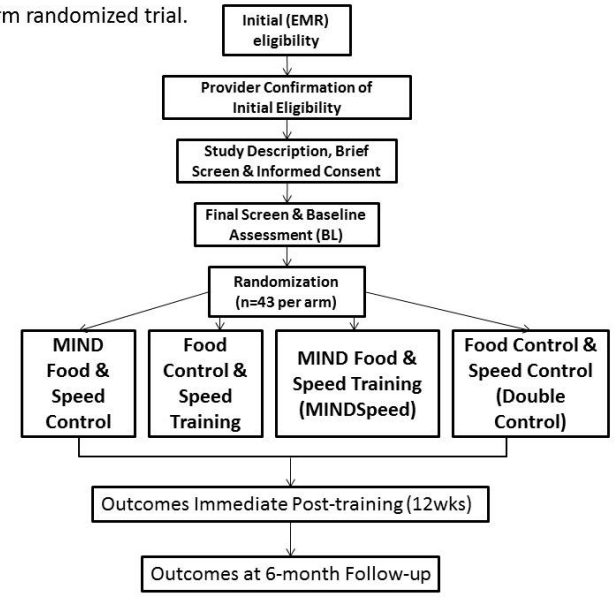
INNOVATION

As far as we are aware, this will be the first study of:

1) cognitive training or MIND food interventions targeting less educated older adults,
2) MIND and speed training as a multi-component intervention in any population,
3) mechanisms of action, including fluid and imaging biomarkers, from this multi-component intervention,

4) MIND food consistent with user preferences and existing nationwide distribution systems, and
5) Speed of processing and MIND foods access, support, and incentives via a single, integrated application.

Figure 2. 4-arm randomized trial.



APPROACH

Figure 2 shows our study design. This is a 4-arm randomized controlled trial. Those eligible and willing will be scheduled for a visit to complete informed consent and baseline assessments. Following completion of baseline assessments, patients will be randomized to two of the four treatment conditions. Tablet devices and the study application will give access, incentives, and support for all interventions including control conditions (see Intervention section). We assess all outcomes and mechanisms immediately post-training (3-months) and at 6 month follow-up. The primary outcome is an executive cognitive composite score.

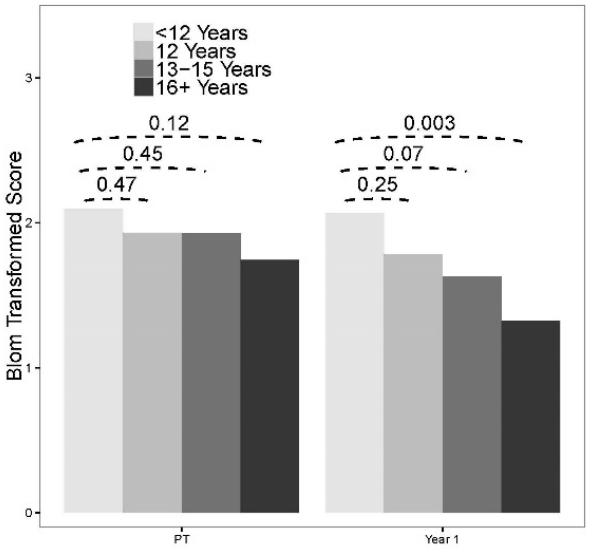
Preliminary Studies and Data. Our team is multidisciplinary with expertise relevant to the proposed study. We have two decades of experience enrolling and retaining vulnerable older adults, particularly lower SES adults from FQHCs, in epidemiological studies and single and multi-site randomized trials(64-68)(22, 25, 69, 70)(71)(72)(73)(74, 75) and in the Indiana Alzheimer Disease Center. Saykin is PI of the Genetics Core of AD neuroimaging initiative (ADNI) and has published studies integrating ADNI's longitudinal cognitive, neuroimaging, biomarker and genetics data,(76-80) reviews of conceptual and methodological issues in MRI neuroimaging and fluid biomarkers,(81) and of differential neuroimaging(82) and neuropsychological assessment.(83) Drs. Unverzagt is expert in trials of cognitive training, Dr. Clark has experience operating large nutrition and health programs in FQHCs, and Dr. Tangney is a leader in clinical nutrition and cognitive health, including the MIND diet.(10, 13) The team is supported by infrastructures of the IU Neuroscience Center, the IU Center for Aging Research, and the Eskenazi Office of Applied Research.

Below we present preliminary data specific to this proposed project. Included here are our efforts at: 1) investigating effects of speed training, 2) nutrition and health promotion in FQHCs, and 4) completing blood draws, and structural and functional brain imaging in older adults.

Speed of processing training and 10yr incident dementia (Edwards, Xu, Clark, Unverzagt, under review). Using data from the Advanced Cognitive Training in Vital Elderly (ACTIVE; NCT00298558) study we examined the effects of cognitive training versus a control condition on 10yr incident dementia. ACTIVE was a multi-site, randomized, controlled clinical trial.(22, 84) Community-dwelling adults aged 65 years and older were eligible. Persons were excluded if they had significant cognitive dysfunction (score < 23 on the MMSE;(85) functional impairment; self-reported diagnoses of Alzheimer disease, stroke within the last 12 months, or certain cancers; current chemotherapy or radiation therapy; or poor vision, hearing, or communicative ability). Enrollment resulted in a sample of 2,802 individuals (average age 74 years, average education 13 years, 74% white and 26% African American, and 76% women). Eligible participants were randomly assigned to one of three treatment arms (Memory, Reasoning, or computer-based Speed training) or a no-contact control group. Interventions were conducted in small groups in ten, 60-75 minute sessions over 5 to 6 weeks. Screening and baseline assessment took place before randomization. Outcome assessments were conducted immediately following and 1, 2, 3, 5, and 10 years post-training. For our analyses, incident dementia was defined using a combination of interview- and performance-based methods. Weibull regression examined intent-to-treat and dose models to predict subsequent dementia incidence, adjusting for risk factors. Intent-to-treat models indicated that participants randomized to speed of processing training were significantly less likely than controls to develop dementia over 10 years (HR=0.67, 95%CI 0.49-0.91, p=.012), but this association became nonsignificant after adjustment for sample characteristics significantly associated with dementia, including age, sex, race, mental status, physical function, depressive symptoms and diabetes (HR=0.73, 95%CI 0.54-1.00,

p=.052). Analyses indicated greater doses of speed of processing (HR=0.91, 95%CI 0.86-0.97, p=.002) were associated with reduced rates of incident dementia relative to controls, and effects remained significant after adjustments for sample characteristics (HR=0.92, 95%CI=0.87-0.98, p=.013).

