2019

The role of cholecystokinin, fasting glucose, and ketone bodies on the pathogenesis of Alzheimer's disease

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The role of cholecystokinin, fasting glucose, and ketone bodies on the pathogenesis of Alzheimer's disease

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Nutritional Sciences

Program of Study Committee:
Auriel Willette, Major Professor
James Hollis
Jennifer Margrett

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2019

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NOMENCLATURE

AD	Alzheimer's disease
CCK	Cholecystokinin
MCI	Mild Cognitive Impairment
CN	Cognitive Normal
DTI	Diffusion Tensor Imaging
CSF	Cerebral Spinal Fluid

ACKNOWLEDGMENTS

I would like to thank my major professor and mentor, Dr. Auriel Willette, for his wisdom and patience as he guided me through many new neuroscience and statistical programs, as well as pushing me to my full potential. I would like to thank my committee members, Dr. James Hollis and Dr. Jennifer Margrett for their guidance and support throughout the course of this research, as well as their expertise and outlook to help me understand and interpret results.

ABSTRACT

Hypometabolism of glucose in the brain is associated with the neurodegenerative disorder, Alzheimer's disease (AD), warranting further study of the relationship between metabolic function and structural integrity of the brain. While demyelination is observed with AD, less is known about the impact of glucose levels in combination with genetic risk factors on myelination. Ketone bodies are the brain's alternative fuel source during periods of low fuel such as in Alzheimer's disease (AD). Demyelination of the brain may lead to accumulations of amyloid beta plaques, associated with the disease (Bartzokis et al., 2007). Individuals with a family history (FH) or the APOE4 allele have been seen to have a predisposition for developing AD. Therefore, in efforts to achieve neuroprotection for people at-risk for developing the disease or during the prognosis of the disease, serum ketone bodies and glucose levels were studied in relation to white matter integrity in the brain from the Alzheimer's Disease Neuroimaging Initiative. Cholecystokinin (CCK) was also examined in the cerebral spinal fluid. CCK is a satiety hormone that is highly expressed in brain regions like the hippocampus. CCK is integral for maintaining or enhancing memory, and thus may be a useful marker of cognitive and neural integrity in participants with normal cognition, mild cognitive impairment (MCI), and Alzheimer's disease (AD). Interactions were also tested with genetic risk factors for AD, family history (FH) and apolipoprotein E ε4 (APOE4) status. Participants with FH or APOE4 allele showed increased myelin integrity as glucose levels increased when examining DTI fractional anisotropy in the fornix. Additionally, participants diagnosed with AD showed more demyelination compared to those who had mild cognitive impairment or who were cognitively normal as measured by DTI radial diffusivity in the fornix. Overall, the

ketone levels predicted improved myelin integrity. The individuals without genetic risk factors showed improved myelin integrity with increases in ketone bodies. Participants with MCI or AD displayed more demyelination with increases in ketones. However, over a period of 2 years, the increases in demyelination for APOE4 carriers, FH positive group, and MCI progressors all show decreased or no association with demyelination. Briefly, higher CCK was related to a decreased likelihood of having MCI or AD, better global and memory scores, and more GM volume primarily spanning parahippocampal gyrus. CSF CCK was also strongly related to higher CSF total tau and p-tau181. Tau levels partially mediated CCK and cognition associations. Participants with FH of AD or the APOE4 allele may show compensatory mechanisms by increasing glucose uptake to protect against degradation caused by the disease. When the disease progresses to full AD diagnosis, the damage may overcome the benefit from hypermetabolism of glucose and thus need to increase the metabolism of ketone bodies. CCK levels also reflect compensatory protection as AD pathology progresses. CCK is released as a response to the ingestion of dietary fats so an increase in ketone body may increase these effects of CCK. The white matter integrity of at-risk groups before symptoms of the disease appear should be studied as these individuals may be predisposed to less myelin integrity and as myelin in being broken down, ketones increase as a product of the demyelination. Over time the brain becomes more efficient at metabolizing ketones and with a greater pool, the demyelination slows.

