Effect of a Ketogenic Diet on Alzheimer's Disease Biomarkers and Symptoms:

"**B**rain **E**nergy for **A**myloid **T**ransformation in **AD** (**BEAT-AD**)" Study

Protocol Identifying Number: ##

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Funded by: National Institute of Aging

Draft / Version Number: d.10.0

16 February 2018

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INTRODUCTION: BACKGROUND AND SCIENTIFIC RATIONALE

Overview

Dietary patterns have been linked to risk of developing Alzheimer's disease (AD) – a progressive fatal disease with no current effective treatments – that places an extraordinary burden on patients, families and society. As recently reviewed, dietary patterns high in saturated fat and simple carbohydrates, also termed "Western" diets, are associated with increased risk of AD and other dementias, whereas diets high in mono- and poly-unsaturated fats, vegetables, fruits and lean proteins are associated with reduced risk.[1,2] It is not surprising that diet is a powerful modulator of brain aging. The brain derives most energetic substrates such as glucose, amino acids and fatty acids directly from dietary sources. Diet also impacts peripheral metabolism and may thereby affect the brain. For example, the Western diet is associated with obesity, diabetes, inflammation, and vascular disease, disorders that increase the risk of AD through multiple mechanisms. Diets have also been used as therapeutic tools to treat medical conditions, such as the use of the ketogenic diet (KD) to treat epilepsy, and the low fat diet to treat cardiovascular disease. In preclinical studies, elevating ketones in AD mice improved memory and reduced levels of the toxic β-amyloid peptide (Aβ), a hallmark of AD pathology.[3-5]. Studies that have elevated ketones with medium chain triglyceride (MCT) supplements in humans with AD have also documented memory improvement.[6,7]

In the following section we describe research investigating the effects of the KD on the brain, and support its investigation as a potential therapy for AD.

The Ketogenic Diet is a Powerful Modulator of Brain Function

The ketogenic diet (KD) is a very low carbohydrate, adequate protein diet developed by the Mayo Clinic in the 1920's to treat refractory epilepsy. Due to carbohydrate restriction, fats replace glucose as a primary energy source; the liver converts fats into fatty acids and ketone bodies (β-hydroxybutryate and acetoacetate) that are readily transported into the brain. The KD is remarkably effective; 50-70% of patients have >50% seizure reduction and 30% have >90% seizure reduction. [8] In recent years, several adaptations of the KD have been developed that improve compliance and reduce health risks associated with prolonged high intake of saturated fats. One such adaptation, the modified Mediterranean ketogenic diet (MMKD), has comparable efficacy to the original KD but allows slightly higher carbohydrate consumption to permit increased intake of vegetables and fruits, and emphasizes fats and proteins derived from healthy sources such as olive oil and fish.

Mechanisms underlying the effectiveness of the KD are not definitively known, but candidates include reduction of neuronal hyper-excitability through glutamatergic inhibition due to increased production of GABA, and KD enhancement of mitochondrial metabolism with corresponding activation of ATP-sensitive K+ channels. These and other potential KD-related mechanisms are discussed below.

KD Effects on Neuronal Excitability. Neuronal hyper-excitability, a cardinal feature in epilepsy, has also been observed in amnestic mild cognitive impairment (aMCI) and early AD, particularly in brain regions prone to early amyloid deposition. Such hyper-excitability has been proposed as a cause or an effect of amyloid deposition, and may represent a compensatory response to reduced local ATP production.[9] Ketones modulate glutamate metabolism, increasing availability of the inhibitory neurotransmitter GABA and may thus restore inhibitory-excitatory balance within the central nervous system (CNS).[10] Supporting this possibility, cerebrospinal fluid (CSF) GABA levels increased following a 4-month KD intervention in epileptic patients, and greater GABA increase was associated with better seizure control.[11]

KD Mitochondrial Effects. Epileptogenesis is associated with disrupted mitochondrial energy metabolism and increased reactive oxygen species (ROS), pathologies also related to AD.[12] Mitochondria are the primary producers of ATP, through carbohydrate oxidation in the TCA cycle, and through β-oxidation of fats. Mitochondria also play critical roles in regulation of calcium and ROS. Rodent studies have demonstrated that mitochondrial dysfunction promotes AD pathology.[13-17] Importantly, mitochondrial bioenergetic deficits precede amyloid plaque formation in mice,[18] and therefore offer a potential target for early intervention. A bioenergetic shift theory of AD has evolved suggesting that the transition from normal aging to AD is associated with increasing reliance on fatty acid oxidation to supply brain energetic needs, in part due to mitochondrial dysfunction.[19, 20]

In *in vitro* and preclinical studies, the KD has potent mitochondrial effects, modulating the expression of enzymes involved in mitochondrial metabolism, as well as increasing mitochondrial biogenesis in key brain regions such as the hippocampus.[21] ROS are produced in the course of normal mitochondrial metabolism, and may function as signaling effectors; they are maintained at non-pathological levels by intact antioxidant defenses. Pathological ROS levels are thought to be both a cause and effect of impaired mitochondrial metabolism, with glutathione (GSH) as a key ROS regulator. Ketones decrease ROS levels in *in vitro* and preclinical models, in part due to increased GSH biosynthesis (for a review see [12]). In preliminary data, we observed that adults with aMCI treated with a 6-week MMKD had increased mitochondrial activity in monocytes that correlated with changes in CSF Aβ42 and memory.

Other KD-Related Mechanisms.

Protein Pathways - HIF-1α, Sirt1 and MTOR: Ketogenic diets have been shown to impact other pathways related to metabolism and autophagy. Ketogenic diets increased levels of hypoxia-inducible factor-1α (HIF-1α) in rodents, possibly due to succinate generation during mitochondrial metabolism of ketone bodies. Increased HIF-1α in turn increased cortical capillary density through enhanced transcription of vascular endothelial growth factor (VEGF), with associated memory improvement.[22] The KD upregulation of proteins Sirt1 and MTOR have also been reported to suppress Aβ production and tau hyperphosphorylation, with increased neuroprotection and autophagy.[23, 24]

KD Epigenetic Effects: A growing body of work documents KD effects on epigenetic mechanisms. Increased neuronal hyper-excitability in rodent epilepsy models is associated with DNA hypermethylation, which is reversed by KD.[25] Such effects are mediated in part through β-hydroxybutyrate which functions as a histone deacetylase inhibitor,[26] and through adenosine-mediated normalization of DNA methylation.[27] In our proposed study, we will examine KD epigenomic effects on pathways related to oxidative phosphorylation, which in preliminary work we have shown to be related to aging and cognitive function.

KD and CNS Insulin Resistance: Markers of brain insulin resistance have been documented in neuropathological and ex-vivo stimulation studies of human AD brain, as well as in AD animal models.[28-30] Insulin plays an important role in memory as well as in regulation of β-amyloid and tau phosphorylation (for a review, see [31]). The KD has dramatic effects on peripheral insulin sensitivity, and in some rodent models also enhances brain insulin response and expression of brain insulin and IGF1 receptor mRNA,[32, 33] although no changes were observed in a short-term diet in C57B6 mice[34]. New methods using neural-derived plasma exosomes have been used to assess CNS insulin resistance; exosome abnormalities indicative of CNS insulin signaling defects in adults with AD have been reported.[35]

KD and Sphingolipid Metabolism: Recent metabolomic /lipodomic studies have identified abnormal brain glycerophospholipids, sphingolipids, fatty acids, and their metabolites in AD and aMCI.[36, 37] Sphingolipids regulate cell membrane structure, function, and apoptosis. The coordinated homeostasis between cholesterol and sphingolipids protects neurons, but also likely underlies some

neurodegenerative processes in AD, as they both modulate amyloid precursor protein (APP) processing.[38-40] Ceramide is a key intermediate for sphingolipid metabolism.[41] Elevated serum ceramide and sphingomyelin predict incident cognitive impairment, dementia and hippocampal volume loss in aging and aMCI.[42-44] CSF ceramide and sphingomyelin also predicted CSF Aβ and tau levels in middle-aged adults, suggesting they may also be biomarkers of early AD pathology.[45] The KD has been shown to reduce levels of ceramides and other toxic lipid metabolites.[46] In the proposed study, we will conduct targeted lipodomic and metabolomic analyses of sphingolipid, fatty acids and other pathways, in addition to proteomic analyses, that may reveal novel biomarkers of early disease or treatment response.

KD Effects in AD.

Preclinical Models and Effects on β-Amyloid: Preclinical studies provide evidence of positive effects of elevating ketones on AD pathological processes, and in particular on regulation of β-amyloid, which has been proposed as an initiator of the AD pathological cascade. Transgenic mice, 3xTgAD, treated with a ketone-inducing intervention showed less amyloid and/or tau pathology, and improved memory performance than chow-fed mice in three studies.[3-5] These results are supported by findings that ketones interact with Aβ metabolism in multiple ways. Aβ may be transported through axons into intracellular compartments, where it interferes with mitochondrial function by binding to mitochondrial proteins, leading to increase ROS. Ketones have been shown to reduce Aβ neurotoxicity through blocking Aβ entry into neurons and reducing amyloid aggregation, with associated improvements in mitochondrial function and memory.[5] Furthermore, a KD has been shown to modulate gene expression of α- and γ-secretases, enzymes involved in Aβ clearance and degradation, and of genes involved in mitochondrial biogenesis.[19, 47] Although there is growing evidence of KD-Aβ interactions, some studies in mice have failed to demonstrate an impact, perhaps due to genetically-based differential susceptibility to KD effects, as different mouse strains have been shown to have different metabolic and seizure modulating effects of the KD.[48,49]

The preponderance of findings from preclinical studies demonstrates that ketones impact Aβ regulation, with associated effects on mitochondrial metabolism, ROS, and memory. Clearly, further study is needed to demonstrate the relevance of these findings to humans. We have documented changes in CSF Aβ42 in adults with aMCI treated with a MMKD. In our proposed study, we will directly address the question of whether MMKD modulates CSF Aβ42 (our primary outcome) in humans.