Older Adults with Low Education Experience Large Effect of Speed of Processing Training (Clark, Xu, Unverzagt, Hendrie, 2016). In other analyses of the ACTIVE trial data, we investigated response to training in participants with less than 12 years of education (i.e., no upper level secondary education).(1) We assessed training effect sizes, defined as the net training effect (change from baseline for intervention – change from baseline for control) divided by the intra-subject standard deviation. Baseline covariates included in the models to obtain adjusted training effects were age, female sex, minority race, married, body-mass index, current smoker, alcohol use, Short-Form 36 physical functioning, CES-D score, hypertension, type 2 diabetes, stroke, congestive heart failure, ischemic heart disease, high cholesterol, myocardial infarction, baseline MMSE score, visual acuity, and field site. Sensitivity analyses were conducted to determine the longevity of differential training gains (2, 3, 5, and 10 years post-training). We found similar response to memory and reasoning training for those with low vs high education but response to speed of processing training was significantly greater in the participants with low education. As shown in the figure, the effect size on the Useful Field of View speed of processing outcome was near 2.0 in those with low education. The training advantage for the less educated persisted to 3 years.

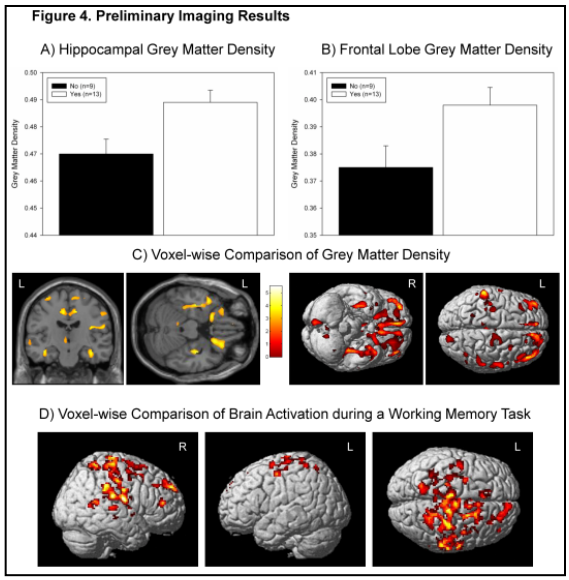


Nutrition Education and Counseling for Obese FQHC Patients (Healthy Me); Fairbanks Foundation, Clark - PI. Healthy Me was a foundation-supported quality improvement program that is now integrated and supported by Eskenazi Health. The Healthy Me program is structured around the 5A's of behavior change model.(86) Enrollment in Healthy Me starts with an FQHC visit. Five Coaches certified in behavior change counseling and fitness instruction are present two or more days per week in each FQHC. Coaches meet in clinic with referred patients to assess their current weight-related behavior, listen to the patient's concerns and goals, and collaboratively set an action plan that may include portion control, food preparation, exercise, or nutrition education classes held in the FQHC meeting rooms. Each patient's action plan is entered and tracked in the Healthy Me portion of Eskenazi's EMR. Dietary and physical activity self-monitoring instruction and logs are provided and a "passport to wellness" incentive program gives participants points for participation that earn them incentives (e.g., t-shirt, produce coupons, gym trial). Healthy Me does not endorse a particular diet strategy or weight loss goal. To date, Healthy Me has had 145,000 visits by over 25,000 adults. Healthy Me recently won the Hulman Health Achievement Award from the Indiana Public Health Association.

Nutrition and Exercise RCT; NIDDK R01 DK092377, Clark - PI. Using an image-based nutrition education program that we developed for lower literacy participants, we are testing video-conference delivery of nutrition education and supervised exercise for FQHC patients in a randomized trial funded by NIDDK. FQHC adults are recruited and randomized to usual care (Healthy Me), in person nutrition and exercise classes, or Internet-delivered nutrition and exercise classes. The purpose of the trial is to test whether eliminating travel and location barriers for urban poor adults can improve weight management outcomes. This is an ongoing trial with no outcomes yet to report. Participants for this trial are recruited from FQHC lists of obese adults via telephone. The total sample size is to be 150. Eight-three percent have completed the 6-month follow-up (3 withdrew from the study and 7 could not be located). Importantly, this trial includes comprehensive dietary assessments using the NCI 24hr dietary assessment tool.(87)

Blood biomarkers (Exercise in Depression pilot). In a recent pilot, Dr. Clark obtained baseline and 3-month blood samples from six older adults with major depression disorder and transported and analyzed the blood. Blood draws were completed in the morning following a night of fasting. Dr. Considine, who has decades of

experience storing and analyzing blood samples, computed C-reactive protein, interleukin-6, interleukin-1ra, tumor necrosis factor, vascular endothelial growth factor (VEGF), and brain-derived neurotrophic factor (BDNF). In separate work, Drs. Saykin, Risacher, and colleagues analyzed the influence of genetic variation on plasma protein levels in older adults(78) and of APOE on Alzheimer's biomarkers.(77)



