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Modified ketogenic diet is associated with improved cerebrospinal fluid biomarker profile, cerebral perfusion, and cerebral ketone body uptake in older adults at risk for Alzheimer's disease: a pilot study

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Abstract

There is currently no established therapy to treat or prevent Alzheimer's disease. The ketogenic diet supplies an alternative cerebral metabolic fuel, with potential neuroprotective effects. Our goal was to compare the effects of a modified Mediterranean-ketogenic diet (MMKD) and an American

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Disclosure

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.neurobiolaging.2019.09.015>.

Heart Association Diet (AHAD) on cerebrospinal fluid Alzheimer's biomarkers, neuroimaging measures, peripheral metabolism, and cognition in older adults at risk for Alzheimer's. Twenty participants with subjective memory complaints ($n = 11$) or mild cognitive impairment ($n = 9$) completed both diets, with 3 participants discontinuing early. Mean compliance rates were 90% for MMKD and 95% for AHAD. All participants had improved metabolic indices following MMKD. MMKD was associated with increased cerebrospinal fluid A β 42 and decreased tau. There was increased cerebral perfusion and increased cerebral ketone body uptake (^{11}C -acetoacetate PET, in subsample) following MMKD. Memory performance improved after both diets, which may be due to practice effects. Our results suggest that a ketogenic intervention targeted toward adults at risk for Alzheimer's may prove beneficial in the prevention of cognitive decline.

Keywords

Alzheimer's disease; Mild cognitive impairment; Ketogenic diet; CSF biomarkers; MRI; PET

1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder characterized by progressive deterioration of cognition, behavior, and ultimate functional decline (Alzheimer's Association, 2018). With no established disease-modifying therapeutic or preventative strategy (Cummings et al., 2014), we must continue to explore unconventional interventions to combat this devastating disorder.

The traditional ketogenic diet (KD) was developed and first implemented to treat intractable childhood epilepsy in the 1920s (Freeman et al., 2007). The KD is a very low carbohydrate, adequate protein, and high fat diet that leads to a state of ketosis (increased systemic ketone bodies). During ketosis the main fuel used by the body shifts from glucose to favor ketone bodies (beta-hydroxybutyrate, acetoacetate [AcAc], acetone) and fatty acids (Hartman and Vining, 2007; Kossoff and Rho, 2009; Maalouf et al., 2009), an adaptation that also occurs with extended fasting. Importantly, ketone bodies may constitute up to 60%–70% of brain energy metabolism during this period, as has been documented during extended fasting (Cahill, 2006). Although the exact mechanism for the KD's efficacy in suppressing seizures had not been definitively identified, several candidate mechanisms have been identified that also have relevance to AD, including inhibition of glutamatergic excitatory transmission with increased production of gamma-aminobutyric acid from glutamate, carbohydrate reduction and decreased glycolytic flux, and activation of adenosine triphosphate-sensitive potassium channels by mitochondrial metabolism (Danial et al., 2013; Maalouf et al., 2009; Stafstrom and Rho, 2012; Wallace et al., 2010). These mechanisms may mitigate neuronal hyperexcitability that has been described in preclinical and early clinical stages of AD and may be related to AD pathology (Vossel et al., 2017).

The KD supplies an alternative metabolic fuel to glucose, which may provide general neuroprotective effects and mitigate the impact of beta-amyloid (A β) on mitochondria, leading to increased efficiency of adenosine triphosphate production with decreased

oxidative stress and glutamate toxicity (Danial et al., 2013; Maalouf et al., 2009; Stafstrom and Rho, 2012). Accumulating evidence in animal models and humans suggests that the implementation of an intervention aimed at inducing ketosis may have benefits in mild cognitive impairment (MCI) and AD dementia. In several mouse models of AD, consuming KD decreased brain A β content (Kashiwaya et al., 2013; Van der Auwera et al., 2005; Yao et al., 2011), although other studies have reported no impact of KD on A β (Beckett et al., 2013; Brownlow et al., 2013). Furthermore, KD has been shown to improve motor performance and learning/memory, while affecting amyloid beta precursor protein, phosphorylated-tau (tau-p181), gene expression of alpha/gamma-secretase, proteins involved in A β clearance/degradation, and genes involved in mitochondrial biogenesis (Beckett et al., 2013; Bough et al., 2006; Kashiwaya et al., 2013; Yao et al., 2011).

Despite the promising evidence of KD in mouse models of AD, there have been relatively few clinical studies exploring ketogenic dietary interventions in adults with cognitive impairment or AD (Brandt et al., 2019; Krikorian et al., 2012; Taylor et al., 2018). A 2012 study by Krikorian et al. (2012) showed that a ketogenic low carbohydrate diet enhanced verbal memory following a 6-week intervention relative to a high carbohydrate diet in older adults with MCI. This study also reported improvements in metabolic measures associated with the ketogenic intervention, including reductions in weight, waist circumference, and fasting glucose and insulin (Krikorian et al., 2012). Two recent small studies (Brandt et al., 2019; Taylor et al., 2018) have reported encouraging preliminary results on the feasibility and efficacy of ketogenic dietary interventions in MCI, but one lacked a dietary control group, and neither investigated diet effects on neuroimaging or cerebrospinal fluid (CSF) biomarkers.

Our goal was to study the effects of a modified Mediterranean-ketogenic diet (MMKD) and a control low-fat American Heart Association Diet (AHAD) on CSF AD biomarkers, neuroimaging measures, peripheral metabolic measures, and cognition. Because such interventions are promising tools for prevention, we examined the effects in adults at risk for AD dementia by virtue of systemic metabolic dysfunction (pre-diabetes) together with subjective memory complaints or amnesic MCI. We hypothesized that MMKD would lead to improvement in peripheral metabolic health, CSF AD biomarker profile, increased cerebral perfusion/ketone body uptake, and cognition.

2. Methods

2.1. Study participants

The protocol was approved by the Wake Forest Institutional Review Board ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02984540) Identifier: [NCT02984540](https://clinicaltrials.gov/ct2/show/study/NCT02984540)), and written informed consent was obtained from all participants and/or their study partners. Participants were medically supervised by clinicians, with safety monitoring overseen by the Wake Forest Institutional Data and Safety Monitoring Committee as detailed in Protection of Human Subjects. All participants had pre-diabetes defined by American Diabetes Association (2016) guidelines (hemoglobin A1c [HbA1c] of 5.7–6.4). Participants were further divided into 2 cognitive subgroups: adults with subjective memory complaints diagnosed using Alzheimer's Disease Neuroimaging Initiative criteria (Risacher et al., 2015) (SMC; Cognitive Change Index score 16 on the first

12 items) and adults with MCI diagnosed by expert physicians and neuropsychologists using the National Institute on Aging at National Institutes of Health and the Alzheimer's Association guidelines (Albert et al., 2011). Exclusion criteria included prior diagnosis of neurological or neurodegenerative illness (except MCI), major psychiatric disorder, stroke, use of diabetes and lipid-lowering medications, or medications with known central nervous system effects including anti-seizure medications, anti-psychotics, and opioids. Participants with well-controlled depression were allowed. Twenty-three adults were enrolled in the study with 3 participants discontinuing diet prior to completion of the study.