Human AD Studies. Several studies have examined the effects of elevating ketones with medium chain triglyceride (MCT) supplements in AD. An early study from our laboratory showed acute memory improvement after MCT consumption in adults with early AD, an effect restricted to participants without the APOE-e4 allele.[7] In a Phase II double-blind, placebo-controlled, 3-month trial of a MCT supplement in 152 adults with early AD, participants without the APOE-e4 allele showed significant improvement on the Alzheimer's Disease Assessment Scale-Cognition (ADAS-Cog). [6] This trial was compromised however by a high rate of gastrointestinal side effects that necessitated a protocol revision mid-trial, and resulted in a high drop-out rate in the active treatment group.

Use of a supplement to increase ketone availability is an attractive strategy due to its relative ease of implementation. However, because supplements are consumed in the context of a normal diet their efficacy may be attenuated. For example, MCT supplements do not reduce seizures in epilepsy. Pathological aspects of the typical diet such as high simple carbohydrate intake may counteract KD effects. Additionally, many nutrient-derived substances enter the brain through competitive diffusion, and thus intake of competitive substrates may attenuate the benefit of ketone supplements. In contrast, the KD promotes endogenous elevations of ketones that are readily transported into the brain. To date, only one small trial has examined the effects of a KD in adults with aMCI.[50] Twenty-three adults with aMCI were randomly assigned to a 6-week KD or AHAD. The KD diet group showed enhanced verbal memory, as well as improved peripheral metabolism.

Summary: Strengths and Weaknesses of Supporting Research. The convergence of research to date indicates that the MMKD, a KD variant designed to improve compliance and nutrition, is a potent therapy for epilepsy and may benefit aMCI via multiple pathways, through: 1) reduction of neuronal hyperexcitability; 2) improved mitochondrial function and cerebral bioenergetics; 3) reductions in oxidative stress, inflammation and lipotoxicity; 4) enhanced autophagy; and ultimately, 5) improved Aβ and tau regulation. Preclinical studies in AD mouse models and preliminary studies that elevate ketones in humans with supplements or brief diet intervention provide promising results. We will build on this solid foundation to conduct a Phase II study that will provide rich data regarding the efficacy, feasibility, safety, and underlying mechanisms associated with MMKD intervention. These data will inform the design of a future Phase III trial and identify novel biomarkers and therapeutic targets that may enhance precision medicine approaches to diet and AD risk.

OBJECTIVES AND PURPOSE

The purpose of this study is to examine the effects of a 4-month MMKD compared with an American Heart Association Diet (AHAD - a regimen that has been shown to reduce the risk for cardiovascular disease). We will investigate diet effects on AD biomarkers, on cognition, on neuroimaging measures of metabolism, vascular function, and on CSF/blood epigenetic, exosome, and omic markers. Our study will extend previous findings in several important ways by: 1) using a MMKD rather than a traditional KD, which has the potential for greater long-term compliance and health benefits; 2) increasing the sample size and duration of the diet intervention; 3) examining potential mechanisms of diet effects that may result in new biomarkers and therapeutic targets; and 4) examining key treatment response variables such as APOE genotype, amyloid positivity and metabolic status that could inform precision medicine approaches to dietary prescription.

Objectives:

1. Assess the safety and feasibility of a 4-month MMKD vs. the AHAD in adults with aMCI, and determine its effects on a key AD biomarker (CSF Aβ42 – primary outcome), as well as on: cognition, cerebral metabolic rate of glucose utilization (CMRg) measured with ^{18}F-FDG PET; brain ketone utilization assessed with ^{11}C-acetoacetate PET; MRI measures of perfusion, hippocampal volume, default mode connectivity, and white matter integrity; and other CSF biomarkers (total tau, p-tau181).
2. Investigate response moderators: APOE genotype, amyloid PET positivity and metabolic status.
3. Investigate proposed mechanisms underlying MMKD effects, including: 1) neuronal activity and synaptic function (MRI, PET); 2) bioenergetic pathways (mitochondrial function assays, plasma neural-derived exosome markers); 3) epigenetic analyses; 4) CSF/blood multi-omic panels (proteomics, metabolomics, lipidomics, transcriptomics).

Importance of the Knowledge to be Gained

In addition to knowledge gained about the potential therapeutic efficacy of a Modified Mediterranean-Ketogenic Diet intervention in adults with early AD, the trial will also provide important knowledge concerning the role of glucose and lipid metabolism in AD and in the effectiveness of diet-induced ketosis. Baseline metabolic status and changes in metabolic profiles may provide insight into the differences between baseline status of the groups, but also response to the proposed intervention. This metabolic profile analysis may ultimately be used as a biomarker to determine potential efficacy of similar interventions in AD. Such knowledge will be of great utility in the design of other therapeutic interventions. Based on these potential contributions, and the minimal potential risks to safety of the participants, the risks of the trial are reasonable.

STUDY DESIGN AND ENDPOINTS

Adults with aMCI (n=120) will be randomized on a 1:1 schedule to receive either a 4-month MMKD or AHAD intervention. The MMKD and AHAD macronutrient profiles will be identical to those tested in a pilot study (IRB# 0029992). Diet interventions will be equicaloric with participants' normal diets. Personalized nutritional guidance and menus will be provided, and compliance will be assessed by a registered dietitian. Amyloid PET will be conducted at baseline. Blood collection and cognitive assessment will be conducted at baseline and after 2 and 4 months of diet. Lumbar puncture (LP), MRI and dual tracer PET will be conducted at baseline and following the intervention for all participants (see Table 1. for a list of study measures).

Participants

Participants will be recruited from the Wake Forest Alzheimer's Disease Core Center (ADCC), and from the community. Participants aged ≥55-85 years will be enrolled who meet adapted NIA-AA criteria for aMCI [51], and adjudicated by expert multidisciplinary consensus. The criteria for aMCI will be based on cognitive and clinical evaluation, and all cognitive and clinical data will be considered in assigning diagnoses. In general, cognitive testing will indicate two deficits, ≥1 SD below age and education-adjusted means, on two memory tests of free and delayed recall, or on one memory and one language or executive function test. In addition to numeric criteria, expert clinician judgment will be used to interpret cognitive performance. The CDR score for study inclusion may be 0 or 0.5, and subjective cognitive complaints will not be required from the participant or study partner. Given that we will conduct amyloid PET, we will be able to determine which participants meet criteria for aMCI due to AD. [51] We will not restrict enrollment on this basis, but rather use this criterion in responder analyses.

Sample Size

120 participants will be recruited for this study. Males and females will be comparably represented, and every effort will be made to enroll an ethnically diverse sample that typifies the geographical region. Participants will be randomized to one of two diets (MMKD or AHAD) for four months, with 60 participants in each diet group.

Length of Intervention

We have observed effects on CSF biomarkers, cognition and imaging in our pilot study with a 6-week MMKD intervention, and in previous month-long diet interventions. [52] We believe that a 4-month period will provide important feasibility and compliance data, as well as potentially reveal other cognitive and biomarker effects useful for the design of a Phase III study.

AHAD as a Control Diet

We believe that the AHAD is a good comparator for the MMKD because it will require the same attention to food intake and thus control for "meta" aspects of the intervention. As a healthy diet it is reasonable to expect it may benefit some participants, and thus will equate expectations across the two diet intervention groups. In our pilot study the AHAD diet produced a distinct profile from the MMKD, and thus will allow us to investigate response predictors that may inform precision medicine dietary prescriptions.

Study Endpoints

CSF Aβ42 as a Primary Endpoint: The convergence of findings from preclinical studies demonstrate that elevating ketones impacts Aβ regulation, and we have documented changes in CSF Aβ42 in aMCI treated with MMKD in our pilot study. Using CSF Aβ42 as a primary outcome will allow us to address the important question of whether the MMKD modulates AD pathological processes. Given that aMCI is

reliably associated with reduced CSF Aβ42, the expected direction of a beneficial response is for CSF levels to increase, as we have observed. We will be able to support the interpretation that increased CSF Aβ42 reflects benefit by examining associations with other outcomes. We have extensive experience in recruitment for studies requiring LPs; we successfully recruited 100 participants for a recent trial of a Western diet that required 2 LPs (R37AG-10880), and our pilot trial required 3 LPs. The fact that 97% of our participants return for follow-up LPs attests to our excellent safety record and the skill of our clinicians in minimizing discomfort.

Preclinical Alzheimer Cognitive Composite (PACC) as a Cognitive Endpoint: Although the PACC was designed for use with preclinical AD, it is reasonable to assume it will also be sensitive in aMCI, as all PACC components are frequently used in aMCI studies. According to PACC developer, Reisa Sperling, MD, it is a useful indicator of cognitive change in aMCI in the Harvard Aging Brain Study (personal communication). Further, we observed significant improvement in our cognitive test composite that includes 2 PACC components following the MMKD.

Selection of Endpoints

We have designed an ambitious study that will collect data on multiple outcomes, to inform future trial design and to enable the identification of novel biomarkers and therapeutic targets. The approach we have taken is to identify a limited number of analytics from each of the proposed methods that are well-supported by our pilot data and that will be analyzed as part of the project (Table 1. below).