Indiana Memory and Aging Study (IMAS, R01 AG19771 - Saykin, PI). Structural magnetic resonance imaging (MRI) measures of brain atrophy in IMAS(88) and ADNI(89, 90) have shown significant hippocampal and medial temporal lobe (MTL) atrophy in patients with MCI and particularly among those that convert to AD.(90) Therefore, hippocampal atrophy is a key variable to examine in interventional studies. In preliminary analyses we examined the impact of physical activity engagement in our IMAS cohort. We determined that amnestic MCI (aMCI) participants who self-reported regular aerobic training had less hippocampal atrophy (**Figure 4A**; p = 0.021) and a trend for less frontal lobe atrophy (**Figure 4B**; p = 0.051) than MCI participants who did not report regular aerobic training. Voxel-wise analysis supported the widespread nature of these differences (**Figure 4C**; voxel-wise threshold p<0.01 (uncorrected for multiple comparisons) and minimum cluster size (k) = 50 voxels). In addition, older adults without MCI who engaged in regular cognitive activity engagement (daily reading and/or puzzles) showed significantly slower annual rate of hippocampal atrophy than those who did not (data not shown; p=0.009). These results are consistent with hippocampal and frontal morphology being sensitive biomarkers for training-induced neuroplasticity. Functional MRI (fMRI) of brain activation is also a sensitive biomarker for cognitive decline (see Risacher & Saykin, 2013(81) for review) and to intervention-induced changes. We have reported improved brain activation in aMCI patients after treatment with donepezil.(81, 91) Preliminary examination in IMAS participants with aMCI indicated an impact of self-reported training on brain activation during a working memory task (Visual-Verbal 2-Back task [see MRI protocols below]). Greater activation was detected in task-specific regions in aMCI patients who self-reported regular training relative to those who did not (**Figure 4D**; voxel-wise threshold p<0.01 and k = 50 voxels).

PROPOSED METHODS:

Participants. The target population is adults aged 60 years or over born in the United States who self-report 12 or fewer years of lifetime formal educational attainment.

Inclusion criteria:

- age 60 years or older,
- ≤ 12 years of education,
- English speaking,
- Marion County (or immediately surrounding county) resident with steady/fixed residence to receive food deliveries,
- natural-born US citizen.
- experience with smart phone and/or tablet [Applicable during COVID-19 restrictions only]

Exclusion criteria:

- living in nursing home
- self-reported diagnosis of dementia, Alzheimer's disease (AD), cancer with short life expectancy, multiple sclerosis, epilepsy, schizophrenia, bipolar disorder, Parkinson disease; current chemotherapy or radiation therapy; history of brain tumor, brain surgery, brain infection; stroke or myocardial infarction within the past 12 months;
- current alcohol consumption ≥8 drinks per week for women or ≥15 drinks per week for men;
- poor vision (self-reported difficulty reading a newspaper) or color blind;

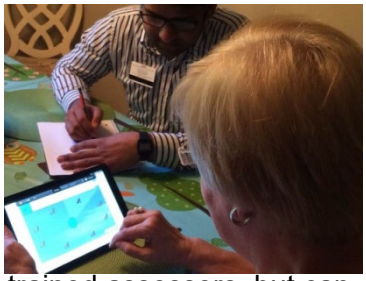
- low communicative ability, functional status, or other disorders (examiner rated) that would interfere with interventions and assessments;
- prior involvement in similar cognitive training studies;
- unable or unwilling to provide blood sample at Baseline* [Applicable during normal study operations (non-COVID-19 restrictions) only]
- unable or unwilling to provide urine sample at Baseline* [Applicable during normal study operations (non-COVID-19 restrictions) only]
- tumor, hemorrhage, aneurysm, hydrocephalus, or other significant clinical finding from Baseline brain MRI [Applicable during normal study operations (non-COVID-19 restrictions) only]

*Additional opportunities will be given to collect blood and urine if the first attempt is unsuccessful. When necessary, a urine collection cup will be left with the subject to collect a sample when they are ready to void. If Baseline blood or urine cannot be obtained prior to randomization, subject will be deactivated from the study.

Participants meeting MINDSpeed eligibility criteria are also eligible for Viromeaging sub-study. [*Enrollment closed 5/31/22 for this sub-study.*]

Recruitment and Randomization. Recruitment will be primarily through Eskenazi Health. Eskenazi is one of the five largest safety-net health systems in the United States and actively provides care to approximately 20,000 adults age 60 and over. Health system data managers or staff with access to EMR may identify patients who meet study demographic and clinical criteria. Providers will give eligibility review and permission to contact potential participants. Potentially eligible participants will be approached at an Eskenazi visit or called by telephone by a research assistant. [During the COVID-19 health crisis, all recruitment will take place via telephone.]

Recruitment through self-referral will also be accepted. Any potentially eligible participant who indicates interest will complete a brief screener including low education eligibility followed by information about the study interventions and assessments, including explanations of blood draw and imaging procedures. [During the COVID-19 health crisis, biospecimen collection and imaging will cease.]