2.2. Procedure

The study consisted of a randomized crossover design in which participants consumed either MMKD or the control AHAD for 6 weeks, followed by a 6-week washout period in which participants were instructed to resume their pre-study diet, after which the second diet was consumed for 6 weeks. Prior to diet randomization, baseline characterization of cognitive status, lumbar puncture (LP), magnetic resonance imaging (MRI), and metabolic profiles were performed. Participants also completed a 3-day record in which they recorded all food consumed on 2 week-days and a week-end day at baseline and during weeks 3 and 6 of the diet intervention. Total calorie and macronutrient intake was calculated from the food records using ProNutra software (Viocare, Princeton, NJ). Cognitive function, LP, MRI, and metabolic parameters were re-assessed after each diet. Fig. S1 shows a representation of study design. Compliance and blood metabolic laboratory profiles were also assessed at the half-way point of each diet period.

2.3. Diet intervention and education

The experimental diet was MMKD, which is a very low carbohydrate diet aimed at inducing ketosis. Our control diet was AHAD, which is a low-fat diet (Krauss et al., 2001). Diets were eucaloric. The proportions of carbohydrates and fat were the main variables manipulated between the 2 diets. The target macronutrient composition (expressed as % of total calories) is approximately 5%–10% carbohydrate, 60%–65% fat, and 30% protein for MMKD; and 55%–65% carbohydrate, 15%–20% fat, and 20%–30% protein for AHAD.

Participants on MMKD worked closely with a registered dietitian to keep the amounts of carbohydrate, fat, and protein within the desired target ranges described above. Throughout the duration of the study, participants on MMKD were encouraged to avoid low-carbohydrate store-bought products and artificially sweetened beverages. MMKD was modeled on a Mediterranean diet in that emphasis was placed on protein sources low in saturated fat (fish, lean meats), healthy fats were emphasized, fruits and vegetable consumption was encouraged within limits, as were whole grains, and 1 glass of wine per day was allowed. Participants were supplied with 1 L of extra virgin olive oil during their Pre-Diet and Mid-Diet visits to use as a source of fat in their diet, and were encouraged to eat plentiful fish, lean meats, and nutrient-rich foods. The results of the PREDIMED trial have shown benefits with extra virgin olive oil supplementation on cardiovascular disease risk and mortality (Guasch-Ferre et al., 2014). Clearly, the carbohydrate restriction imposed for the study limited the amount of grains and fruits that might be associated with a classic Mediterranean diet. However, the dietary pattern was consistent with a Mediterranean-like

diet. Participants on AHAD were encouraged to limit their amount of fat intake to <40 g/d, while eating plentiful fruits, vegetables, and carbohydrates containing adequate fiber, as well as proteins from healthy sources such as fish and lean meat. They did not receive supplementary olive oil.

A registered dietitian developed daily meal plans for each study participant based on their food preferences and caloric needs as determined by a 3-day food diary, activity level, and macronutrient requirements for the respective diets. Participants had weekly inperson and phone diet education/compliance visits starting 1 week prior to start of each diet and continuing throughout the remainder of the intervention. Participants maintained a food record that was reviewed at these visits. Capillary ketone body measures were collected at all major time points and during diet education visits using the Nova Max Plus (<http://www.novacares.com/nova-max-plus/>) capillary glucose and ketone body (beta-hydroxybutyrate) monitoring system. Prior studies have reported that less frequent blood ketone body measures are just as accurate a measure of ketosis as daily urine ketone body test strips (Gilbert et al., 2000). Subjective measures of compliance were also recorded by the study dietician. Participants were asked to keep their exercise and physical activity level stable throughout the study.

Participants were required to supply their own food based on a daily meal plan, food list, and other educational material provided. A food stipend of \$25/wk was provided to help defray the cost of foods. Participants received a daily multivitamin supplement (Centrum Silver) while on diet. Moreover, participants were asked to discontinue the following supplements for the duration of the study: resveratrol, coenzyme Q10, coconut oil/other medium chain triglyceride-containing (e.g., Axona) supplements, or curcumin, as they may impact bioenergetic status and interfere with interpretation of study results.

2.4. Cognitive protocol

Study participants completed assessments of immediate and delayed memory at baseline and after each diet. Tests included the Free and Cued Selective Reminding Test (FCSRT) (Grober et al., 2010), story recall (modification of the episodic memory measure from the Wechsler Memory Scale-Revised) (Wechsler, 1987), and the ADAS-Cog12, a test of general cognition that includes items related to executive function, attention, verbal abilities, and memory (Rosen et al., 1984). Cognition was assessed before and after each diet intervention as seen in Fig. S1. Different versions of selected tests were utilized to mitigate the impact of learning on cognitive performance.

2.5. Plasma biomarkers

Fasting blood for metabolic measures was collected before and after each diet. Samples were 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 were aliquoted into separate storage tubes and flash frozen at 80 C until analyzed. Specific measures analyzed include the following: HbA1c, glucose, insulin, triglycerides, and total, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) cholesterol.

2.6. Lumbar puncture and cerebrospinal fluid biomarkers

Participants completed LP after 12-hour fast at baseline and after each diet for collection of CSF. Participants were placed in the seated or lateral decubitus position per study clinician preference. Using a 25-gauge needle, the L3–4 or L4–5 interspace was infiltrated with 1% lidocaine for local anesthesia. Using a 22-gauge Sprotte needle to drip, up to 25 mL of CSF was withdrawn into sterile polypropylene tubes. The first 3 mL was sent to the local laboratory for analysis to include cell count, protein, and glucose. CSF was then transferred in 0.2 mL aliquots into pre-chilled polypropylene tubes, frozen immediately on dry ice, and stored at -80°C until analysis. First thawed CSF was used for $\text{A}\beta$ and tau measures. $\text{A}\beta_{42}$, total tau, and phosphotau (tau-p181) were measured using a Luminex-based INNO-BIA AlzBio3 assay, and $\text{A}\beta_{40}$ was measured by standard enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions, in each case using kits from Fujirebio (Malvern, PA; formerly Innogenetics, Belgium). We also quantified CSF neurogranin using an in-house sandwich ELISA, as previously described (Portelius et al., 2018), sTREM2 using an in-house Meso Scale Discovery assay, as previously described (Gisslén et al., 2019), YKL-40 using a commercial ELISA kit (R&D Systems, Minneapolis, MN), and NF-Light using a commercial ELISA kit (Uman-Diagnostics, Umeå, Sweden) to assess the impact of diet on newly emerging CSF AD biomarkers (Blennow and Zetterberg, 2018). The measurements were performed by board-certified laboratory technicians who were blinded to clinical data. Baseline and follow-up samples were analyzed side-by-side on the same measurement plates and intra-assay coefficients of variation were below 10%.