Table 1. All Study Endpoints

Primary	
CSF	AB42
Secondary	
CSF	Total tau, p-tau 181, ceramides, fatty acids, redox proteomic profile
PET	Global 11C-AcAc uptake, 18F-FDG ALZ/PMOD
MRI	Hippocampal volume, AD signature cortical thickness, Default Mode Network connectivity, PCASL posterior cingulate perfusion
Blood	Mitochondrial basal, maximal, spare respiratory capacity, OXPHOS transcriptomic index, OXPHOS methylation index, exosome insulin resistance index
Cognition/Function	PACC (Primary), ADAS-COG 12, CDR, Executive function tests, Cogstate tests, ADCS-ADL
Mood/Sleep	NPI-Q, Sleep Quality Assessment (PSQI), Epworth Sleepiness Scale, WatchPAT

Setting: All study visits will take place at Wake Forest Baptist Medical Center.

STUDY ENROLLMENT AND WITHDRAWAL

Participant Inclusion Criteria:

1. Male or post-menopausal female;
2. Age 55 to 85 years inclusive;
3. Diagnosis of amnestic mild cognitive impairment (aMCI; single or multi-domain);
4. An informant (study partner) able to accompany the participant to at least 3 visits, including at least two diet education visits;
5. Stable medical condition (generally 3 months prior to screening visit) at the discretion of study physician;
6. Stable on medications (generally 4 weeks prior to screening visit) at the discretion of study physician;
7. Able to complete baseline assessments;
8. Fasting glucose within the normal range.

Participant Exclusion Criteria:

1. Diagnosis of neurodegenerative illness (except for MCI);
2. History of a clinically significant stroke;
3. Current evidence or history in past year of focal brain lesion, head injury with loss of consciousness or DSM-IV criteria for any major psychiatric disorder including psychosis, major depression, bipolar disorder, alcohol or substance abuse;
4. Sensory impairment (i.e.: visual or auditory) that would preclude the participant from participating in the protocol;
5. Diabetes that requires current use of diabetes medications;
6. Clinically significant elevations in liver function tests;
7. Active neoplastic disease (stable prostate cancer and non-melanoma skin cancer is permissible);
8. History of epilepsy or seizure within past year;
9. Contraindications for MRI (claustrophobia, craniofacial metal implants, pacemakers);
10. Significant medical illness or organ failure, such as uncontrolled hypertension or cardiovascular disease, chronic obstructive pulmonary disease, liver disease, or kidney disease;

11. Use of the following medications: anticonvulsants, drugs with potential interfering CNS effects (other than cholinesterase inhibitors or memantine), medications with significant anticholinergic activity, anti-parkinsonian medications or regular use of narcotic analgesics; small stable doses may be permitted at the discretion of study clinician

12. If female, menstruation in the past 12 months or hysterectomy and current hormone replacement therapy medication;

13. Major digestive disorders, absorption issues, or surgeries that may be exacerbated by diet changes;

14. Untreated hypothyroidism or B12 deficiency;

15. Participants currently using resveratrol, CoQ10 (coenzyme Q10), coconut oil/other medium chain triglyceride-containing (ie: Axona) supplements, or curcumin will be excluded unless they are willing to discontinue them 2 weeks prior to the start of baseline visits and remain off for study duration.

Strategies for Recruitment and Retention

A total of 120 participants (age 55-85 years) will be recruited for completion of the proposed study. Subjects that meet inclusion criteria will be randomized into one of two diet groups, the MMKD or AHAD. The AHAD group will serve as the control group and the MMKD the intervention group, each with a target of 60 participants.

A standardized phone screen questionnaire will be used to determine basic eligibility. Potential participants who pass this initial screen will then receive an in-person screening, consisting of physical/neurological exam, medical history, and neuropsychological exams to confirm an aMCI diagnosis. All clinical data will be reviewed by expert study physicians and neuropsychologists. Clinical laboratories and physical/neurological exam will be performed, or may be reviewed from a clinic visit within the last 6 months, in order to exclude patients with major medical disorders.

Additionally, participants that gave blood in the past 56 days must agree to abstain from donating blood for the duration of the study. Study visits will not be scheduled until 56 days after the most recent donation.

Subject Recruitment Methods

Our lab has had ample success in recruitment for its clinical studies, thus current recruitment methods for other lab studies will be used. These methods include newspaper advertisements in the Winston Salem Journal and The Chronicle, radio advertisements, postcards, website, the Memory Assessment Clinic (MAC) at Wake Forest Baptist Medical Center and community talks in churches and retirement communities. Males and females will be comparably represented, and every effort will be made to enroll an ethnically diverse sample that typifies the geographical region. Recruitment through talks and print/radio ads will be targeted at the minority populations and women in order to ensure representative enrollment in these populations.

Once participants have been identified as potential study candidates, they will be contacted by telephone by the study coordinator. The study coordinator will explain the nature of the study and the study procedures by reviewing the material presented on the consent form. Potential participants who express interest in the study will be asked to meet with the coordinator and a study nurse, at which time the consent form will be reviewed again and questions or concerns about the study will be addressed.

Potential participants for this trial may also be recruited through other studies ongoing in our lab, including the Alzheimer's Disease Core Center (ADCC). Participants who have participated in other clinical trials, such as the ADCC study, within the past 3 months will not have to repeat clinical labs, screening cognitive tests, MRI, LP, and DEXA during their screening/baseline visits. The data previously collected for the ADCC study will be used.

DIET INTERVENTION

Interventions and Interactions
All potential participants for the study will first undergo a screening visit. Upon enrollment participants will have a total of three Core study assessments (data may be collected over several days for each Core assessment as long as visits are completed within two weeks – imaging, LP, etc.) occurring at baseline, 8 and 17 weeks (on-diet assessments), and 24 weeks (post-diet follow-up assessment). In addition to Core study visits participants will have weekly diet education visits for the first eight weeks, then return at 12 weeks to assess diet compliance over the course of the diet intervention. On week's 9-11 and 13-16 participants will have weekly telephone contact with the study dietitian to discuss food selection, meal preparation and any issues with diet compliance.

Experimental Diet
We will use a Modified Mediterranean-Ketogenic Diet (MMKD), which is a very low carbohydrate diet (<20 grams/day) aimed at inducing ketosis, as the experimental diet in the proposed study. We will use a modified American Heart Association Diet (AHAD), which is a low fat diet (<40 grams/day) as the control diet. The amount of carbohydrate (and fat) will be the main variable manipulated between the two diets. The target macronutrient composition (expressed as % of total calories) is approximately 5-10% carbohydrate, 60-65% fat, and 30% protein for the MMKD; and 55-65% carbohydrate, 15-20% fat, and 20-30% protein for the AHAD. Participants on the MMKD will be asked to keep their daily carbohydrate consumption below 20 grams per day throughout the 4-month intervention and the amount of fat and protein may be variable. Higher fat foods (preferably low in saturated fats) will be added liberally to the diet plan. Throughout the duration of the study, participants on the MMKD will be encouraged to avoid low-carbohydrate store brought products and artificially sweetened beverages. The MMKD group will be supplied with extra virgin olive oil during their in person visits to use as a source of fat in their diet, and will be encouraged to eat plentiful fish, lean meats, and nutrient rich foods that meet the requirement of <20 grams total carbohydrates per day. The results of the recent PREDIMED trial have showed benefits with extra virgin olive oil supplementation on cardiovascular disease risk and mortality. Participants on the AHAD will be encouraged to limit their amount of fat intake to <40 grams/day, while eating plentiful fruits, vegetables, and carbohydrates containing adequate fiber. Participants will be encouraged to consume the same amount of calories as their pre-study diet.

Participants on both diets will receive the same daily multivitamin supplement (Centrum Silver) over the course of the study and instructed to take 1 tablet each day while on both diets. Moreover, the following supplements should be discontinued for the duration of the study: resveratrol, CoQ10 (coenzyme Q10), coconut oil/other medium chain triglyceride-containing (ie: Axona) supplements, or curcumin, as they may impact bioenergetic status and interfere with interpretation of study results.

Diet Implementation and Monitoring: A dedicated registered dietician will develop daily meal plans for each study participant based upon their food preferences and caloric needs as determined by resting metabolic rate assessment, pre-study food records, and activity levels. The dietitian will work with an existing portfolio of more than 400 recipes that meet the macro-nutritional requirements for each diet. Participants will have weekly in-person visits for the first two months where a food diary will be reviewed and compliance rated, followed by monthly in-person visits, with phone calls on the off

weeks; food diaries will be completed at least 3x per week and include food/drink description and volume. Capillary ketone and glucose levels will also be measured in person weekly for the first two months, then monthly. If compliance is not satisfactory based on ketone measurements or food diary review, weekly in-person visits will be reinstated. Participants will be required to supply their own food based upon a daily meal plan, food list, and other educational material provided. A food stipend of $25 per week will be given to participants to help defray food cost, and a food scale to aid in food preparation. Participants will be asked to keep their exercise stable throughout the study. *We have used these methods in our pilot study and found that they were highly successful in achieving dietary targets and maintaining compliance.* Less than 10% (2 of 21) of participants discontinued our pilot study and the remaining participants showed good compliance. Because participants will be encouraged to maintain their normal caloric intake, they will experience minimal weight change throughout the intervention. We have defined weight stable as ±5 pounds. Weight will be monitored at each study visit, and caloric recommendations adjusted to ensure weight stability.

Storage and Distribution of Study Supplements
The multivitamins and olive oil provided to participants in the study diet groups will be stored within the Sticht Center at Wake Forest University School of Medicine. The supplements will be kept within a locked area on the first floor Kulynych Research Center. Participants on the MMKD will receive olive oil as needed at their in-person study visits. The olive oil will be used as an added fat source. All participants in the diet study will receive Centrum Silver multivitamin tablets at baseline to take throughout the study.