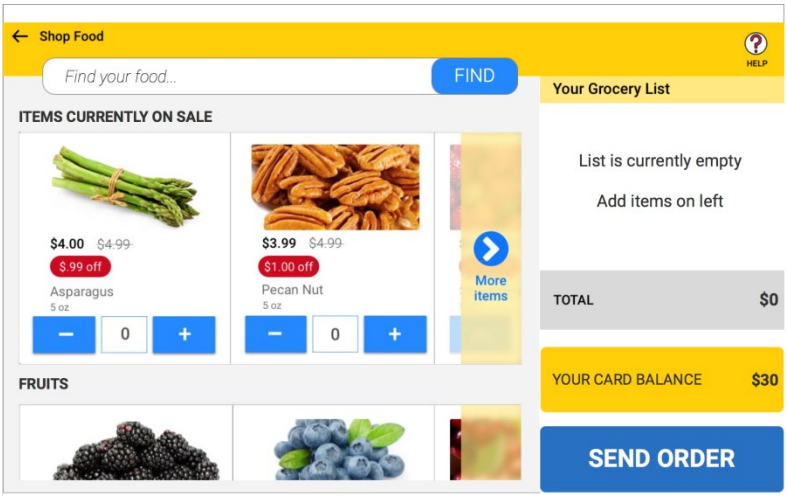
Those who remain interested and eligible will schedule an appointment to complete informed consent and baseline assessment including fluid biomarker samples and optional imaging; consenting participants will be presented with the option to participate in the Viromeaging sub-study at baseline assessment as well [Enrollment closed 5/31/22 for this sub-study.]. Study information sheets and/or appointment letters will be mailed to participants as requested prior to the appointment. [During the COVID-19 health crisis, verbal consent will be obtained by phone, and Viromeaging substudy enrollment will cease.] Assessments will be completed primarily at the IU Health Neuroscience Center by trained assessors, but can be broken down into multiple shorter visits or completed in part by phone, if desired. [During the COVID-19 health crisis, all study visits will be completed by phone or Zoom.] Participants completing all baseline assessments will be randomized. Randomization will be stratified by age (60-69; 70 and over) and MRI completion. To address frequent moves and service changes in the population, we will obtain contact information for 2 relatives/friends at enrollment.

[After the IU Research Restart beginning June 1 2020, study visits will be completed by a combination of in-person and telephone measures until full in-person measures are allowed to resume. Biospecimen collection, imaging, and Viromeaging substudy enrollment will be included; written consent will be required.]

Interventions. As noted, a professional UX design team led a user-centered design research effort to integrate the speed of processing and MIND food interventions. First, five users evaluated application screens and functions rendered on Sketch (https://www.sketchapp.com/). Second, these same users experienced

tablet-operated, interactive prototypes created through an application called Axure (http://www.axure.com/). Third, the design was evaluated by an outside expert team including human factors and software engineers and design researchers. Finally, the MindSpeed app was evaluated in a different group of five users for one week (ages 52-76yrs). The pilot study involved a combination of interview sessions, iterative design and usability evaluation of the prototype, food delivery and reporting, and game play (BrainHQ). Some older adults with low education have limited reading skills, low computer familiarity, and limited eyesight. Thus, a major objective of the design work was to make the application easy for the target group to use. This appears to have been achieved. First time users reported "this is self-explanatory" and "I will know how to use after first time, pretty simple." Findings from the pilot indicate that participants will shop through the app, accept home delivered foods, play speed training games, and track incentives.

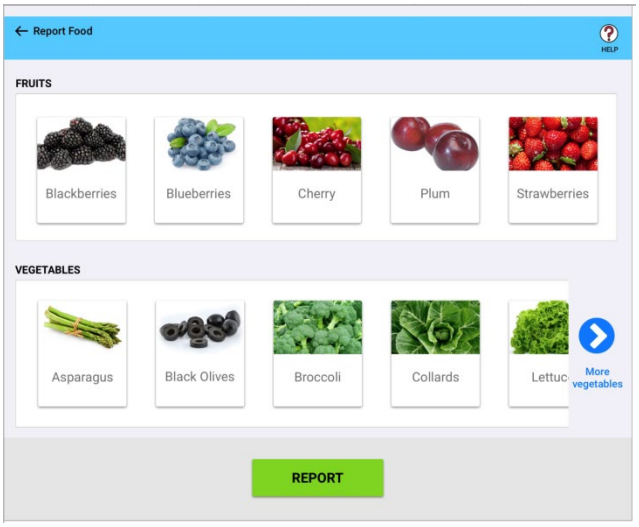
Speed of processing training is provided by the Internet-based BrainHQ program from Posit Science, Inc., which we are using in older adults with MCI in our ongoing NIH-funded CARB trial (R01 AG045157). BrainHQ contains an enhanced version of the original ACTIVE trial speed of processing module. Five different speed training modules (Hawk Eye, Visual Sweeps, Fine Tuning, Eye for Detail, Sound Sweeps) tap time-order judgment, visual discrimination, spatial-match, forward-span, instruction-following, and memory.(93) The Posit program systematically reduces stimulus presentation time to maintain an 85% accuracy rate and launches each new session based on the stopping point of the preceding session. Participants receive performance ratings relative to their prior trial upon completion of each “exercise.” Participants will be rewarded for their weekly game play. The goal is 75 minutes per week, but subjects will be compensated as such: $5 for completing at least 30 minutes of weekly game play, or $10 for at least 60 minutes of weekly game play. The cumulative incentive amount is displayed at the top of the home screen in the app (see image on Response to Reviewer’s page). Through Posit Science research platform, we have real-time, objective records of training time per module, per session, for each participant (see letter from Posit’s Dan Tinker).



The logic of making MIND food “easy” was noted earlier. Many FQHC patients experience monthly food insecurity and are on the supplemental nutrition assistance program (SNAP). We know from decades of work that every food dollar is stretched in this population and that locating affordable MIND food is a serious challenge. In fact, it has been demonstrated that the urban poor travel to many locations within a city to access lower priced food stores and “on-sale” items.(94) The frail in this population may even receive home-delivered meals. Our app is designed to be consistent with this behavior, USDA efforts to make produce more accessible,(95) and the Older Americans Act Nutrition services program.

We refer to our *MIND* intervention as MIND foods rather than MIND diet to emphasize that participants randomized to this arm will shop for and receive MIND food through the app. Participants will select foods from within the app (options can be modified based on reported food allergies). Selected foods will be delivered to the participant's place of residence or available for pick-up at Eskenazi Health locations once per week. The food options within the app will be restricted to those created from the Phenol-Explorer database(96) and approved by Dr. Tangney. Having ordered the week's food, participants will be prompted each day of the week to report the type and amount of purchased food consumed. An incentive of $1 for each daily report will be added to the cumulative incentive total.