2.7. Magnetic resonance imaging

Magnetic resonance images were acquired on a 3-Tesla Siemens Skyra scanner with a high-resolution 32-channel head coil (Erlangen, Germany) for the following sequences: (1) high-resolution T1-weighted images were used to provide gray matter and white matter volumes for the partial volume correction (Asllani et al., 2008) and warp perfusion images into a common space. High-resolution 3-dimensional T1-weighted images were obtained using a magnetization-prepared rapid gradient echo sequence: repetition time (TR) = 2300 ms; inversion time (TI) = 900 ms; echo time (TE) = 2.98 ms; slice thickness = 1 mm no gap; and in-plane resolution = 1.1 mm. (2) Perfusion imaging (pseudo continuous arterial spin labeling or PCASL) (Jung et al., 2010) was used to estimate cerebral perfusion. Quantitative data processing includes data cleaning (Tan et al., 2009), realignment, and quantification of cerebral blood flow into the physiological unit (mL/100 g tissue/min) using a kinetic model (Buxton et al., 1998). PCASL was collected with the following parameters: a single-shot echo-planar imaging (EPI) sequence; tagging duration = 1700 ms; post-labeling delay = 1500 ms; TR = 4000 ms; TE = 12 ms; repetitions = 80; field-of-view (FOV) = 22.22 cm; matrix size = 64 × 64; no. of slices = 24; slice thickness = 5 mm; and acquisition time = 5 minutes 24 seconds.

2.8. PET acquisition and processing

An indwelling catheter was placed into a forearm vein in both arms. A heating pad was wrapped around 1 arm 30 minutes prior to the positron emission tomography (PET) scan. About 10 mL of blood was obtained for glucose, AcAc, and beta-hydroxybutyrate

quantification. This blood was stored on ice and immediately processed for analysis after the PET scan. Prior to PET acquisition, a low-dose computed tomography scan of the head was obtained for attenuation correction. For the first scan (acetoacetate or AcAc) participants were injected with an intravenous bolus of up to 5 mCi ($\pm 10\%$) of ^{11}C -AcAc over 2 minutes and for the second scan [fluorodeoxyglucose (FDG)] participants were injected with an intravenous bolus of up to 5 mCi (370 MBq) ($\pm 10\%$) of ^{18}F -FDG over 20 seconds, standard doses used in previous studies (Castellano et al., 2015; Nugent et al., 2014a,b). The AcAc scan acquisition began immediately after the tracer injection and lasted for a total of 30 minutes. Time frames used for analysis were from the first 10 minutes of scan acquisition, 12×10 seconds, 8×30 seconds, and 1×4 min. Immediately after the AcAc scan, a washout period occurred from 30 to 60 minutes post injection of the first tracer. This was used to ensure that most of the first tracer was excreted prior to injection of the ^{18}F -FDG tracer and second part of the dual-tracer scan. The FDG scan acquisition began immediately after the tracer injection and lasted for a total of 60 minutes. Time frames for scan acquisition were 12×10 seconds, 8×30 seconds, 6×4 minutes, and 3×10 minutes.

Blood was drawn during the ^{11}C -AcAc and ^{18}F -FDG scans and processed for gamma radiation estimation immediately after the scan was completed. Up to 2 mL of blood was sampled from the forearm vein at 3, 6, 8, 12, and 20 minutes post ^{11}C -AcAc tracer injection; and at 3, 8, 16, 24, 35, and 55 minutes after ^{18}F -FDG tracer injection. Whole blood was spun at 6000 rpm for 5 minutes at room temperature. Three hundred microliters of plasma for each time point was placed in a counting tube. Plasma radiation estimation was performed in an automated fashion with a Wallac 1480 Wizard 3' (Perkin Elmer) gamma counter. The estimates were used for calibration of brain counts. See Fig. S2 for representation of dual-tracer PET protocol.

PMOD software version 3.5 (PMOD Technologies Ltd, Zurich, Switzerland) was used to process PET data. Both AcAc and FDG data were first co-registered to each participant's T1-weighted structural MRI using the PFUS tool in PMOD. Co-registered PET images were corrected for partial volume effects with the partial volume correction (brain-based) feature in PMOD using principles of the modified Müller-Gartner method. An arterial input function was determined by tracing 3–4 regions of interest (ROIs) within the internal carotid artery: first on the MR images and then applied to the PET images. The activity calculated in the internal carotid artery was then corrected using plasma radioactivity measures taken from each individual during the PET acquisition at time intervals indicated in the PET protocol. Cerebral metabolic rates (CMRs) were quantified with the Patlak model feature in the PKIN tool of PMOD. The lumped constant for determination of CMR AcAc was set to 1.0 and CMR glucose was set to 0.8, as previously published (Castellano et al., 2015).

2.9. Statistical analyses

CSF biomarker values, cognitive scores, and metabolic values were submitted to repeated measures analysis of covariance using Proc GLM from SAS v9.4. Time (Pre vs. Post) was the repeated factor, with group (SMC vs. MCI) as the independent factor, and age, apolipoprotein E4 allele (APOE4) carriage (yes or no), and order of diet intervention (MMKD first vs. AHAD first) as covariates. If covariates did not contribute significantly (p

> 0.15) they were dropped from the model. Separate repeated measures analyses were conducted for pre and post MMKD data, and for pre and post AHAD data. Partial eta squared effect sizes and post hoc power estimates were determined with GPower. Partial eta squared values of 0.01, 0.06, and 0.14 are considered small, medium, and large (Cohen, 1988). For CSF values, analyses were conducted only for participants who completed LPs and had valid data for both baseline and post-diet LPs. Two CSF analytes (tau and neurofilament light) had non-normal distributions and were log-transformed prior to analysis. For MR gray matter volume values, a meta-ROI shown to discriminate between AD and controls was constructed as the average of the following regions: inferior temporal gyrus, caudate, paracentral lobule, superior temporal pole, posterior cingulate, amygdala, hippocampus, entorhinal cortex, angular gyrus, and mid-temporal pole (Rondina et al., 2018). Main effects of diet, cognitive group, and the interaction of diet by cognitive group on PCASL perfusion were examined utilizing a voxel-wise multiple regression in SPM12. An a priori anatomical mask was constructed using the WFU PickAtlas toolbox (Maldjian et al., 2003) and was applied to all analyses. The mask was composed of regions involved in memory and regions shown to be negatively affected by AD including the frontal, parietal, and temporal cortices, cingulum, postcentral gyrus, amygdala, parahippocampal gyri, and hippocampus (Fig. S3). Data were corrected for multiple comparisons with a voxel-wise threshold level of $p < 0.005$ holding alpha at 0.05 for a minimum cluster size of 112 contiguous voxels. This a priori threshold was derived via Monte Carlo simulations (3dClustSim, AFNI, <http://afni.nimh.nih.gov>). To further explore the regions of significance yielded from voxel-wise analyses, beta weights were extracted for pre and post MMKD diet PCASL for both cognitive groups utilizing the SPM12 toolbox MarsBaR (version 0.44).

For dual-tracer PET values, meta-ROIs that characterized prototypical AD hypometabolism were constructed for the ^{11}C -AcAc and ^{18}F -FDG scans from the average of the following regions: left and right angular gyrus, left and right temporal lobe, and posterior cingulate (Landau et al., 2011). Seven participants completed the dual-tracer PET at baseline and at the end of the MMKD and AHAD. For 2 participants, a scanner malfunction resulted in uninterpretable data. Exploratory analyses were conducted for the remaining 5 participants. The PET meta-ROI for ^{11}C -AcAc and ^{18}F -FDG tracers was subjected to repeated measures analysis of variance, with time (Pre-Diet, Post-MMKD, Post-AHAD) as the repeated measure.