***Blinding*:** Although it is not possible for participants and the dietitian to be blinded to the study diet, all study personnel involved in performing cognitive testing or other outcome procedures or data analysis will be blinded. Participants will receive equal attention and instruction regarding their diets, and must expend similar amounts of effort to comply with dietary requirements. It will also be emphasized to participants that both diets may reasonably be expected to provide health benefits.

STUDY PROCEDURES AND SCHEDULE

Screening and Enrollment: Following a 12-hour fast, prospective participants will arrive at the Clinical Research Unit at the Sticht Center for Healthy Aging and Alzheimer's Prevention and sign informed consent. Participants will then undergo the following screening procedures to confirm eligibility: brief cognitive screening assessment, blood draw for screening labs (CBC, CMP, LFTs, lipid panel, calcium, thyroid stimulating hormone (TSH), B12, and PT/INR), blood pressure, height/weight, and a brief physical exam and questionnaires.

Following the screening visit, the eligibility of the participant for enrollment will be determined by the study physician and an expert consensus panel. Once a participants has been enrolled they will be asked to complete a physical activity log and 3-day food diary prior to their first diet education visit. These measures will be used to determine basic information about physical activity level, chronic caloric intake and typical pre-study dietary habits. Participants will continue to adhere to a self-selected, ad-libitum diet until the diet intervention begins.

Note: Screening data from other center studies may be used if a prospective participant is interested in the study. These data are valid as long as it was acquired within 6 months of the baseline visits for this diet study.

Core Study Visits: The Core study visits will be scheduled to begin within a two-week period before the diet intervention begins. The Core study visits include the Pre-Diet visits, Mid-Diet visit, the Post-Diet visits, and the Post-Diet Follow-up visit. The Core study visits will include a combination of the

following study measurements: DEXA scan, amyloid and dual tracer PET, structural/functional MRI, lumbar puncture (LP), cognitive testing, blood draw, capillary glucose/ketone testing, and stool collection.

Pre-Diet Study Visits: The Pre-Diet Study Visits will occur during 2 weeks prior to the start of the assigned study diet. At these visits, cognitive assessment, blood testing, capillary glucose/ketone testing, MRI, PET, DEXA, stool collection, and LP will occur. If the participant has not had the Apolipoprotein E (APOE) genetic test performed in a previous study, we will ask that they agree to the test and draw a sample for that purpose at the Pre-Diet visit. Once participants complete the Pre-Diet Lumbar Puncture (Visit 3) they will be randomized to a diet group.

Mid-Diet Study Visit: The Mid-Diet visit occurs at week 8 (Visit 13) and will include diet education and compliance check, cognitive testing, stool and blood sample collection. At this visit additional measures will be recorded such as capillary glucose/ketone testing, weight and metabolic outcomes.

Post-Diet Study Visits: The Post-Diet Study Visits will occur during week 17 (Visits 15-17). At these visits, cognitive assessment, blood testing, capillary glucose/ketone testing, MRI, PET, DEXA, stool collection, and LP will occur.

Post-Diet Follow-up Visit: The Post-Diet Follow-up Visit will occur during week 24 (Visit 19) after participants have discontinued the diet and returned to an adlib diet. At this visit, cognitive assessment, blood testing, and capillary glucose/ketone testing will occur.

Diet Educational Study Visits: Over the course of the diet study, participants whether on the MMKD or AHA diet, will participate in a series of individual, group and phone educational visits to inform participants of diet specifics, develop personalized meal plans, ensure adherence to their diet, and answer any questions. The study dietician will lead these educational sessions in the clinic or by phone.

In-Person Diet Education Sessions: The Pre-Diet Education session will take place within 1 week prior to the start of the diet (90 min session). After diet initiation, for both diets, participants will meet with the study dietitian weekly for the firest 8 weeks of the study for continued diet education and assessment of compliance. Participants will return to the clinic at week 12 for diet education and compliance check.
If participant enrollment and scheduling permit group sessions, participants may meet as a group or individually during later weeks. These sessions will last from 30 min (individual) to 60 min (group) each and will provide participants support and the opportunity to ask questions. Diet guidelines will be reviewed at each session. However, if necessary to improve diet adherence and understanding, individual sessions with the study dietitian may take place as needed during the remainder of the study. The need for these sessions can be determined by the study dietitian and/or by participant request.

Phone Sessions: Each participant will have a phone session in addition to diet education with the study dietitian 3-4 days after starting the diet and again in week 2. These session will allow the participants and caregivers to ask questions and receive support as needed. Phone sessions will also occur in weeks 9-11 and 13-16, and may be increased in frequency as needed to optimize compliance.

Note: Data will be recorded at Diet Education Visits, including capillary glucose/ketone testing, weight, adverse events, diet compliance, and nutritional intake.

Table. 2. Schedule of Study Visits and Procedures

Clinic Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Visit Week	-12	Baseline				1	2	3	4	5	6	7	8	12	17			20	24
Visit Name	Screen	Pre Cog	Pre LP	Pre dtPET	Pre amyPET	Edu	Edu	Edu	Edu	Edu	Edu	Edu	Mid Cog	Edu	Post Cog	Post LP	Post dtPET	Interm. F/U	F/U Visit
Informed Consent	X																		
Demographics	X																		
Medical History	X																		
Phys/Neuro Exam	X																		
Screen Cognitive Battery	X																		
Concomitant Meds	X												X		X				X
Resting Metabolic Rate		X													X				
Vital Signs	X	X	X	X	X								X		X	X	X	X	X
Diet Cognitive Battery		X											X		X				X
Weight	X	X			X	X	X	X	X	X	X	X	X	X	X			X	X
Capillary Gluc/KB	X				X	X	X	X	X	X	X	X	X	X	X			X	X
Blood Draw:	X	X	X						X				X	X	X	X			X
APOE Genotyping		X																	
Clinical / Safety Labs	X												X		X				X
Metabolic Outcomes			X						X				X	X	X				X
Mito, Epign & Exo		X													X				
Blood Banking		X	X										X		X	X			X
Stool Banking		X											X		X				X
CSF Banking			X													X			
LP			X													X			
DEXA Scan		X													X				
MRI		X													X				
dtPET				X													X		
amyPET					X														
Diet Edu. / Compliance					X	X	X	X	X	X	X	X	X	X					
Food Diary		X				X	X	X	X	X	X	X	X	X	X				
Compliance Call						X	X						X	X					
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Phone Check			X													X			
Sleep Battery/Monitor.		X											X		X				X

Study Visit Measures

***Clinical/Physical Evaluation*:** During eligibility screening, participants will receive standardized clinical cognitive evaluation (ADCC UDS3), a clinical interview and physical/neurological exam, clinical labs, and depression screening with the Geriatric Depression Scale (GDS). [53]

Metabolic Outcomes: Fasting plasma or serum will be sent out for assay by LabCorp diagnostic company for glucose, insulin, and lipids (total cholesterol, LDL, VLDL, HDL, triglycerides) at screening, baseline, month 2, and month 4. Quick analysis of metabolic outcomes done in week 1 and 12 or to ensure participant safety may be delivered to the Wake Forest CLIA-certified laboratory for expedited processing. Capillary ketone levels and weight will be measured at each in-person visit in the center. Body composition will be measured using DXA at baseline and at the end of the diet intervention.

Cognitive Testing Visits: For all cognitive visits, participants will be asked to eat a standard meal the night before and fast for 12 hours prior to start of visit. Upon arrival, weight and vital signs will be obtained and then blood will be drawn (about 3 tablespoons). A breakfast meal will then be provided (standard meal for baseline visit and study meal (MMKD or AHAD) for other visits) and the cognitive protocol will begin. All cognitive test administration will be audio-recorded.

Cognitive Battery: The cognitive protocol will consist of both computerized and paper-based assessments and will include all components of the memory composite as well as additional assessments. Cognitive testing will be audiotaped using a digital recorder for quality assurance and random checks will occur for quality control and to correct drift. Recordings will be erased once review has been completed. The total cognitive battery will take approximately 60-75 minutes to administer at both screening and Core Study Visits. Methods for cognitive assessments and other evaluations are described in this section.

Screening Cognitive Battery:

Participants will undergo a cognitive assessment at screening using the Uniform Data Set (UDS) version 3 test battery. Objective tests of cognitive function will include the following: global cognitive function (MOCA, MMSE), CDR, premorbid cognitive function (AMNART, Rey Auditory Verbal Learning Test), memory (Craft Story), visual memory (Benson Complex Figure), attention/concentration (forward and backward Number Span, Trails A/B), and language/verbal fluency (Verbal/Category Fluency, Multilingual Naming Test).

Diet Cognitive Battery

Once participants have been enrolled in the study they will complete another cognitive battery that will measure the effect of diet on cognition. The Diet Cognitive Battery will consist of the Preclinical Alzheimer Cognitive Composite (PACC), CogState computerized test battery, the Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog) version 12, the Alzheimer's Disease Cooperative Study – Activities of Daily Living Scale (ADCS-ADL), and the Geriatric Depression Scale (GDS). The major components of each battery are outlined below:

1. **PACC** (primary cognitive endpoint):
    i. **Free and Cued Selective Reminding Test – Total Recall:** The FCSRT measures verbal memory through controlled learning of a list 16 words that are introduced to participants by both visual and auditory presentations. Once the participant has learned the list they immediately recall each item. Then after a

delay of 20 minutes participants are asked to recall the items over three trials. The total score is the sum of free and cued words recalled over all three trials.

ii. **Logical Memory IIa – Delayed Paragraph Recall:** Participants will be read a story divided into 'bits' of information containing main content words. The participant recalls the story immediately, then again after a 20 minute delay. The total number of 'bits' remembered after the delay will be calculated as the delayed recall score.

iii. **Digit Symbol Substitution Test:** The participant will be introduced to a sheet of paper with a key that matches numbers 1-9 with an unfamiliar symbol. The participant must work as fast as possible for 90 seconds to match a random list of numbers 1-9 with their associated symbol.

iv. **MMSE – Total Score:** The MMSE is a questionnaire read to participants and tests multiple areas of cognition. The total number of questions right indicate general cognitive ability with higher numbers representing higher cognitive function.

v. **Category Fluency:** Participants will be given a category then asked to verbally generate nouns that belong to that category. The total number of correct items generated in 60 seconds will be summed.