The Control interventions are designed to mimic the activities of the MIND and Speed interventions. The food control participants will experience all of the features of the MIND food participants with the exception that the foods available to order will be limited to whole foods very low in polyphenols (e.g., corn, rice, potatoes, iceberg lettuce, pears, etc). Speed control participants will experience the same app features as Speed participants but in place of speed training will play inert games such as tic-tac-toe. All participants will be eligible for up to $17/wk of incentives.



All participants will be asked to sign an iPad user agreement when they receive the iPad at the Randomization Visit. Intervention calendars and other "reminder" tools will be utilized to increase engagement with weekly game play and food reporting.

Intervention Maintenance. After the 12-week post-training assessment, incentives and food deliveries will cease but participants will have continued access to BrainHQ. Those in the MIND or MINDSpeed arm will be encouraged to maintain MIND food consumption with utilization of the MINDSpeed Cookbook which details the foods and recipes used in the trial. Maintenance was chosen over total cessation of the intervention because it holds potential to maximize treatment potency, provides information on the rates of self-managed adherence (speaks to reach and effectiveness of the interventions), and should enhance subject retention. This phase of the study will provide information on the degree to which the trainings were incorporated into subjects' lifestyle in a sustainable manner. We will use intent-to-treat principles when analyzing the 6 month follow-up data. The adherence information from this phase of the study will be used in exploratory analyses. Subjects in the Food Control arms will receive the MINDSpeed Cookbook at the end of study participation.

Strategies for Treatment and Measurement Fidelity. Treatment fidelity and monitoring as defined by the NIH Behavior Change Consortium Strategies(97) and checklist(98) is enhanced by app-delivery and daily food and objective game/video data. A help call button on the app allows the participant to text research staff or request a call back (8 to 5, M-F). Staff will have access to the participant's treatment assignments. The staff on call will conduct supervised practice with the BrainHQ programs, and MIND food and food control interventions with access to our UX designer, Dr. Srinivas, clinical nutritionist, Christy Tangney, and training expert, Dr. Unverzagt and, if needed, Mr. Tinker. The staff will complete a 3-step certification process prior to working with enrolled subjects including: written test (passing score of 80%), individual module check out, and proctored mock counseling sessions with older adults using structured performance rating forms to judge quality. After certification, ongoing quality assurance checks will include observed counseling sessions once per month for first 1 month and every 3 months thereafter. A similar program of training, certification, and ongoing quality will occur for the Assessor, who will be blinded to treatment.

Safety. We have outlined our protocols for identifying and responding to events and injuries in the Protection of Human Subjects section of the proposal (see Adequacy of Protection Against Risk). We have described standard regulatory reporting procedures in the Data and Safety Monitoring Plan (DSMP) and the operation of our Data Safety and Monitoring Board (DSMB). Cognitive training is relatively safe but anxiety and burden can occur and will be monitored. MRI is a safe procedure for people who do not have metal implants or other contraindications. Study personnel will screen participants for these risk factors prior to enrollment. Before

entering the MRI environment, participants will be re-screened for MRI contraindications per IU standards. Physical confinement or noise in MRI can be uncomfortable. All efforts are made to assure participant's comfort. Blood draws will be conducted by trained and certified staff. Finally, a small number of participants may develop depression, dementia, or physical dysfunction during the study. Should this occur, we will direct the subject to appropriate clinical care (i.e., PCP or specialty clinic as needed). We will also direct subjects to appropriate clinical care based on blood pressure and pulse values obtained during Baseline, Post-Training, or Follow-Up appointments in clinic. This is not a study procedure, but rather a process for addressing potential safety issues within the hospital environment.

Measures. All assessments will be completed by a trained, certified, and blinded Assessor; all data will be entered into Research Electronic Data Capture (REDCap). We have decades of experience completing assessments with older adult participants.

Unless otherwise noted, measures are taken at baseline (BL), immediate post-training (PT), and 6 month follow-up (FU). BL, PT and FU assessments may be conducted at Eskenazi, IU Health Neuroscience Center, participant's homes, or via telephone with abbreviated outcome measures as necessary. [During the COVID-19 health crisis, all study visits will be completed by phone or Zoom, and biospecimen collection, imaging, and Viromeaging substudy enrollment will cease.]

"Off-line" training is tracked via subject-report at PT and FU of all non-study training, either cognitive or physical. Participants will indicate frequency and duration of leisure cognitive, physical, and formal educational activities.(99)

Feasibility Outcome Measures. We will track the number of potential participants approached, screened, and enrolled to gauge recruitment success using ≥ 50% of eligible participants enrolled as the criterion. We will also monitor the safety, acceptability, and adherence of the app-delivered interventions and outcome measurements. Safety will be assessed by comparing adverse event rates, both serious (i.e., hospitalizations, falls, episodes of angina) and non-serious (i.e., anxiety) across treatment groups. The acceptability of the experimental and control interventions will be measured using self-report surveys assessing satisfaction (i.e., 1 = very satisfied, 3 = neutral, 5 = very dissatisfied) with scores from 1-2 indicating acceptability. Adherence will be documented by dates, episodes, and time spent in completing speed training modules and food reports. Maintenance will be determined from training data and diet scores from PT to FU. Creation of RCT logistical structure will be measured as printed manuals for app delivery and support (experimental and control conditions) and Assessor certification and quality assurances.

Cognitive Outcome Measures. Consistent with the conceptual model, the primary outcome is a cognitive function composite. Our executive cognitive composite (ECC) consists of verbal fluency (phonemic and semantic fluency),(103) Symbol Search from the WAIS-IV, (104) Trail Making Test (Parts A & B, sec to complete),(105) and Stroop Interference (number completed in 45 seconds on color-word trial)(106) for each participant at each occasion of measurement. Raw scores for each test are standardized using the baseline mean and standard deviation. A composite is then formed as an average of z-scores. In addition, List Learning from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) will also be administered to assess new learning and memory. Cognitive outcome measures may be modified to accommodate illiterate participants, including but not limited to, removal of Stroop and COWA and modification of MMSE.