3. Results

A total of 20 participants completed both diets. Regarding attrition, 3 of 23 participants discontinued the study early. Mean compliance rates assessed by dietician assessment of daily food records were 90% for the MMKD and 95% for AHAD. Baseline demographic characteristics are shown in Table 1. Our sample had a mean Mini-Mental State Examination score of 28.7, mean years of education of 16.1, and APOE4 positivity of 30%. No serious adverse events occurred, nor any adverse event deemed related to diet intervention by the study clinician.

3.1. Macronutrient, weight, and peripheral metabolic measures

Total calories and net carbohydrate, protein, and fat grams were calculated from the 3-day food record administered at baseline, as well as from data averaged across 2 on-diet time points. Calorie intake was reduced from baseline for both diets, and did not differ between diets (mean calorie intake prior to MMKD = 1981.6 ± 802.6 [standard deviation, SD] vs. on-diet = 1681.6 ± 517.9, prior to AHAD = 1976.3 ± 753.9 vs. on-diet = 1456.5 ± 402.8; overall time effect $p = 0.0021$, partial $\eta^2 = 0.401$, power = 0.907). Carbohydrate consumption (g) was significantly reduced for MMKD relative to AHAD (mean intake prior to MMKD = 191.1 ± 91.8 [SD] vs. on-diet = 40.2 ± 99.0, prior to AHAD = 191.4 ± 99.1 vs. on-diet = 170.5 ± 68.7; diet by time effect $p = 0.0002$, partial $\eta^2 = 0.538$, power = 0.991). Fat consumption (g) was significantly increased for MMKD and reduced for AHAD (mean intake prior to MMKD = 86.6 ± 45.9 [SD] vs. on-diet = 114.4 ± 29.3, prior to AHAD = 88.4 ± 51.6 vs. on-diet = 42.4 ± 33.3; diet by time effect $p = 0.0001$, partial $\eta^2 = 0.659$, power = 0.999). No change in protein intake was observed with either diet. These results demonstrate that the dietary intervention successfully modulated macronutrient profiles.

Metabolic outcomes are presented in Table 2. MMKD successfully elevated fasting ketone body levels ($p = 0.008$, partial $\eta^2 = 0.424$, power = 0.847), with the SMC group showing greater increases (time by group $p = 0.015$, partial $\eta^2 = 0.378$, power = 0.774). Participants lost weight with MMKD, despite having calorie targets that were determined by pre-study caloric intake. The MCI group showed greater percent weight loss than the SMC group ($p = 0.004$, partial $\eta^2 = 0.427$, power = 0.847). All participants had reduced fasting glucose and HbA1c levels, with no group differences noted (glucose and HbA1c by time $p = 0.03$, partial $\eta^2 = 0.287$, power = 0.632 and $p = 0.004$, partial $\eta^2 = 0.514$, power = 0.984). Fasting insulin levels were also reduced ($p = 0.03$, partial $\eta^2 = 0.241$, power = 0.617). Total cholesterol was unchanged by the MMKD. VLDL cholesterol levels were reduced by MMKD ($p = 0.02$, partial $\eta^2 = 0.274$, power = 0.693), particularly for the MCI group (time by group $p = 0.02$, partial $\eta^2 = 0.281$, power = 0.709), who also showed a trend increase in HDL (time by group $p = 0.096$, partial $\eta^2 = 0.146$, power = 0.382). Triglycerides were lowered with MMKD ($p = 0.02$, partial $\eta^2 = 0.273$, power = 0.69) with the greatest reduction demonstrated by the MCI group (time by group $p = 0.02$, partial $\eta^2 = 0.288$, power = 0.549).

The AHAD did not affect peripheral ketone body, glucose, insulin, HbA1c, total cholesterol, VLDL cholesterol, or triglyceride levels. HDL cholesterol levels were lowered for both groups ($p = 0.02$, partial $\eta^2 = 0.277$, power = 0.699), and both groups had reduced percent body weight after the diet ($p = 0.008$).

3.2. CSF biomarkers

CSF A β 42 levels increased following MMKD independent of group (time $p = 0.04$, partial $\eta^2 = 0.404$, power = 0.700; Fig. 1). This effect was moderated by age (time by age interaction $p = 0.04$, partial $\eta^2 = 0.406$, power = 0.704) such that older participants showed greater increases in A β 42 following the diet (Spearman rho = 0.51, $p = 0.06$). No MMKD-associated changes were observed for A β 0, or for the ratio of A β 42/A β 40 (data not shown).

MMKD-induced changes in CSF tau levels differed according to group (time by group interaction $p = 0.007$, partial $\eta^2 = 0.571$, power = 0.935; Fig. 2). The MCI group showed decreased tau after MMKD and the SMC group's tau levels were unchanged. Age also moderated this interaction (time by group by age interaction $p = 0.008$, partial $\eta^2 = 0.563$, power = 0.927) with younger participants in the MCI group showing greater reductions (data not shown). No effects were observed for tau-p181 levels.

MMKD showed trend-level lowering of axonal injury marker NFL for the MCI group [time by group interaction $p = 0.097$, partial $\eta^2 = 0.23$, power = 0.38; Pre vs. Post means with (standard error of the mean) for the SMC group were 2.71 (0.06) vs. 2.72 (0.06); and for the MCI group were 2.66 (0.08) vs. 2.61 (0.08)]. For the synaptic protein neurogranin, an age-moderated trend for reduced levels was observed following the MMKD independent of group (time by age interaction $p = 0.09$, partial $\eta^2 = 0.279$, power = 0.386), reflecting greater reduction for older participants (Spearman rho = 0.65, $p = 0.03$; Fig. 3). Although sTREM2 levels were reduced following MMKD independent of group and age, this effect did not achieve significance ($p = 0.12$, partial $\eta^2 = 0.203$, power = 0.303). YKL-40 levels were unchanged by MMKD.

There were no changes in CSF A β 42 or A β 40 after AHAD for either group. AHAD reduced tau levels ($p = 0.02$, partial $\eta^2 = 0.511$, power = 0.778), an effect that trended greater for the MCI group (time by group $p = 0.056$, partial $\eta^2 = 0.383$, power = 0.559; Fig. 2), and was moderated by age (time by age $p = 0.03$, partial $\eta^2 = 0.465$, power = 0.701), with younger participants showing greater reductions regardless of group (Spearman rho = 0.74, $p = 0.01$). AHAD did not affect CSF levels of NFL, neurogranin, YKL-40, or sTREM2.

3.3. Imaging measures

Gray matter volume meta-ROI values did not change following either diet for either group. Voxel-wise analyses detected increased perfusion in response to MMKD in the left parahippocampal and the right temporal lobe while there were no changes in response to AHAD. When this change in response to MMKD was divided by cognitive group, the MCI group displayed increased perfusion in left inferior parietal, medial and superior frontal, medial temporal, and parahippocampal as well as right medial temporal, precentral, and angular gyrus, compared to the SMC group (Fig. 4). The SMC group showed no regions of greater perfusion than the MCI group. The PCASL in these regions were extracted and demonstrated that the interaction was driven by an increased perfusion in the MCI group while the SMC group showed no change (Fig. 4).