2. **CogState Computerized Test Battery:**
   - **i. Tests of Learning and memory**
     - **i.** Object Pattern Separation Task (OPST), target pictures of common objects are presented along with highly similar "lures" and participants must identify targets;
     - **ii.** One Card Learning (OCL) Test, a playing card is presented and the participant must decide whether he/she has been shown that card before. The arcsine proportion correct is calculated
   - **ii. Tests of psychomotor speed:**
     - i. Detection Test (DT), as soon as a playing card is presented, the participant must press the "Yes" key;
     - ii. In the Identification Test (IDT), the participant must decide whether the card presented is red. Number correct and/or mean correct response time in log (msec) are recorded.
3. **ADAS-Cog 12:** The ADAS-Cog 12 will also be administered to provide data to inform future trial design. The ADAS-Cog 12 is a psychometric instrument that evaluates memory, attention, reasoning, language, orientation, and praxis. A higher score indicates more impairment. Scores from the original portion of the test range from 0 (best) to 70 (worse) and then number of items not recalled ranging from 0-10 is added for a maximum score of 80. A positive change indicates cognitive worsening. The ADAS-Cog12 version will be used in the current study, which includes Delayed Word Recall - a measure of episodic memory.
4. **ADCS-ADL:** The Alzheimer's Disease Cooperative Study - Activities of Daily Living Scale (ADCS-ADL) is an activities of daily living questionnaire aimed at detecting functional decline in people with Mild Cognitive Impairment (MCI). It was adapted from an inventory developed by the ADCS to assess functional performance in participants with Alzheimer's disease. In a structured interview format, informants are queried as to whether participants attempted each item in the inventory during the prior 4 weeks and their level of performance. The ADCS-ADL-MCI scale discriminates well between normal controls and patients with mild AD or MCI. It has good test-retest reliability. The questions focus predominantly on instrumental activities of daily living scales (e.g. shopping, preparing meals, using household appliances, keeping appointments, reading).

5. **Geriatric Depression Scale:** The Geriatric Depression Scale (GDS) is a well-validated self-report questionnaire used frequently in clinical and research settings to assess mood in older adults. This questionnaire includes 15 questions and takes about 5-7 minutes to complete. The GDS will be used as a part of the screening cognitive battery to determine whether participants should be excluded due to a significant presence of depressive symptoms.
6. **Neuropsychiatric Inventory Questionnaire:** The neuropsychiatric inventory questionnaire (NPI-Q) is widely used to assess the presence of mood disorders in geriatric populations. The NPI-Q measures 12 symptom categories and is scored on a scale of 0-36. An informant or caregiver for the participant must answer each question by indicating Yes or No for the presence of a symptom and when present they rate the severity on a scale of 1-3.

Imaging Procedures
Imaging for this study will include PET, MRI and a DEXA scan. The protocols for each are described below.

Positron Emission Tomography (PET)
Dual Tracer PET scans (dtPET): A separate consent form for dual tracer PET administration and data collection will be administered to all participants (IRB# 00033365). ^{11}C-AcAc will be synthesized as described previously.[54, 55] PET scans will be performed on a GE Discovery PET/CT scanner with an isotropic voxel size of 2 mm^3, field-of-view of 25 cm, and axial field of 18 cm. Imaging with ^{11}C-AcAc will be conducted first with acquisition frames of 12x10 sec, 8x30 sec, and 1x4 min (total scan 10 min), followed by a 50-min wash-out. FDG imaging is then conducted, using the time frames 12x10 sec, 8x30 sec, 6x4 min, and 3x10 min (total scan 60 min). Indwelling catheters will be placed in forearm veins for the injection of 5-10 mci of tracer. Blood samples will be obtained at 3, 6, and 8 min after the ^{11}C-AcAc infusion and at 3, 8, 16, 24, 35 and 55 min after FDG infusion. Radioactivity in plasma samples is counted in a gamma counter cross-calibrated with the PET scanner. PET images are preprocessed and co-registered to each participant's MR using a cross-modality 3D image fusion tool in PMOD 3.5. Co-registered PET images are corrected for PVE with the modified Müller-Gartner method.[56] Using the PMOD 3.5 pixel-wise kinetic modeling tool, parametric images of CMRg and CMRa will be produced for each participant. CMR quantification requires an arterial input function, determined by tracing regions of interest (ROIs) on the internal carotid arteries with the aid of co-registered MR images as previously validated.[57] The calculated activity within the ROI will be corrected using the radioactivity of the plasma samples obtained during image acquisition. The lumped constant will be set to 0.89 for the CMRg calculation,[58] and to 1.0 for the CMRa calculation. CMRg and CMRa will be expressed as mmol/100 g/min using the graphical Patlak model.[59] CMRa will be corrected for loss of 5.9% of the dose of ^{11}C-AcAc that is catabolized to $^{11}CO_2$.[60] *Key outcomes for analysis will be global ^{11}C-acetoacetate uptake and the PALZ PMOD index for ^{18}F-FDG PET*. [61]

***β-Amyloid PET Imaging (amyPET)*:** All study participants will receive an amyloid PET scan. A separate consent form for amyloid PET administration and data collection will be administered to all participants (IRB# 00027675). Amyloid PET data will be used to confirm Alzheimer's pathology and will be added to the PET imaging repository of the ADCC. Participants will be injected with an iv bolus of up to 15mCi (370 MBq) (+/- 10%) of ^{11}C-Pittsburgh compound B (PiB, over 5-10 seconds), followed by a 40 min uptake period (+/- 10%). Brain emission images will be acquired continuously for a 30 min to quantify amyloid uptake in MRI-defined regions, acquired in six 5-minute frames. Participants will be contacted 24-72 hours after injection with ^{11}C-PiB to inquire if any adverse events occurred within 24 hours of injection.

Aβ deposition in the brain will be quantified by ^{11}C-PiB uptake using standardized uptake volume ratio (SUVR) of 5 primary cortical areas (anterior cingulate; posterior cingulate/precuneus; frontal, lateral temporal and parietal cortex) relative to cerebellar gray matter. The unweighted average of these 5 regions will be used to determine amyloid positivity. Averages<1.35 will be considered amyloid negative.[62]

Magnetic Resonance Imaging (MRI)

Magnetic resonance images will be acquired on a 3 Tesla Siemens MAGNETOM Skyra scanner with a high-resolution 32-channel head coil (Erlangen, Germany) located in the MRI building at the Wake Forest Baptist Medical Center. The MRI session will last 45 -60 minutes and include 6 unique sequences that assess multiple levels of brain structure, connectivity, function, and cerebral blood flow. The acquisition parameters of each sequence are detailed below:

Image Acquisition Parameters:

- Resting-State BOLD: For the resting state paradigm the subject will be instructed to rest quietly with eyes open. High resolution BOLD images will be acquired using the following parameters, time of recoil (TR) = 868 ms; time of echo (TE) = 30.6 ms, flip angle (FA) = 52°; field of view (FoV) = 208 mm, and a total of 72 slices with a voxel size of 2.0 mm^3. Slices will be collected in an interleaved series and oriented in the transverse plane.

- Anatomical MPRAGE: Anatomical T1 images will be obtained using a 3D volumetric MPRAGE sequence. Acquisition parameters are as follows; TR = 2300 ms; TE =2.98 ms; TI = 900 ms, FA = 9°, FoV = 256 mm and a total of 192 slices with a voxel size of 1.0 mm^3. Slices will be collected in an ascending order and oriented in the transverse plane.

- T2 FLAIR: The T2 FLAIR paradigm will measure brain structure under different contrast of brain fluids compared to the T1 image. T2 FLAIR images will be acquired using the following parameters; TR = 5000 ms; TE = 383 ms; TI = 1800 ms, FoV = 256 mm and a total of 192 slices with a voxel size of 1.0 mm^3. Slices will be collected in an interleaved series and oriented in the transverse plane.

- *Neurite Orientation Dispersion and Density Imaging (NODDI)- DTI:* The NODDI-DTI sequence will measure brain white matter structure. Acquisition parameters are as follows, TR=3500 ms; TE=106 ms; FA=90° with multiple diffusion weighting shells (b=1000, 2500, and 4000 s/mm2 with 15 b=0) that provides orientation dispersion index, intra-cellular volume fraction as well as the conventional diffusion tensor maps**.** *Diffusion* parameters include a 30-direction scan with a 2 mm isotropic spatial resolution. Slices will be collected in an interleaved series and oriented in the transverse plane.

- Susceptibility weighted imaging - quantitative susceptibility mapping (SWI-QSM): The SWI-QSM sequence is an enhanced image contrast to measure cerebral venous architecture. The acquisition parameters are as follows, TR=51 ms; multiple TE 1-6 = 9.8 ms, 16.6 ms, 23.5 ms, 30.4 ms, 37.3 ms, 44.2 ms; FA=20°, FoV = 240 mm and a total of 64 slices with a voxel size of 0.6x0.6x2.0mm. Slices will be collected in an interleaved series and oriented in the transverse plane.