MIND Diet Score. At each assessment (BL, PT, and FU), we will complete an interviewer-administered food frequency questionnaire (FFQ). At BL, study personnel will use the subject's FFQ report generated by Viocare to guide a discussion on incorporating selected study foods into their diet during the 12 week intervention.

In addition, we will use urine samples to obtain "biomarkers of compliance" as was done in the recently completed PREDIMED multisite RCT.(109) We will use the "fast and simple" rapid Folin-Ciocalteu method to determine total polyphenols at BL and PT.(110)

Participants may be asked to repeat the urine collection at any assessment if the first collected samples become compromised (i.e., not useable) in any way.

Self-reported Outcomes. Geriatric Depression Scale (GDS) will measure depression, a modified CSRQ-25 will measure cognition and engagement, and portions of the SF-36(112) will measure health status and/or QOL. IADL will be measured by self-report using portions of MDS-Home Care.(22) These will be assessed at BL, PT, and FU.

APOE Collection and Assay. Genomic DNA will be extracted from blood samples using the DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. Approximately 50ng of genomic DNA will be used for amplification. *APOE* genotypes will be determined by restriction enzyme digestion of amplified DNA.(114)

Treatment and Adherence Modifiers. Individual difference factors with potential to modify response to treatment and adherence will be measured at BL including: age, race, gender, years of education, employment status and history, current exercise, *APOE* ε4 allele status, baseline cognitive status (via the MMSE), height & weight to compute body mass index, blood pressure & pulse, Charlson Comorbidity Index (collected/calculated by health system data manager via EMR;115), and reading ability (via Wide Range Achievement Test-IV Reading).

Blood Biomarkers. Fasting blood samples will be obtained at BL, PT, and FU in all subjects to explore the mechanisms of action. The conceptual model holds that the cognitive benefit of combined training is achieved through modulation of physiologic process related to neural proliferation, oxidative balance, and metabolic function. As a result, we will measure circulating levels of the pro-inflammatory cytokines IL-1α, IL-1β, IL-6, and TNF-α; the anti-inflammatory cytokines IL-1ra and IL-10; the acute-phase reactant CRP; VEGF and BDNF; and also metabolic processes reflected in insulin resistance and blood glucose. We will also measure plasma biomarkers for levels of amyloid-β as a predictor for amyloid-β deposition in the brain, which is the earliest pathological sign of Alzheimer's disease.

Oxidative stress will be determined from Thiobarbituric acid reactive substances (TBARS), a byproduct of lipid peroxidation. We will obtain 3 six-ml purple top EDTA tubes of blood taken in the fasting state. Blood samples will be centrifuged within 10 minutes of collection. Plasma aliquots will be stored at -80°C until the time of assay. Measures will be performed in the Analyte Lab (Considine Laboratory) at the Center for Diabetes and Metabolic Diseases at Indiana University (1P30DK097512 Mirmira R [PI]) using Human Quantikine ELISA Kits (R&D Systems, Minneapolis, MN). Both Drs. Clark and Considine are members of the Center, which provides analyte services at reduced rates.

Participants may be asked to repeat the blood collection at any assessment if the first collected samples become compromised (i.e., not useable) in any way.

Viromeaging procedures [only available to consented subjects through 5/31/22]. At Baseline only, we will obtain additional fasting blood in purple top EDTA tube(s) totaling 10 ml for participants consenting to this sub-study. These samples will be delivered to Dr. Twigg's lab for immunologic and inflammation assays. Dr. Twigg will send deidentified samples to The Jackson Laboratories for RNA and DNA Sequencing and virome analysis. Consented subjects will also complete measures of grip strength, repeated chair stand, and a modified CES-D (depression) questionnaire.

MRI Acquisition Protocol. Participants agreeing to MRI will have two scans; one at BL and one at PT. Scans will be read and interpreted by the IU Center for Neuroimaging team consisting of Dr. Saykin, Professor of Radiology, and his assistant, Dr. Shannon Risacher. Clinically concerning findings will be communicated to the subject and their primary care provider by our study physician, Dr. Christopher Callahan. In these cases, we will also send the patient's physician a radiologist report. Significant findings (i.e., tumor, hemorrhage, aneurysm, hydrocephalus, or other significant clinical finding) may lead to withdrawal of subject. The following scans will be performed on the same research-dedicated new generation Siemens Prisma 3T scanner with a 64CH receiver-only head coil: 1) Sagittal survey 3-plane localizer; 2) ADNI T1-weighted MP-RAGE 3D anatomical volume; 3) ADNI High resolution T2-weighted hippocampal sequence; 4) ADNI Axial T2-weighted FLAIR sequence; 5) Diffusion tensor imaging (DTI); 6) Blood Oxygenation Level Dependent (BOLD) functional MRI (scene encoding); and 7) A GRE Field map. The first four sequences will be collected following ADNI protocols (http://adni.loni.usc.edu/methods/documents/mri-protocols/). For DTI, a single-shot spin-echo echo-planar imaging (EPI) multi-band 3 sequence is used with standard parameters, 69 slices, 2mm^3 voxels, 60

diffusion directions, 3 b0 images, and b-value: 1000 s/mm^2; parallel imaging with iPAT=2 will be used to reduce susceptibility artifact (116). The BOLD fMRI task protocol is a 2D T2*-weighted multiband EPI with 39 axial contiguous slices, 3.0x3.0x3.5 mm^3 voxels, TR/TE=2250/29ms, parallel imaging with iPAT=2 and 32 reference lines to reduce susceptibility artifact (116), and 3D prospective motion correction algorithm to detect head motion (123). The GRE field map is obtained at the beginning of the functional MRI scans to correct for distortion due to field inhomogeneity with two TEs (3.98ms, 6.44ms). Scene Encoding and Recognition, modified from Detre and colleagues(124-126) which elicits reliable bilateral MTL activation. Briefly, during the blocked-design encoding trial subjects encode complex scenes for later recognition. The control condition consists of repetition of a degraded version of one of the encoding stimuli. During the event-related recognition trial the subject views target scenes intermixed with foils and is asked to decide which were presented during the encoding trial. Approximate scan time is 30 minutes.