CHAPTER 1. GENERAL INTRODUCTION

Brain utilization and metabolism is studied for many neurological diseases because the human brain weighs 2% of the body's total weight, but consumes 25% of the body's energy. Alzheimer's disease is the 6th leading cause of death in the United States, and out of the top 10 leading causes of death in the U.S., AD is the only one that we cannot treat, prevent, or even slow down. Alzheimer's disease is a degenerative brain disease seen with a decline in memory, executive function, and atrophy of gray matter in the brain. There are many genetic factors of the disease, but the cause is unknown. This research looks to see how nutrition and brain utilization is involved in the pathogenesis of Alzheimer's disease.

Purpose of Study

The purpose of this research aims to examine nutritional biomarkers that may predict the onset and prognosis of Alzheimer's disease. Studying a satiety hormone, released with the ingestion of fats and proteins, CCK, as well as studying glucose and ketone bodies in the serum in a large cohort of elderly participants highlights the important of diet and fuel sources in the brain.

CHAPTER 2. CHOLECYSTOKININ AND ALZHEIMER'S DISEASE: A BIOMARKER OF METABOLIC FUNCTION, NEURAL INTEGRITY, AND COGNITIVE PERFORMANCE

Modified from a manuscript published in Neurobiology of Aging

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Introduction

Cholecystokinin (CCK) is a 33-amino acid satiety hormone secreted in the small intestines during digestion that binds to CCK-A receptors (CCKAR). CCK is secreted to allow the uptake of nutrients, most specifically fat uptake and metabolism of fatty acids (Pietrowsky et al. 1994). CCK is stimulated by fat and protein ingestion to signal the pancreas to release pancreatic enzymes into the duodenum, as well as to signal the secretion of bile salts from the gall bladder into the duodenum. A main function of CCK is to slow gastric emptying to allow time for proper digestion. Persons with AD have shown changes in their eating behavior, including both increased and decreased food intake, suggesting instability in weight regulation. People with AD also manifest changes in food variety preferences and their eating patterns (Morris, Hope and Fairburn 1989). Malnutrition is common and weight loss is seen in 40% of persons with AD (Wallace et al. 1995). Dietary changes, due to food preferences of persons with AD, tend to contain a higher proportion of carbohydrates and a reduced intake of proteins (Greenwood et al. 2005). Hyperphagia is also found in a third of all individuals with AD (Morris et al. 1989). The reason for hyperphagia is unknown, but there may be a link to decreased satiety hormones or decreased sensitivity to

these hormones (Adebakin et al. 2012). In concert, a decline in body mass index (BMI) is associated with an increased risk of developing AD (Buchman et al. 2005). This change in body mass could be due to muscle wasting (i.e., sarcopenia) or a result of decreased food uptake.

Interestingly, CCK receptors are found not only in the gut as CCK-A receptors, but also in the brain as CCK-B receptors (Pietrowsky et al. 1994). **Figure 1.1** illustrated the function of CCK peripherally as well as centrally. CCK is also the most abundant neuropeptide in the brain and selectively binds to CCK-B receptors, or CCKBR (Pietrowsky et al. 1994). Indeed, CCK-B receptors are highly expressed in the hippocampus (Dockray et al. 1978) (Innis et al. 1979, Zarbin et al. 1983), a brain region integral in memory formation that is adversely affected early in Alzheimer's disease, or AD (Braak, Braak and Bohl 1993). Hippocampal injection or cell culturing with CCK agonists or antagonists respectively improves or impairs long-term potentiation and memory in rodents by acting on CCKBR (Sebret et al. 1999, Wen et al. 2014). Memory impairment in aged rodents also corresponds to less CCK expression (Croll et al. 1999). Further, cerebral cortex has the highest concentration and CCK-specific binding in the brain (Saito et al. 1980), where endogenous CCK activity may produce long-term potentiation in medial prefrontal cortex akin to hippocampus (Liu and Kato 1996). Thus, it is important to observe if metabolic biomarkers related to body weight and dietary regulation dynamics are associated with neural, cognitive, and other behavioral outcomes relevant to AD.