Baseline meta-ROI values were contrasted with each of the post-diet values. ^{11}C -AcAc meta-ROI values increased for each of the 5 participants following MMKD, resulting in a significant effect of time ($p = 0.02$, partial $\eta^2 = 0.759$, power = 0.552; Fig. 5). No significant changes in ^{11}C -AcAc meta-ROI values were observed following AHAD. There were no differences in ^{18}F -FDG after either diet (data not shown).

3.4. Cognition

Cognitive test score differences are presented in Table S1. Both groups showed better performance on FCSRT after MMKD (time $p = 0.03$, partial $\eta^2 = 0.233$, power = 0.6). No

MMKD-associated changes were observed for total story recall or ADAS-Cog12 scores. For AHAD, the MCI group showed greater improvement on FCSRT (time by group $p = 0.01$, partial $\eta^2 = 0.3$, power = 0.748). No changes were observed for total story recall or ADAS-Cog12 scores.

4. Discussion

The present study examined the effects of a modified KD intervention on CSF AD biomarkers, cerebral blood flow, cerebral metabolism, peripheral metabolic measures, and cognition in adults with metabolic and cognitive risk factors for AD. Both MMKD and AHAD diets achieved good mean compliance (>90%) and safety. Only MMKD improved peripheral metabolic profile, cerebral perfusion, and cerebral ketone body uptake. Both diets altered CSF AD biomarkers, but in different ways.

4.1. Ketogenic diet is associated with improvement in peripheral metabolic measures

The MMKD increased levels of capillary ketone bodies for all participants, confirming that the dietary intervention was successfully implemented. Interestingly, lower ketone levels were observed for the MCI group relative to the SMC group, despite similar dietary compliance as determined by daily food records. This pattern may reflect greater ketone uptake into target tissues in MCI, leading to lower circulating levels. This pattern would also be consistent with the possibility that adults with MCI are less able to generate ketone bodies as a result of ketogenic interventions. Additionally, the MCI group had higher fasting insulin and triglyceride levels at baseline, indicating they may have had greater insulin resistance, which may necessitate longer exposure to MMKD to generate ketone levels comparable to the SMC group.

Only MMKD had a beneficial impact on peripheral metabolic measures related to lipid and glucose metabolism; a significant reduction in HbA1c, glucose, insulin, triglycerides, and VLDL cholesterol were observed. These findings support metabolic data from other studies of dietary ketosis showing improvement in lipid profile and insulin sensitivity secondary to KD (Hu et al., 2012; Yancy et al., 2004). Our striking metabolic results are important as they show the potential for MMKD to beneficially impact metabolic dysfunction in older adults with and without cognitive impairment. The majority of published reports targeting ketosis have focused on supplementation of ketogenic compounds (medium chain triglycerides) (Croteau et al., 2018; Henderson et al., 2009; Reger et al., 2004). Although supplements may be easier to implement than dietary modification, our study demonstrates that a ketogenic dietary intervention is feasible in our target population—aided by robust education materials, meal plans, and the support of dietitians. Given that systemic metabolic dysfunction increases risk for the development of Alzheimer's and cognitive decline (Craft, 2009; Di Paolo and Kim, 2011; Razay et al., 2007; Whitmer et al., 2005, 2008), the improvement in peripheral glucose and lipid metabolism through ketogenic interventions may provide a therapeutic tool for the prevention of age-related neurodegenerative disorders.

4.2. Ketogenic diet is associated with improvement in CSF biomarker profile

A primary goal of the study is to examine the effects of MMKD on CSF AD biomarkers. We observed that MMKD led to improvement in CSF AD biomarker profiles as evidenced by increased CSF A β 42 (independent of group), decreased CSF tau (only in MCI), and increased CSF A β 42/tau ratio after MMKD (greater in MCI). These promising results suggest that a targeted 6-week dietary intervention aimed at ketosis can positively affect CSF AD biomarker profile. To our knowledge, this is the first published report showing an impact of dietary ketosis on CSF amyloid and tau levels in humans. These results suggest that dietary interventions can have significant impact on AD pathological markers and as such support our previous work showing that a 4-week low saturated fat/low glycemic index diet also increased CSF A β 42 in MCI (Bayer-Carter et al., 2011).

There is a general lack of knowledge concerning the impact of ketosis on the 2 main biomarkers of Alzheimer's, with all current literature coming from animal models, and providing contradictory results. For example, several studies in mouse models of AD reported that ketosis leads to decreased brain A β content (Kashiwaya et al., 2013; Van der Auwera et al., 2005; Yao et al., 2011) and hyperphosphorylated tau (Kashiwaya et al., 2013), while other studies have shown no impact of ketosis on A β (Beckett et al., 2013; Brownlow et al., 2013). Mechanistically, ketone bodies may be neuroprotective by blocking A β entry into neurons (Yin et al., 2016), which may decrease oxidative stress, improve metabolic function, and decrease glutamate excitotoxicity (Hertz et al., 2015; Maalouf et al., 2009; Yin et al., 2016). In the present study, the fact that lowering of tau was only observed in adults with MCI raises the possibility that MMKD is affecting process relating to tau aggregation and neurodegeneration, which occurs around the time that clinical symptoms manifest. Supporting this possibility, we also found trend-level reduction of CSF neurogranin and NFL after MMKD, suggesting diet may have impact on measures of dendritic/synaptic injury and neurodegeneration (Blennow and Zetterberg, 2018; Zetterberg et al., 2016).

Intriguingly, AHAD also led to a reduction in CSF tau that was greater in MCI. We hypothesized that improvement in systemic metabolic health would promote a better CSF biomarker profile, yet only MMKD showed significant impact on peripheral metabolic measures. The positive change in CSF tau levels after AHAD may be driven by weight loss alone or other unknown diet-related effects, rather than the specific metabolic changes found after MMKD intervention. Another possible explanation for the post-diet changes in CSF measures could relate to a general diet-induced decrease in systemic inflammation, which might promote positive CSF AD biomarker outcomes. Unfortunately, this was not assessed in the present study and should be explored in future work.

4.3. Ketogenic diet is associated with increased cerebral perfusion

Cerebral blood flow or perfusion is an important measure of overall brain health (Chen et al., 2011, 2013) and has been shown to be negatively impacted on neurodegenerative disorders (Alsop et al., 2010; Heron et al., 2014; Zhang et al., 2017). In Alzheimer's, decreased cerebral perfusion has been related to disease progression (Zhang et al., 2017), although there may be regional compensatory increases in cerebral blood flow early in the disease (Dai et al., 2009). In the present study, we used PCASL to determine how diet impacts

cerebral perfusion. We found increased perfusion on the pre-specified meta-ROI only following MMKD (greater in the MCI group). Previous work has shown that metabolic dysregulation is associated with reduced perfusion (Birdsill et al., 2013; Zhang et al., 2017). Thus, it is possible that MMKD-induced metabolic improvement contributed to enhanced perfusion. We have an incomplete understanding of dietary impact on cerebral perfusion, especially in older adults at risk for Alzheimer's. Prior reports have shown that dietary supplementation may impact cerebral perfusion (Presley et al., 2011; Taheri et al., 2016; Zerbi et al., 2014), while a study by Lin et al. (2015) reports that caloric restriction and ketosis preserved cerebral blood flow in aging rat brains. Our results provide important clinical data concerning diet effects on cerebral perfusion, and suggest that dietary ketosis may improve cerebral perfusion rather than a low-fat diet. The potential impact of diet on cerebral blood flow after a 6-week trial is intriguing but must be interpreted cautiously. There is a need for additional study of interventions aimed at ketosis to better understand their benefits and risks.