- Pseudo Continuous Arterial Spin Labeling (PCASL): Whole-brain resting baseline cerebral blood flow (CBF) will be acquired with a pseudo-continuous ASL (PCASL) sequence.[63] Participants will be asked to sit still with their eyes open then hold their breath during a second brief pcASL acquisition. The acquisition parameters are as follows, TR = 4000 ms, TE = 11 ms,

TI = 3000 ms, FA = 90°, FOV = 240 mm, with a total of 36, 4 mm axial slices with a voxel size of 3.0x3.0x4.0mm. Slices will be collected in an ascending series and oriented in the transverse plane. Quantitative perfusion maps (mL/100 gm tissue/min) for each voxel are computed as previously described.[64]

Lumbar Puncture (LP) Procedure and CSF Assays: All participants will undergo LP before 11 a.m. after fasting for ≥12 hours. Using a 22-gauge Sprotte needle to drip, up to 25ml of CSF will be withdrawn into sterile polypropylene tubes. CSF will be transferred in 0.2 ml aliquots into pre-chilled polypropylene tubes, frozen immediately on dry ice, and stored at -80°C until assay. CSF will be analyzed for AD biomarkers (Aβ42, tau, p-tau^{181}) using the AlzBio3 multiplex assay (FujiRebio Europe). Participants will be fed following the LP. The participant will rest in the recumbent position for 30 minutes post-LP. A recent Cochrane Review did not find that bed rest or IV fluid administration reduced the risk of post-LP headache. Study participants or authorized caregivers will be contacted by phone within 24 hours of the LP to inquire about any adverse events. Participants will sign a repository-consent for storage of samples at the time of study consent.

***Redox Proteomic/Lipidomic Assays*:** For the determination of free fatty acids (FFAs), 200 µl of CSF will be extracted using chloroform/methanol according to a modified method of Bligh and Dyer,[65] in which the aqueous phase is adjusted to pH 3 or lower with HCl prior to extraction. Pentadecanoic and heneicosanoic acids (Nu Chek Prep) will be added to each sample prior to extraction as internal standards. The lipid extracts will be evaporated under a stream of argon, and the FFAs isolated by the solid phase extraction method of Kaluzny et al.[66] using 500 mg amino-SPE columns (Thermo Scientific). FFAs will then be derivatized to their fatty acid methyl esters by treating with 1 ml of 14% BF_3 in methanol (Sigma-Aldrich) under Argon, and heating to 100°C for 10 minutes. After cooling, the samples will be treated with 1 ml of hexane and 4 ml of saturated, aqueous NaCl. The hexane phase will be transferred to a sample vial, and 1 µl analyzed on a TSQ Quantum XLS GC/MS/MS interfaced to a Trace GC Ultra (Thermo Electron Corp.), automated by a TriPlus auto-sampler. Separation will be accomplished using a 30 m × 0.25 mm diameter DB-WAX ETR column (Agilent Technologies) with a 0.25-µm coating as reported previously.[67] Sphingolipids and ceramides will be analyzed using published methods.[68]

DEXA Procedure: Participants will receive body fat assessments as part of the Pre-Diet and Post-Diet Study Visits. Total fat mass, fat-free mass, and their relative distributions will be determined using dual energy x-ray absorptiometry (DEXA) measurements. For this test, participants cannot have removable metal objects on their body, such as snaps, belts, underwire bras, and jewelry. This test will last about 20-30 minutes.

Blood, CSF, and Tissue Biomarker Methods

Blood Collection Procedures/Measures: Participants will fast overnight prior to blood draw before 10 a.m. Whole blood will be collected and processed as described.[69] Fasting blood for neuroendocrine measures will be collected multiple times during the study period (visits 1, 3, 6-13). The total volume of blood to be collected for neuroendocrine measures of stored blood ranges from 40-70mL (about 3-5 tablespoons) at cognitive Pre/Post Diet and Follow-up visits, and 25mL (about 2 tablespoons) at the lumbar puncture visits. Included in this amount is blood to be stored for use in future studies. Participants will sign a repository-consent for storage of samples at time of study consent. Additionally, 8.5mL of blood will be collected during the first week and again at 12 weeks for safety labs and measurement of metabolic outcomes. The total amount of blood to be collected in this study will not exceed 400mL over the 24-week study.

Samples will be immediately placed on ice and spun within 30 minutes at 2200 rpm in a cold centrifuge for 15 minutes. Plasma, serum, and red blood cells will be aliquoted into separate storage tubes and flash frozen at -80°C until assays are analyzed. The following assays will be performed.

Metabolic assays:
Metabolic measures will be assessed at the Pre-Diet, Mid-Diet and Post-Diet visits. These will include, glucose, insulin, lipids (HDL, LDL, VLDL, and triglycerides) will be measured by LabCorp diagnostics.

APOE genotyping:
Additional blood (up to 10 ml, Visit 2) will be drawn for confirmation and validation of ApoE genotyping by the Laboratory of Donald Bowden, PhD at Wake Forest School of Medicine. Participants that have already had APOE genotyping done as a part of another ADCC stuidy will not have to repeat this procedure. The total amount of blood to be drawn at this visit, including blood for the repository, mitochondrial assay and genotyping, is 70 ml (~5 tablespoons).

Mitochondrial respiration assays:
To determine bioenergetic profiles of intact cells, we will assess basal respiration (Basal-OCR), maximal respiration (MAX), ATP coupled respiration (ATP-OCR), proton leak (Leak), and non-mitochondrial respiration (non-mito).[70-72] The spare respiratory capacity (SRC) will be calculated by subtracting Basal-OCR from MAX. A Seahorse XF96e will be used to run samples in triplicate. Basal-OCR measurements will be performed with standard fuel conditions designed to examine glucose oxidation (pyruvate and glucose). Oligomycin will be added to block ATP synthase. The resultant decrease in oxygen consumption (ATP-OCR) reflects the amount of oxygen consumed to fuel the conversion of ADP to ATP. FCCP will be added to uncouple mitochondria and induce maximal oxygen consumption. The resultant increase in oxygen consumption represents MAX. Antimycin-A and rotenone will be added to block electron transport chain activity. Residual respiration is non-mitochondrial. Oxygen consumption not linked to ATP synthesis (Leak) will be measured by subtracting non-mito OCR from the rate left after the blockage of ATP synthase by oligomycin.

Epigenomics/Transcriptomics
Read Mapping/Counting: Illumina sequencing runs will be processed to generate FastQ files using the Illumina provided *configureBclToFastq.pl* script to automate running CASAVA, using default parameters for removal of sequencing reads failing the chastity filter and mismatches in the barcode read. Reads will be trimmed for QC using *Btrim* (5 base sliding window average with $Q > 15$)[73] and to remove any adaptor sequence using ea-utils. The current *Ensembl Homo Sapiens* reference file, annotations and Bowtie2 indexes will be used for mapping of the sequencing reads to the genome and read counting. *Bowtie2* and *TopHat2* will be used to map the sequencing reads to the genome using a mate-inner-distance of 100 bp and '*firststrand*' options.[74,75] Following alignment, read counts per gene will be obtained using *HTSeq(88)*.Pre-Processing and QC of mRNA Raw Count Data will be performed in *R* using *Bioconductor* packages. Counts will be converted to Counts Per Million (CPM) using the *cpm* function of the *edgeR* package,[76] and all features with CPM ≤ 0.25 in ≥90% will be removed. We will use the *biomaRt* package and the *Ensembl BioMart* database to obtain *Entrez Gene ID*s, Gene Symbols, genome coordinates, gene length and percent GC content for detectable features. We will perform differential isoform expression analysis.[77] To use linear modeling in *R,* we will transform the raw count data to log CPM,[78] and use the Trimmed Mean of M-values (TMM) normalization method[79] implemented in the *calcNormFactors* function in the *edgeR Bioconductor* package.[80] We will adjust for flow cell effects. Pre-Processing and QC of DNA Methylation Data: The raw DNA methylation microarray data will be pre-processed with published pipelines for expression and methylation implemented in *R* using *Bioconductor* packages. The final methylation value for each probe is computed as the M-value (log ratio of methylated to unmethylated intensity).[81] Filters applied to the probes for the CpG sites include detected methylation levels in <90% of the samples using a p-value of

0.05, 65 probes that assay highly polymorphic single-nucleotide polymorphisms (SNPs) rather than CpG sites, and overlap with a repetitive region. We will flag probes containing any SNPs within 10 base pairs of the targeted CpG site.

Neural-Derived Exosome Markers of Insulin Resistance
Exosome isolation and L1 CAM enrichment methods have been described.[35, 82, 83] Plasma will be mixed with Thromboplastin-D followed by ExoQuick precipitation solution (System Biosci). Exosome suspensions will be incubated, centrifuged, re-suspended with protease and phosphatase inhibitors inhibitor cocktails and immunoprecipitated using mouse anti-human CD171 (L1 CAM) biotinylated antibody (eBiosci). After pellet re-suspension and biotin elution, exosomes will be lysed. To quantify p-panTyr-IRS-1, we will use MesoScale Discovery plates for IRS-1 with Anti-Phospho-Tyr Antibody Sulfo-TAG Labeled detection antibody, and for Ser312-IRS-1, Phospho-IRS-1 (Ser312) Kit. To normalize, we will use measurements of exosome marker human CD81 (Hölzel Diagnostika-Cusabio), with verification of the CD81 antigen standard curve using human purified recombinant CD81 antigen (Origene). The mean value for CD81 in each assay group will be set at 1.00 and the relative values for individual samples will be used to normalize their recovery. The insulin resistance index R will be the target outcome.

Nutritional Data Information: Nutritional data for this study will be collected by two methods. First, participants will complete food diaries. These will be completed at least 3 times per week during the first week on a diet and once a week thereafter. Food/drink description, amount (in ounces), time consumed will be recorded. Second, 24-Hour food recalls will be utilized at several points throughout the study (pre and post both diets). The ASA24 (Automated Self-Administered 24-hour Recall) system from the National Cancer Institute will be used for data collection. Participants will complete these 24-hour recalls with help from the study dietitian, or other study staff, and may complete the recalls themselves once they receive training on how to use the ASA24 program. Similar information to the food diaries will be recorded with the 24-hour recalls (food/drink description, amount (in ounces), time consumed).