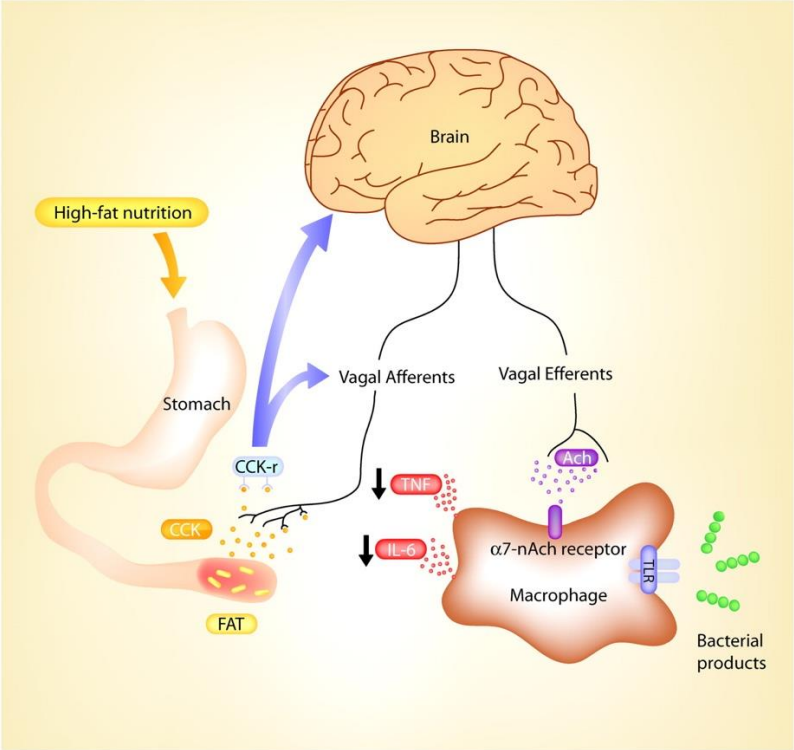


Figure 2.1 Bi-directional CCK pathways in the periphery and brain. This diagram is displayed with permission from the original publisher.

Despite a rich animal literature showing consistent enhancement or amelioration of memory by CCK-B activation, its role is virtually unknown in AD. AD-related changes in brain include progressive structural atrophy and decreased functional integrity (Klöppel et al. 2018), leading to forgetfulness and progressively worsening memory loss (Azuma et al. 2018). These changes occur in the presence of amyloid beta (Aβ) plaques and hyperphosphorylated tau (p-tau) tangles, as observed in brain tissue at autopsy or antemortem through cerebrospinal fluid (CSF). While CCK-B receptor binding does not differ in cognitively normal vs. AD persons (Löfberg et al. 1996), regional differences in post-mortem CCK concentration suggest an AD-like pattern of decreased expression (Mazurek and Beal 1991).

Thus, we examined if levels of CSF CCK were associated with onset and severity across the AD spectrum, and determined if CCK was related to AD-like changes in cognition, neuroimaging, and classic AD biomarkers like Aβ and tau.

Materials and Methods

Participants

Data from late middle-aged to aged adults, ages 55-90, were obtained from the ADNI database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org. Written informed consent was obtained from all ADNI participants at their respective ADNI sites. The ADNI protocol was approved by site-specific institutional review boards. All analyses used in this report only included baseline data, however measures were taken periodically for the database spanning a time of 90 months. Baseline CSF data for CCK was available for 287 subjects: 86 CN, 135 MCI, and 66 AD.

Participants with MCI had the following diagnostic criteria: 1) memory complaint identified by the participant or their study partner; 2) abnormal memory as assessed by the Logical Memory II subscale from the Wechsler Memory Scale- Revised, with varying criteria based on years of education; 3) Mini-Mental State Exam (MMSE) score between 24 and 30; 4) Clinical dementia rating of 0.5; 5) Deficits not severe enough for the participant to be diagnosed with Alzheimer's disease by the physician on site at screening. Participants with AD met similar criteria. However, they were required to have an MMSE score between

20 and 26, a clinical dementia rating of 0.5 or 1.0, and NINCDS/ADRDA criteria for probable AD.