4.4. Ketogenic diet is associated with increased cerebral ketone body uptake

We used a novel dual-tracer PET imaging technique in a subset of participants to assess the impact of diet on both cerebral ketone body and glucose metabolism. We found that ^{11}C -AcAc uptake increased after MMKD, while cerebral ^{18}F -FDG uptake was unaffected. These results suggest that MMKD may increase peripheral ketones and thereby make greater concentrations available for uptake into the brain. These results complement previous work showing that a ketogenic intervention (medium chain triglyceride supplementation) increases cerebral ketone body uptake as assessed by ^{11}C -AcAc PET, without impacting glucose uptake (Croteau et al., 2018). Although our sample size is too small to identify direct relationships among peripheral ketone bodies, cerebral ketone body uptake, and CSF biomarker/cognitive outcomes, or differences between SMC and MCI groups, our results clearly support the hypothesis that a ketogenic dietary intervention promotes cerebral ketosis. Future work should focus on larger samples to directly test this hypothesis.

4.5. Ketogenic diet is associated with better performance on FCSRT but not ADAS-Cog

Improved memory has been observed in some studies of ketogenic interventions in MCI and AD (Henderson et al., 2009; Krikorian et al., 2012; Reger et al., 2004; Van der Auwera et al., 2005). In a previous small uncontrolled pilot study, improvement on ADAS-Cog was observed following a 3-month KD intervention that was reversed following a washout period (Taylor et al., 2018). A second small controlled study did not find improved memory in an intent-to-treat analysis, although results were in a direction favoring the KD group (Brandt et al., 2019). We found that both diets were associated with improved memory performance as assessed by FCSRT, but not on story recall or ADAS-Cog12 scores. However, it is likely that improvement on FCSRT was affected by practice, a tendency that could have been exacerbated by the crossover design. Similarly, the 6-week intervention period may have been an insufficient length of exposure to impact global cognitive measures such as ADAS-Cog, particularly for adults with subjective memory complaints or MCI, whose scores are close to normal and thus have little room for improvement. Future controlled studies with parallel group designs, longer durations, more sensitive tests, and larger samples are needed to determine possible ketogenic-induced cognitive benefits.

4.6. Limitations and future directions

The present study has several key limitations, despite promising results. First, the small sample size limits generalizability, as well as the ability to examine subgroup response factors such as APOE genotype or sex. Second, our crossover design was susceptible to metabolic carry-over effects of diet and practice effects for cognitive tests. Furthermore, participant drop-outs, while infrequent, may potentially have biased results in this small sample. Although we saw significant diet-associated changes in CSF biomarkers, metabolic measures, imaging, and cognition after only 6 weeks, a longer study would likely strengthen our outcomes. The post-intervention weight loss may have contributed to diet-related effects. Although weight loss was observed with both diets, the MCI group showed greater weight loss following MMKD than did the SMC group. Participants in our study prepared their own food with close supervision by a registered dietician, so the dietary intervention may not have been as consistent as when food is supplied by a metabolic kitchen. However, this design improves applicability to a broader population. Finally, a potential drawback of ketogenic interventions is difficulty with compliance over prolonged periods. We observed good compliance in our study, but even minor lapses may have compromised results given the relatively brief intervention. Despite these limitations, we observed robust effects on CSF and brain imaging biomarkers that await replication in larger, longer studies.

5. Conclusions

In conclusion, our results demonstrate that the MMKD intervention was well-tolerated with good compliance, and associated with improved CSF AD biomarker profile, improved peripheral lipid and glucose metabolism, increased cerebral perfusion, and increased cerebral ketone body uptake. These effects suggest that further studies are warranted to determine whether a ketogenic intervention targeted toward adults at risk for AD dementia may prove beneficial in the prevention of cognitive decline. Future longer, larger studies may elucidate the mechanisms underlying therapeutic effects of ketogenic interventions, and may have broad applicability to Alzheimer's and other neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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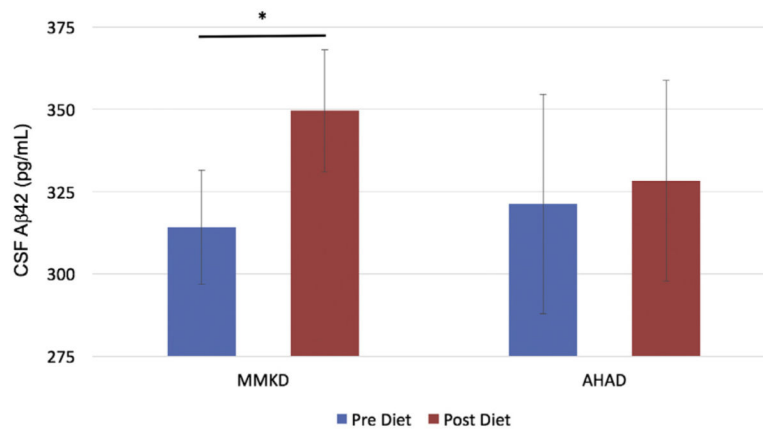


Fig. 1. MMKD effects on CSF A β 42. CSF A β 42 increased with MMKD independent of group ($p = 0.04$). Mean values, with error bars representing ± 1 standard error from the mean. *Significant effect of MMKD, $p < 0.05$. Abbreviations: AHAD, American Heart Association Diet; CSF, cerebrospinal fluid; MMKD, modified Mediterranean-ketogenic diet.

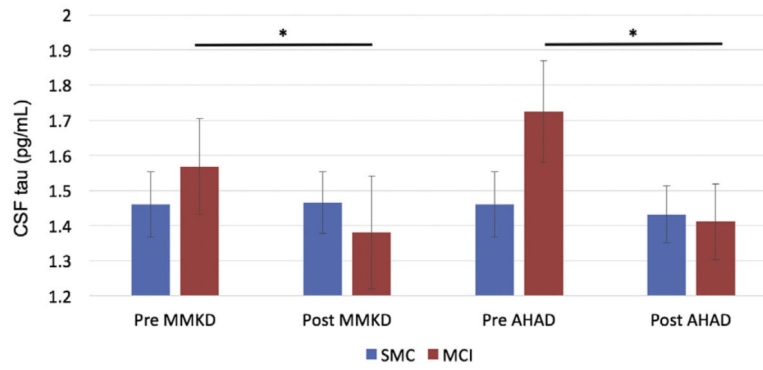


Fig. 2.

Diet effects on CSF tau. CSF tau decreased with MMKD in MCI ($p = 0.007$), and after AHAD independent of group ($p = 0.02$). Mean values, with error bars representing ± 1 standard error from the mean. *Significant effect of diet in MCI, $p < 0.05$. Abbreviations: CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMKD, modified Mediterranean-ketogenic diet; SMC, subjective memory complaints.