STATISTICAL CONSIDERATIONS

Statistical Analysis and Power
Statistical analyses will be overseen by Iris Leng, PhD, Wake Forest ADCC Data Core member, who has extensive experience in the analyses of clinical trials and in using results from Phase II studies to design efficient Phase III trials. We will examine change in our primary outcome CSF Aβ42, using analysis of covariance to compare mean levels at 4-months between intervention groups with adjustment for baseline level, age, and MMSE. Two-sided type 1 error for the primary outcome will be controlled at 0.05 and the primary analysis (intention to treat) will include all data on participants grouped according to intervention assignment, regardless of adherence. If residuals from this model are markedly skewed, we will perform supporting analyses after data transformation. Similar approaches will be used for other secondary outcomes of our primary aim (e.g. PET, MRI, CSF). Mixed effect models will be used for repeated measures (cognition). To examine whether any differences in these outcomes are moderated by APOE genotype, amyloid PET positivity, insulin resistance (secondary aims), or by gender, we will use tests of their interaction with intervention assignment. Additional aims involve examining how changes among outcomes differ between intervention groups and whether they track with markers of hypothesized mechanisms. We will explore the intercorrelations among changes using multivariate decomposition and assess whether these differ between intervention groups. We will also assess mediation, using models in Emsley et al. 2010[84] to identify potential underlying mechanisms.

***Power Analysis for Primary Endpoints*:** Sample size estimates for the proposed study were based on the results of our small pilot study showing 6-week mean MMKD vs AHAD diet changes in CSF

Aβ42 of 89 vs 11 units (pooled SD of changes 102). With 2-sided type 1 error of 0.05, if attrition is as large as 15% (our observed pilot attrition was 10%), and allowing a 10% attenuation of effect related to possible non-adherence, enrolling 53 participants/group provides >90% power: thus, we target N=60/group. The proposed longer 4-month intervention and covariate adjustment may increase power to detect a true difference.

Power analyses for Secondary and Exploratory Endpoints: The exploratory nature of these aims in this Phase II trial are necessary to identify potential outcomes, biomarkers and therapeutic targets for follow-up and confirmation in future trials; thus we have not applied formal power analyses to these aims. However, we note that enrolling N=60 per group provides 80% power to detect mean differences (t-tests) of 0.56 SD in other outcomes.

ASSESSMENT OF SAFETY

Safety and Compliance Monitoring. All participants will be medically supervised by Co-investigator Benjamin Williams, MD. Safety monitoring will be overseen by a Data and Safety Monitoring Board approved by the study sponsor, the National Institute on Aging, as detailed in Protection of Human Subjects. Compliance will be assessed by regular capillary ketone measurement and dietitian-rated compliance based on food records. In our 4-month pilot study, mean compliance rates were 4.5 and 4.75 out of 5 (perfect compliance) for the MMKD and AHAD diets

Data and Safety Monitoring
The principal investigator will be responsible for the overall monitoring of the data and safety of study participants, with assistance by members of the study staff, and the Data and Safety Monitoring Board (DSMB), which will be responsible for monitoring the safety of research participants. The DSMB will consist of a neurologist and statistician from another institution who have been approved by the study sponsor (NIA) and by the Wake Forest IRB. Information regarding serious adverse events (SAEs) will be presented to the DSMB every six months, collated in A-B format by an independent statistician. The DSMB may recommend stopping the trial before its planned conclusion if convincing evidence is observed of a treatment difference in adverse events. Recommendations of the DSMB after each review will be presented to the study PI and IRB. Participants will be screened at the beginning of the study and will have their clinical labs and other results (i.e. neuroimaging) monitored carefully at each study visit.

Reporting of Unanticipated Problems, Adverse Events or Deviations
Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

All participants will be evaluated for adverse events (AEs) during each study visit. All AEs will be recorded on an adverse event case report form. Based on the nature of the AE, study physicians will determine the severity of the event and the association with the study. All SAEs will be recorded on an IRB "Serious Adverse Event" case report form. SAE are defined as events that are (1) life-threatening or fatal; (2) result in severe or permanent disability; or (3) require hospitalization, other than brief emergent care for non-life threatening conditions. All SAEs will be reported to the IRB within seven calendar days of the investigator or other members of the study team becoming aware of the event. SAEs will be followed until resolution, even if study participation has been terminated.

The "severity" of events will be graded according to the following guidelines:

1. Mild: The patient is aware of, but can easily tolerate, the event;

2. Moderate: The discomfort of the event is severe enough to interfere with some usual activities;

3. Severe: The patient is incapacitated, and unable to perform most or all usual activities.

The "study relatedness" will be assigned according to the following guidelines:

1. Definitely Related: The reaction follows a reasonable temporal sequence and is known to be an effect of the medication or procedure;

2. Probably Related: The reaction follows a reasonable temporal sequence with reasonable suspicion that the study medication or procedure caused or contributed to the event;

3. Possibly Related: The reaction follows a reasonable temporal sequence, and current medical knowledge does not preclude a relationship between the medication or procedure and the event;

4. Unlikely: Current medical knowledge precludes a causal relationship between the medication or procedure and the event;

5. Definitely Unrelated: The cause of the reaction is known to be other than the medication or procedure.

The following adverse events would preclude further participation in this study: (1) fasting plasma glucose >165 mg/dl on two consecutive visits; (2) evidence of symptomatic hypoglycemia, on two occasions following appropriate dietary and physical activity counseling; (3) clinically significant changes in neurological or medical status, including, but not limited to, stroke, myocardial infarction, new onset of kidney or liver disease, etc. The study physician will review all medical records to determine whether changes in medical status are significant and warrant removal from study participation.

ETHICS/PROTECTION OF HUMAN SUBJECTS

Informed Consent

Signed informed consent will be obtained from each participant. Once participants have been identified for potential inclusion into the study, a study team member will explain the nature of the study and the study procedures by reviewing the material presented on the consent form. Potential participants who express interest in the study will then be asked to meet with the study coordinator in person, at which time the consent form will be reviewed again, and questions and concerns related to the study will be solicited. Once informed consent has been obtained, potential participants will undergo clinical evaluation to ensure they meet the inclusionary criteria for the study. To avoid coercion, the study team will stress, both in the initial telephone contact and in follow-up appointments: 1) that all research participation is voluntary, 2) that participants may withdraw at any time, 3) the decision to participate or not participate will not affect the participants' medical care or any benefits to which the participant is entitled, and 4) encourage questions about the study.

Suzanne Craft, PhD, the study coordinator, and project collaborators will develop the consent forms. The consent form will include all of the required elements of informed consent required by the FDA. The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented.

Information will be given in both oral and written form. Participants, their relatives, guardians, or authorized representatives and informants will be given ample opportunity to inquire about the details of the study. All investigators will attend a training meeting prior to the start of participant recruitment. Informed consent will be documented by the use of a written consent form approved by the IRB and signed by the participant and/or a legally authorized representative.

Potential participants for this trial may be recruited from other ongoing studies in affiliation with the ADCC; data from blood, CSF, cognitive testing, and imaging measures may be utilized for this trial as long as a participant is enrolled within 6 months of the start of the diet intervention.

Confidentiality and Privacy
Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify participants, and maintaining all study information in a secure manner. To help ensure participant privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, stored separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection participant identifying information will be destroyed within three years of study completion, producing an anonymous analytical data set. Paper documents will be placed in Cintas bins and electronic data will be deleted. Data access will be limited to study staff and personnel from collaborators that will help with data analysis. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

DATA HANDLING AND RECORD KEEPING

Sources of Materials
Research material obtained from participants will consist of a) data collected from the cognitive protocol administered during each cognitive testing session, b) brain images obtained during MRI and PET scans, c) blood, CSF, and stool samples collected during experimental sessions that will be assayed for specified peptides, neuroendocrine markers, and other measures. This study will be conducted in accordance with Good Clinical Practice (GCP) guidelines, as defined by the International Conference on Harmonisation (ICH) Guideline, Topic E6, the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50) – Protection of Human Subjects and Part 56 – Institutional Review Boards (IRBs), HIPAA, State and Federal regulations and all other applicable local regulatory requirements and laws.

Biomarker/APOE data handling: Blood, CSF,DNA and stool samples will be handled in the following manner using a protocol developed by ADNI (Alzheimer's Disease Neuroimaging Initiative); a database will be created and used for the inventory of stored samples in conjunction with a bar code reading system. Bar code labels affixed to each sample vial will contain the following information: sample ID# (to preserve confidentiality), date of collection and processing, total initial volume collected, sample type (plasma, CSF, stool), volume, aliquot number, freezer, shelf, rack, box, location in the box. A bar code label will be used on the sample tracking form. There will be no link to participants' names. Samples will be catalogued and maintained at -80 °C.

Neuroimaging data storage: a) Image data collection and PHI: All imaging data will be uploaded and stored at the Radiology Informatics and Image Processing Laboratory (RIIPL) at Wake Forest, under the direction of co-investigator Dr. Whitlow. Only study number is entered into the scanning console interface at the time of the scan. Names are never used. As an extra check, when scans are uploaded, the images are quarantined while header fields are checked to ensure compliance with this policy, and

if in the rare case names are found they will be replaced with the study number and the site notified. b) Image management and access: Once uploaded and after initial quality and PHI checks, the images are inserted and catalogued into an auto-populating imaging database based on DICOM fields and metadata accompanying the upload. The images are backed up multiple times to prevent technical loss of data. At the scan console the exam is backed up to DVD and kept at the local site. The transferred images are maintained on a primary 40TB RAID6 server with nightly synchronization to a second server located in a separate building (both servers are housed in climate controlled specialized server rooms with secure physical and electronic access). The images are served back to the local sites or other authorized users via a secure web interface and the data access policy and granted access will be under the purview of the PI.