MRI Analysis Methods. Scans will be processed using standard techniques and normalized to standard space for analysis (i.e. Montreal Neurologic Institute, MNI). Structural MRI scans will be processed using voxel-based morphometry (VBM) in Structural Parametric Mapping (SPM8 or SPM12 which we are testing now) and FreeSurfer as previously described.(88-90, 127) Each DTI image volume will be motion- and eddy current-corrected prior to preprocessing. The diffusion tensor, fractional anisotropy (FA), and mean diffusivity (MD) will be processed voxel-by-voxel. The directional measure will also be generated for white matter (WM) tractography and tract-specific ROI. Structural connectivity between gray matter regions with significant fMRI/rsfMRI signal will also be obtained. Task-related fMRI scans will be processed using methods described our prior work.(82, 91, 99) Briefly, contrast images of task-related activation relative to control conditions will be generated using pre-processing techniques implemented in SPM8.(82, 91) These contrast images will be used for further analysis. (99) Briefly, pre-processed RS-fcMRI scans will be temporally concatenated and analyzed using independent component analysis (ICA) to generate whole group-level components using FSL.(128) Reconstructed participant-specific component maps will be calculated using dual regression (129-134) with the 20 group components as templates. The resulting maps for each component of interest (i.e., DMN, and other cognitive networks), will be further analyzed. ASL images will be corrected for motion, pair-wise subtracted between label and control images and averaged to generate a mean difference image. Quantitative CBF maps will be calculated using a single compartment model with *in vivo* measurement of blood T_1 and labeling efficiency.(121, 122) CBF maps will be co-registered with MPRAGE, which will be segmented into gray (GM), white matter (WM), and CSF compartments and normalized to the MNI template. CBF in standard regions of interest (ROIs) will be extracted for further statistical analysis to test study hypotheses.

VBM grey matter density (GMD) images, DTI FA and MD images, task-related contrast images, RS-fcMRI component maps, and ASL CBF images will be compared between those receiving the combined intervention, a single intervention, and control cross-sectionally and longitudinally on voxel-wise and ROI levels. Cross-sectional voxel-wise analyses will compare the scans between groups at BL, PT, and FU. Longitudinal voxel-wise analysis will model time-by-group interactions using a two-way Analysis of Covariance (ANCOVA) model. All voxel-wise analyses will be performed in SPM8 (or SPM12). For regional analysis, we will extract a mean value for ROIs from the final stage preprocessed scans. Specifically, cross-sectional and longitudinal change in regional brain atrophy (i.e., MTL, frontal lobe), WM integrity (i.e., FA, MD from MTL WM tracts), task-related fMRI activation (i.e., activation from task-specific regions), resting-state connectivity (e.g., DMN connectivity from medial parietal lobe and MTL, etc.), and cerebral perfusion (i.e., whole brain, frontal lobe, etc.) will be compared between groups using values extracted from hypothesized target ROIs (see Analytic Plan).

Cost. We will track all personnel, equipment, technology, and support costs associated with delivering the interventions thus allowing an estimation of intervention costs on a per subject basis.

Power and Sample Size. The primary analysis of the study focuses on the evaluation of the effectiveness of the interventions on the cognitive outcome, ECC. We therefore determined the sample size to ensure adequate power for this analysis. In these prior studies, speed of processing training was found to have an effect size (ES) of 1.4 standard deviation (SD) at immediate post-training (PT) and 1.2 SD at one year PT on processing speed outcomes.(22) MIND diet is a brain health optimized hybrid of the Mediterranean-DASH diets, and the latter were found to have an effect size of 0.44 to 0.56 SD on executive function and speed of processing(136). There are no published trials of speed of processing training combined with MIND diet but we expect a large effect size of the combined intervention. With an overall alpha level of 0.05 for six Bonferroni-

adjusted two-sided comparisons, a conservative estimate of the correlation of 0.7 between baseline and follow-up based on prior studies, and 15% attrition rate (in our recent GRACE trial of 951 older adults—62% had < 12yrs of education—91% completed the 6-month follow-up assessment)(73), the sample size of up to 50 participants per treatment arm will yield 80% power to detect an effect size of >0.5 SD based on the constrained longitudinal data analysis (cLDA).(137) The cLDA (138) has been shown to produce greater efficiency and handle missing data more flexibly with valid inference when data are missing at random.(137, 139). Cognitive aging studies support an effect size of over one-half standard deviation as clinically meaningful.(140-142)

Exploratory Aims and Power: Outcomes include inflammation, metabolic function, oxidative stress, and hippocampal structure and function (via MRI). For non-imaging outcomes, we will have a full sample and adequate power to detect effect sizes of 0.6. Imaging creates anxiety and discomfort for some, however. Thus, in addition to using information and communication strategies shown to reduce imaging anxiety(143) we will complete imaging on the minimum necessary. From the literature, we anticipate large effect sizes on the brain outcomes of hippocampal structure and function. Cognitive training has resulted in changes in brain structure with approximate effect size of 0.56 in left hippocampus volume following spatial navigation training among healthy younger and older men(144) and an approximate effect size of 0.47 in gray matter density (GMD) after the completion of a multidomain computerized cognitive training among healthy older adults.(20) In addition, our preliminary results showed an effect size of 0.5 in annual percent change in hippocampal volume and 0.8 in annual percent change in hippocampal GMD among older adults without MCI who are frequently engaged in reading and puzzle-solving compared to those who are not. Food interventions alone have had an effect size of 0.7 on hippocampal volume.(9, 56) Other studies support effect sizes of 0.64 to above 1.00 on functional connectivity.(145, 146) A one-half subsample of each arm (n=22 per arm) with an alpha level of 0.05 and a correlation of 0.7 between baseline and post-training provides 80% power to detect an effect size of 0.7 or larger for the brain imaging outcome.