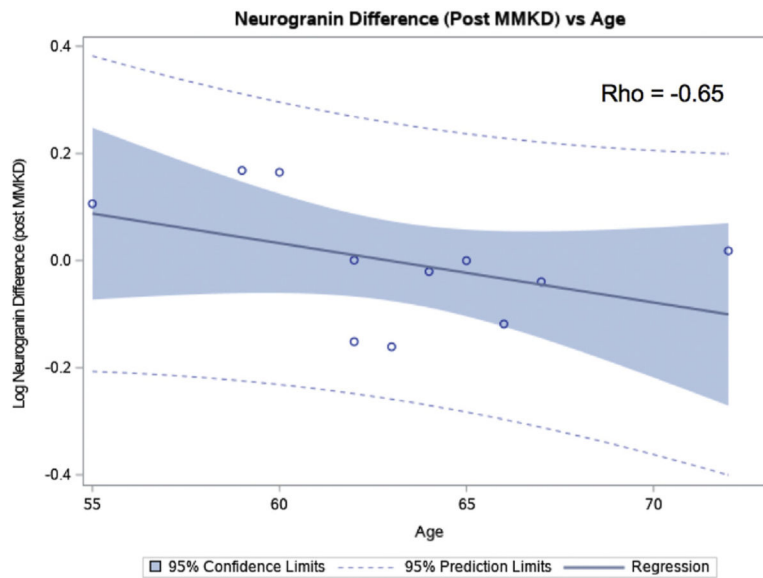
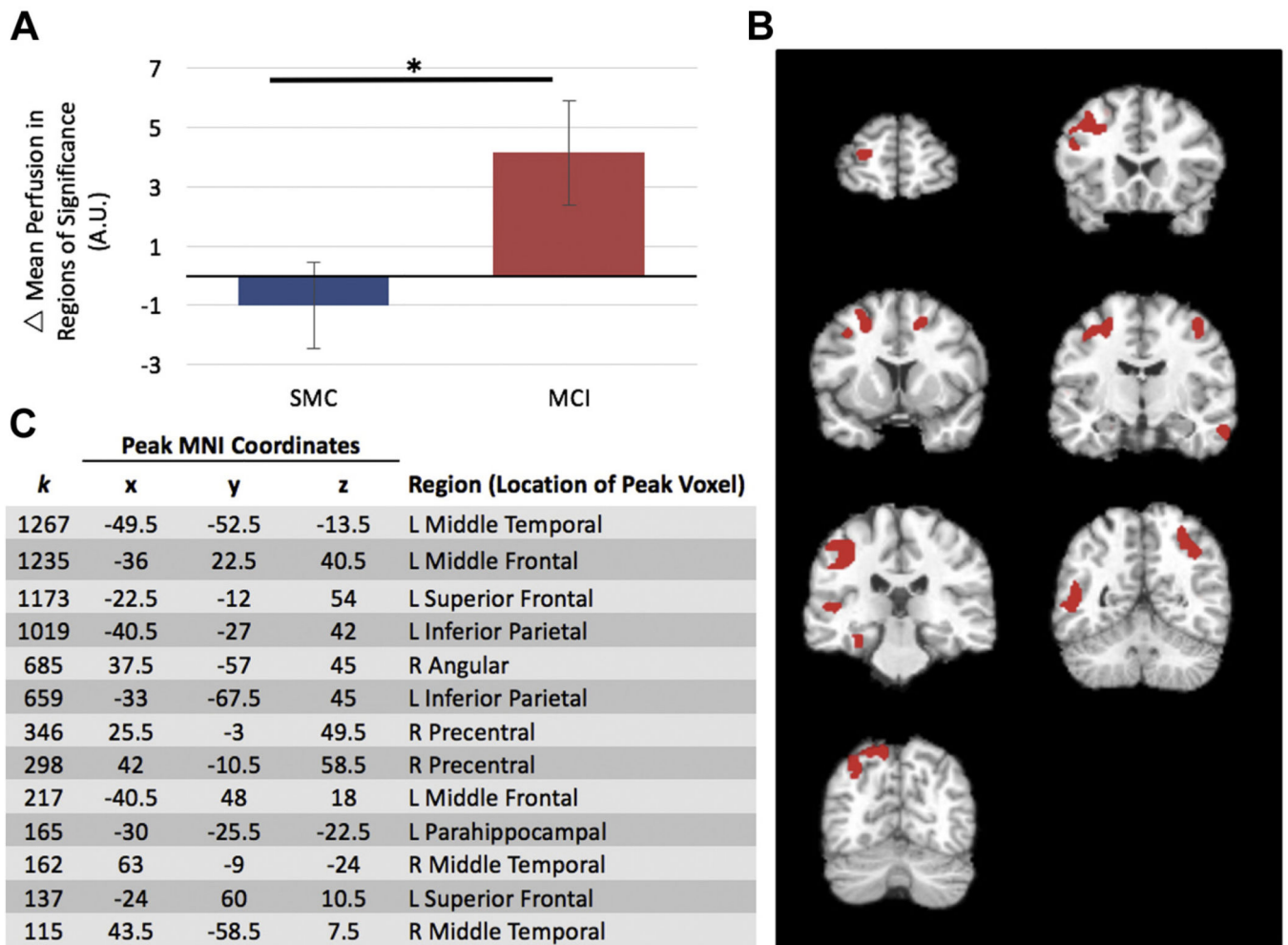


Fig. 3. MMKD effects on neurogranin by age. Neurogranin levels were reduced for older participants after MMKD independent of group ($p = 0.03$). Older participants showed greater reductions in NG levels (Spearman $Rho = -0.65$). Abbreviations: MMKD, modified Mediterranean-ketogenic diet; NG, neurogranin.

**Fig. 4.**

(A) Change in mean perfusion (post-pre diet PCASL) across all regions of significance after the MMKD. Error bars represent ± 1 standard error from the mean. *Significant at $p < 0.05$. (B) Regions where MCI showed greater perfusion compared to SMC in response to MMKD ($p < 0.005$, $k = 112$). All images presented in neurological orientation. (C) Regions where MCI showed greater perfusion than SMC in response to MMKD ($p < 0.005$, $k > 112$); k = number of contiguous voxels, and MNI coordinates for peak voxels within significant cluster. Abbreviations: MCI, mild cognitive impairment; MMKD, modified Mediterranean-ketogenic diet; MNI, Montreal Neurological Institute; PCASL, pseudo continuous arterial spin labeling; SMC, subjective memory complaints.