The following individuals involved in conducting this study may have access to individually identifiable private information about human participants: research team members and collaborators, federal oversight agencies including the National Institute of Aging, Food and Drug Administration (FDA), the Office for Human Research Protection (OHRP), and the Government Accountability Office (GAO); Institutional Review Boards from Wake Forest that oversees the safety and ethics of studies. All study personnel will be qualified by education, training, and experience to perform their respective task(s).

POTENTIAL RISKS AND BENEFITS

Potential Risks and Protections Against Risks

Risk Associated with Diet Interventions: The two diets utilized in this study, Modified Mediterranean -Ketogenic Diet (MMKD) and a modified American Heart Association Diet (AHAD), mainly differ based upon macronutrient ratios (carbohydrate and fat content). Participants on the MMKD will be asked to keep their daily carbohydrate consumption below 20 grams per day throughout the 4 month intervention and the amount of fat and protein may be variable. High fat foods (preferably low in saturated fats) will be added liberally to the diet plan. Throughout the duration of the study, participants on the MMKD will be encouraged to avoid low-carbohydrate store brought products and artificially sweetened beverages. Participants on the AHA will be encouraged to limit their amount of fat intake to <40 grams/day, while eating plentiful fruits, vegetables, and carbohydrates containing adequate fiber.

Participants on both interventions may lose weight depending on their baseline metabolic status. This is not an outcome measure of the intervention and will be carefully monitored as described below. We do not foresee a significant problem with safety based upon the common use of similar interventions in the general public and minimal safety issues reported in other literature using similar interventions.

Although symptomatic hypoglycemia (blood glucose <60 mg/dl) is rare with the MMKD, lowered blood glucose will be expected to occur as a result of the MMKD intervention, due to the restriction of carbohydrates. Some participants may experience fatigue or difficulty concentrating for the first few days on diet. However, if there is an adequate increase in circulating fatty acids/ketone bodies (as expected) for participants on the MMKD, these symptoms will resolve. Safety monitoring will occur at all scheduled study visits and education visits as well as during safety/adherence phone calls. At each study visit, participants will also be queried for any adverse events, changes in health status, or changes in medication. Blood chemistries will be obtained at regular intervals throughout the study. Participants and study partners will receive education regarding the symptoms of hypoglycemia, and instructed to consume their next scheduled meal/snack if they experience any symptoms. Although not anticipated, symptomatic hypoglycemia may occur in the first week of the MMKD intervention, as participant's preferred metabolic substrate is shifted from glucose to fatty acids/ketone bodies. Thus, additional safety checks will be scheduled during this time in concordance with the individual education visit day at week one. Decreased blood glucose is expected with the MMKD intervention, due to the change in metabolic fuel from glucose to fatty acids and ketone bodies. If study participants in either group become lethargic, dizzy, or have other significant symptoms medical history, blood

glucose/ketone levels, and Complete Metabolic Panel (CMP) results will be assessed by a study clinician to determine probable cause. Due to the higher fat/protein content of the MMKD dietary intervention there may be an increased risk for an exacerbation of gout in participants on the diet. Participants will be queried at baseline regarding any history of gout and informed of a potential exacerbation based on the MMKD intervention. Moreover, plasma uric acid levels will be monitored 4 times throughout the study at the screening visit and at weeks 8, 17 and 24. Study participants in the MMKD group may also experience dehydration and constipation. Each will be queried and monitored throughout the study at all interactions. Participants will be encouraged to drink plenty of water and report any significant symptoms to study personnel. MiraLAX may be suggested for participants with persistent constipation. The constraints of the MMKD specifically may lead to an inadequate intake of necessary vitamins and micronutrients. Thus, participants in the MMKD and AHAD groups will each receive the same daily multivitamin supplementation to ensure vital nutrients are consumed, especially in the MMKD group. Participants will be asked not to vigorously exercise during the first week for both interventions and not change their exercise pattern from their habitual pre-study pattern after the first week.

Risks Associated with Head Imaging using MRI: MRI is a non-invasive technique with no known side effects other than possible physical or psychological discomfort. No contrast agent is required. Participants with the following should not participate in MRI studies: metallic implants, such as prostheses, shrapnel, aneurysm clips, or persons with electronic implants, such as cardiac pacemakers. The magnetic field generated by the MRI machine can cause a displacement or malfunctioning of these devices and all participants will be thoroughly screened prior to entering the MRI room. Also, participants who have anxiety or claustrophobia will not be recommended to participate since the head must be placed fully inside the MRI scanner tube. In addition, fatigue and physical discomfort are possible. Earplugs are used in all participants due to the loud noise in the MRI scanning environment. These will not eliminate all sound, so that communication with the participant is still possible.

All participants will be rigorously screened by MRI personnel to be certain that they do not have any medical contraindications for MRI, which include metallic foreign bodies in the brain or eye or cardiac pacemaker. This safety screening is part of routine clinical practice at MRI centers and is performed before any participant is permitted to enter the scanning room. There is a slight risk of anxiety due to claustrophobia. Any participant who experiences anxiety when placed into the MR scanner will be removed from the scanner, offered reassurance by the MRI technician doing the scan, and offered the option of continuing or terminating the scan. If the participant decides that the anxiety associated with MRI is uncomfortable for them and they wish to terminate the scan, then the examination will be ended at that time. There will be no attempt to coerce participants to complete exams that they are uncomfortable with. Participants uncomfortable with MRI scans should preferably not be included in this study. Use of anxiolytic agents for completion of MRI scans is discouraged, but allowed at the discretion of study clinicians.

Risks Associated with PET imaging: PET imaging scans involve exposure to radiation. The amount of radiation exposure is considered low and comparable to everyday risks. For the three PET scans participants will receive about 3 Rem of exposure to radiation. This is the below the annual dose limit per patient of 5 Rem.

Risks Associated with DEXA: DEXA scans involve exposure to radiation. Although it can vary from person to person, whole-body radiation exposure from the two DEXA scans will be less than 6 mRem. The average annual exposure a person in the United States receives from natural background radiation is about 300 mRem. The risk of harm from this amount of radiation is low and no harmful health effects

are expected; however, risk of harmful effects may increase if the participant is exposed to more procedures that involve radiation within a short period of time.

Risks Associated with Blood Draw: Transient discomfort and bruising may occur when plasma is drawn, and there is a small risk of infection with any blood draw. In our experience, the discomfort associated with blood draws is usually minimal; every effort will be made to minimize any discomfort.

Risks Associated with LP: The risk for serious adverse events related to lumbar puncture (e.g., post-LP headache, infection, nerve root damage) is very low. Lidocaine may cause transient stinging and rare dermatitis. Rarely, patients may experience transient local pain at the site of the puncture or in the distribution of the sciatic nerve, if the nerve roots of the cauda equina are stimulated. Although very rare, it is possible that there may be an allergic reaction to the local anesthetic (lidocaine 1%) used for the LP. This reaction would cause swelling and a rash on the skin where the anesthetic was injected.

The LP is performed well below the termination point of the spinal cord. Because the nerve roots readily move aside from the spinal needle, the possibility of permanent nerve root damage is extremely remote. Post-LP headache has been commonly reported (~20% in young persons). The incidence of post-LP headache is a function of the diameter of the spinal needle rather than the amount of CSF drawn, and it may be related to age. The removal of up to 30 ml of CSF does not increase the risk of a post-LP headache. In order to minimize these risks even further, participants will be asked to hydrate well the day before the LP. Participants will remain supine for 30 minutes following the LP. Participants or authorized study partner will be contacted by the study nurse the day following the LP. They will also be told to avoid strenuous physical exercise and to call our clinic in the event of any discomfort. In the unlikely event of a post-LP headache that does not respond to conservative management, participants will return to the clinic and a trained anesthesiologist will perform a blood patch procedure. The blood-patch immediately terminates LP headaches.

Other Risks: Cognitive testing may produce frustration in some participants. If the participant appears distressed during cognitive testing, they will be comforted and encouraged. If the distress persists, testing may be discontinued. Stool collection does not pose any significant risks. Participants will be instructed on proper methods of collection and hygiene will be emphasized pre and post collection

Potential Benefits of the Proposed Research to Human Subjects and Others

There is an urgent need to identify promising strategies to treat and prevent Alzheimer's disease. The proposed trial targets AD pathology that may be accumulating several years prior to the development of the debilitating cognitive symptoms of AD in MCI patients. There are significant potential scientific and clinical benefits for the participant population. Currently, there have been relatively few studies examining the effects of ketogenic interventions in MCI/AD. Thus, results gained from this study will provide valuable information concerning the impact of an intervention aimed at ketosis (and alternate metabolic fuels) on cognition and other measures in AD. The results of this study will also provide insight into an important indicator of AD diagnosis/progression, as there is little known concerning how a ketogenic intervention may impact the pathophysiologic features of AD (AD biomarkers) in humans. Moreover, the MMKD may improve insulin sensitivity and overall metabolic status in participants, which may significantly decrease the risk for development of AD. All participants will be given information as to the outcome of their safety labs and physical exams.

The proposed work has the potential to identify significant mechanisms related to eventual cognitive decline and suggest novel therapeutic strategies for patients designed to prevent irreversible neurodegeneration prior to the onset of cognitive decline. The relatively minor risks posed by the diet-based interventions, cognitive testing, MRI, and LP are outweighed by the value of the scientific investigations outlined in this proposal.

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