In order to test the mediating effect of blood biomarkers, brain structure, and brain function variables we need to establish the association between the mediating variable and primary outcome, after controlling for the intervention effect. Such an association is evaluated using a partial correlation. With our sample size, we have more than 80% power at the 0.05 alpha level to test a partial correlation of 0.3 between mediating variables and the primary memory composite outcome. Effect modifiers include adherence, percent body fat, age, baseline cognitive status, comorbidity, and *APOE* ε4 carrier status. Evaluation of moderating effects is an exploratory aim and detection of such effects requires large sample sizes. Our study is therefore only able to explore treatment moderators. In addition, our sample size provides accuracy with no more than 8% margin of error at the 95% confidence level for feasibility outcomes.

Analytic Plan: *Preliminary Analyses.* Baseline characteristics of participants will be compared across treatment arms. We will also compare the baseline scores on outcome measures across treatment arms. For continuous variables, means and standard deviations will be reported. Frequency tables will be reported for categorical variables. Normality of continuous outcomes will be examined using a normal probability plot. For highly skewed measurements, appropriate transformations will be applied to achieve approximate normality. Repeated measurements over time will be graphed as spaghetti plots and examined for outliers. All data will be examined for completeness. The baseline characteristics will be compared across treatment arms and between participants who do and do not complete the study. If any significant differences exist, we will control for differences in the subsequent analyses. Analysis will be performed under the intention-to-treat paradigm.

The *primary analysis* of the intervention effect on ECC will be performed using the constrained longitudinal data analysis (cLDA) proposed by Liang and Zeger.(138) Repeatedly measured ECC outcome at BL, PT, and FU will be modeled as dependent variables with the baseline mean responses constrained to be the same across intervention arms due to randomization. This model is flexible in handling missing data and has been shown to provide greater power than other models when testing intervention effects and yields valid results when data are missing at random. The clustering effect of repeated measures within a subject will be accommodated using a random subject effect. Time of measurement (BL, PT, FU) and its interaction with intervention arm will be included in the model. The time of measurement is considered as a categorical variable to allow non-linear time effect. Intervention effects are captured by the significant interaction effect. Comparison of outcome measures across intervention arms at PT and FU will be estimated based on the

model using appropriate contrasts. In order to assess whether the intervention effect is affected by physical activity change, weight change, or other factor, we will examine change patterns across intervention arms. If the change pattern differs across intervention arms, that factor will be controlled by including its main effect and interaction with the intervention arm in the primary analysis for intervention effect on memory.

Exploratory Analyses. Feasibility hypotheses are 80% or greater intervention adherence and acceptability, and no serious adverse events. These will be estimated using the 95% confidence intervals. To compare safety, we will estimate the difference in rates of adverse events across the intervention arms using 95% confidence intervals. Hypotheses of improved self-reported outcomes including depression, cognition and engagement, quality of life, and IADL will be examined using the cLDA methods described immediately above.

Mechanism Analyses. Mechanistic hypotheses are that, relative to control, intervention arm participants will have greater hippocampal volume, enhanced DMN and CEN connectivity at PT, better metabolic function, higher levels of neurotrophic growth factors, and lower levels of inflammation and oxidative stress. Hypotheses will be analyzed using cLDA. With regard to MRI, scan analysis described immediately above will be performed on targeted pre-specified ROIs and followed by spatially unbiased whole brain voxel-based analyses. Supplemental analyses will compare across scan modalities to address the relative incremental contribution of each modality (e.g. hippocampal volume) to capturing the overall neuroimaging outcome.

Moderator and Mediator Analyses. Analyses will be performed to determine the role of adherence, years of education, age, baseline cognitive scores, comorbidity, and *APOE* ε4 carrier status (an indicator of elevated ADRD risk) in moderating intervention outcomes using cLDA. We hypothesize in particular that participants with greater adherence, more education, and lower comorbidity will experience larger ECC score gains. A three-way interaction between potential moderators, intervention arm, and time of measurement will be included in the model and a significant three-way interaction indicates the moderating effect. To evaluate hypothesized mediators of the speed training and MIND foods (e.g., inflammation, growth factors, oxidative stress, hippocampal volume), we will use the approach proposed by Krull and Mackinnon (2011) (147) that allows the testing of mediating effects in clustered data. Using the change in ECC outcome from baseline as the dependent variable, a series of three mixed effects models, as outlined by Krull and Mackinnon, will be fit and regression coefficients from these models will be used to estimate the direct and indirect treatment effects.

The cLDA provides valid inference when data are missing due to random dropouts. Sensitivity analyses will be performed to examine to what extent the results are affected when dropouts are not missing at random. Analyses based on complete cases, mean imputation, and worst case imputation will be performed. In addition, a pattern mixture model will be used to allow different intervention effects for subjects with different missing data patterns. Results based on these models will be compared to results from cLDA.

Study Considerations. A key feasibility challenge is treatment and assessment adherence. If adherence via the MINDSpeed app drops below 80%, we will institute, first, increased incentives and, second, telephone reminder and support calls. Study retention is also a feasibility challenge. With fair subject payments in past work, we have completed one-half day assessments at the IU Center for Neuroimaging. We have a "step-down" assessment battery if a participant is not able to tolerate the full outcome assessments. The timeline shown below will allow us to accomplish the aims of our proposed MINDSpeed pilot trial, which includes time in Year 5 for development of a later stage AD prevention trial should feasibility and efficacy be supported.