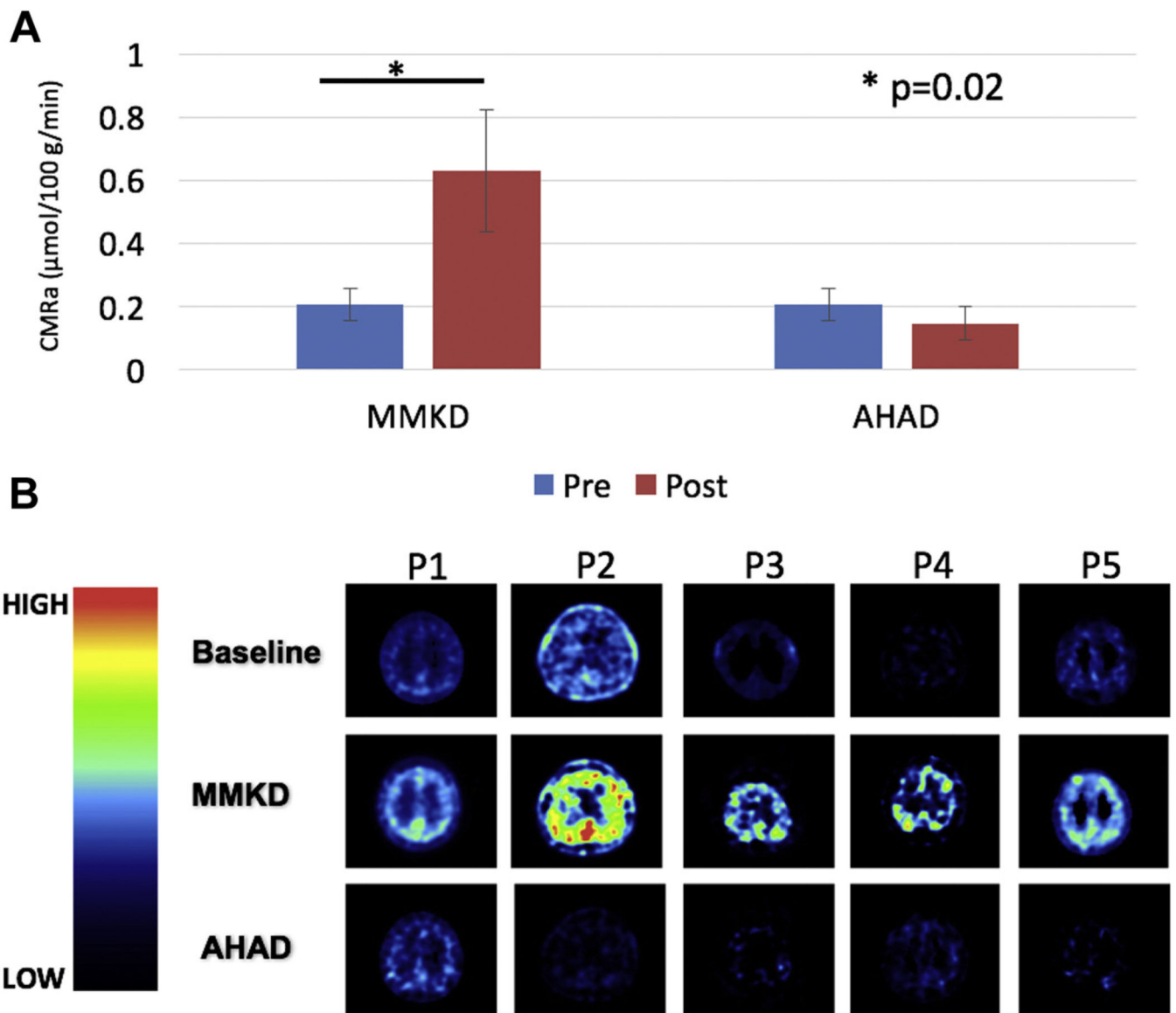


Fig. 5. Dual tracer PET. (A) ^{11}C -AcAc uptake increased after MMKD ($p = 0.02$), but not the AHAD independent of group. No effects were observed for FDG. CMRa or the mean cerebral metabolic rate of acetoacetate is plotted ($\mu\text{mol}/100 \text{ g}/\text{min}$). (B) ^{11}C -AcAc uptake at baseline, and after MMKD and AHAD for 5 individual participants. Abbreviations: AHAD, American Heart Association Diet; MMKD, modified Mediterranean-ketogenic diet.

Table 1:

Descriptive statistics: baseline descriptive statistics (means and standard deviations) for the total sample, and SMC and MCI groups

Variable	All (n = 20)	SMC (n = 11)	MCI (n = 9)
Sex (male/female)	5/15	2/9	3/6
APOE4 (±)	6/13	2/8	4/5
Age (y)	64.3 (6.3)	64.9 (7.9)	63.4 (4.0)
Education (y)	16.1 (2.5)	16.5 (2.3)	15.7 (2.9)
BMI (kg/m ²)	28.4 (5.7)	26.9 (6.2)	30.3 (4.7)
MMSE (out of 30)	28.7 (1.1)	28.9 (1.0)	28.3 (1.2)
Glucose (mg/dL)	97.6 (18.3)	93.9 (11.4)	102.1 (24.4)
BHB (mmol/L)	0.23 (0.27)	0.35 (0.31) ^a	0.1 (0.14) ^a
Insulin (μIU/mL)	8.3 (6.1)	5.2 (3.4) ^a	12.0 (6.8) ^a
Hemoglobin A1c (%)	5.9 (0.3)	5.9 (0.2)	6.1 (0.4)
Total cholesterol (mg/dL)	215.2 (43.7)	200.5 (42.1)	233.2 (40.6)
HDL cholesterol (mg/dL)	67.4 (25.8)	69.5 (25.8)	64.8 (27.1)
VLDL cholesterol (mg/dL)	20.0 (11.1)	15.2 (6.2) ^a	25.8 (13.2) ^a
LDL cholesterol (mg/dL)	121.5 (41.2)	104.1 (45.6) ^a	142.7 (24.2) ^a
Triglycerides (mg/dL)	99.8 (55.7)	75.5 (30.5) ^a	129.4 (66.4) ^a

Key: BHB, capillary beta-hydroxybutyrate; BMI, body mass index; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; SMC, subjective memory complaints; VLDL, very-low-density lipoprotein.

^aMCI different than SMC, $p < 0.05$.

Metabolic outcomes: mean change from baseline (post-pre diet) for metabolic outcomes after MMKD and AHAD for total sample and the SMC and MCI groups

Table 2:

Metabolic indices	MMKD		AHAD			
	All (n = 20)	SMC (n = 11)	MCI (n = 9)	All (n = 20)	SMC (n = 11)	MCI (n = 9)
Weight (lbs)	-8.5 ^a	-3.9 ^b	-14.2 ^b	-4.8 ^a	-3.2	-6.8
Glucose (mg/dL)	-13.5 ^a	-13.9	-13.1	0.3	2.4	-2.1
BHB (mmol/L)	0.7 ^a	1.0 ^b	0.4 ^b	0	0	0
Insulin (μIU/mL)	-2.1 ^a	-0.5	-4	-1.6	-0.2	-3.3
Hemoglobin A1c (%)	-0.1 ^a	-0.2	-0.1	0	0	0
Total cholesterol (mg/dL)	12.3	20.9	1.7	-24.1	-24.3	-23.8
HDL cholesterol (mg/dL)	5.8	10.8 ^c	-0.3 ^c	-8.8 ^a	-8.3	-9.3
VLDL cholesterol (mg/dL)	-4.6 ^a	0.1 ^b	-10.2 ^b	-0.8	0.1	-1.8
LDL cholesterol (mg/dL)	17.4 ^a	21.6 ^b	12.2 ^b	-14.6	-16.1	-12.7
Triglycerides (mg/dL)	-23 ^a	1	-52.3 ^b	-3.4	0.6	-8.3

Key: AHAD, American Heart Association Diet; BHB, capillary beta-hydroxybutyrate; MCI, mild cognitive impairment; MMKD, modified Mediterranean-ketogenic diet; SMC, subjective memory complaints.

^aSignificant effect of diet, $p < 0.05$.

^bMCI different than SMC, $p < 0.05$.

^cMCI different than SMC, $p < 0.10$.