Mass Spectrometry and Fasting Glucose

Data was downloaded from the Biomarkers Consortium CSF Proteomics MRM dataset. As described previously (Spellman et al. 2015), the ADNI Biomarkers Consortium Project investigated the extent to which selected peptides, measured with mass spectrometry, could discriminate among disease states. Briefly, Multiple Reaction Monitoring-MS (MRMMS) was used for targeted quantitation of 567 peptides representing 221 proteins in a single run (Caprion Proteome Inc., Montreal, QC, Canada). Analyses for this report focused on CCK levels, which were assayed in the CSF proteomics panel, for which the peptide AHLGALLAR was chosen because it performed better in most analyses (data not shown).

Amyloid and Tau CSF Biomarkers

CSF sample collection, processing, and quality control of p-tau-181, total tau, and $A\beta_{1-42}$ are described in the ADNI1 protocol manual (http://adni.loni.usc.edu/) and (Shaw et al. 2011).

Apolipoprotein E ε4 genotype

The ADNI Biomarker Core at the University of Pennsylvania conducted APOE genotyping. We characterized participants as being "non-APOE4" (i.e., zero APOE ε4 alleles) or "APOE4" (i.e., one to two APOE ε4 alleles).

Neuropsychological Assessment

ADNI utilizes an extensive battery of assessments to examine cognitive functioning with particular emphasis on domains relevant to AD. A full description is available at http://www.adni-info.org/Scientists/CognitiveTesting.aspx. All subjects underwent clinical and neuropsychological assessment at the time of scan acquisition. Neuropsychological assessments included: The Clinical Dementia Rating sum of boxes (CDR-sob), Mini-Mental

Status Exam (MMSE), Auditory Verbal Learning Test (RAVLT), and AD Assessment Schedule - Cognition (ADAS-Cog). A composite memory score encompassing the RAVLT, ADAS-Cog, MMSE, and Logical Memory assessments was also utilized (Crane et al. 2012). Additionally, a composite executive function score comprising Category Fluency—animals, Category Fluency—vegetables, Trails A and B, Digit span backwards, WAIS-R Digit Symbol Substitution, Number Cancellation and 5 Clock Drawing items was used (Gibbons et al. 2012). These composite scores were used in formal analyses to represent global memory and executive function among subjects.

Magnetic Resonance Imaging (MRI) Acquisition and Pre-Processing

T1-weighted MRI scans were acquired within 10-14 days of the screening visit following a back-to-back 3D magnetization prepared rapid gradient echo (MP-RAGE) scanning protocol described elsewhere (Jagust et al. 2010). Images were pre-processed using techniques previously described (Willette et al. 2013). Briefly, the SPM12 "New Segmentation" tool was used to extract modulated gray matter (GM) volume maps. Maps were smoothed with a 8mm Gaussian kernel and then used for voxel-wise analyses.

^{18}F-fluorodeoxyglucose Positron Emission Tomography (FDG-PET)

FDG-PET acquisition and preprocessing details have been described previously (Jagust et al. 2010). Briefly, 185 MBq of [18-153-F]-FDG was injected intravenously. After 30 minutes, six 5-minute frames were acquired. Frames of each baseline image series were coregistered to the first frame and combined into dynamic image sets. Each set was averaged, reoriented to a standard 160 x 160 x 96 voxel spatial matrix of resliced 1.5 mm^3 voxels, normalized for intensity, and smoothed with an 8 mm FWHM kernel. In order to derive the standardized uptake value ratio (SUVR), pixel intensity was normalized according to the

pons since it demonstrates preserved glucose metabolism in AD (Dowling et al. 2010). Normalization to the pons removed inter-individual tracer metabolism variability. The Montreal Neurological Institute (MNI) template space was used to spatially normalize images using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). A subset of subjects underwent FDG-PET scans and analyses included in this report.

Statistical Analysis

All analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY) or SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Binomial logistic regression was used to assess the odds ratio of a given participant being diagnosed as AD versus MCI or CN reference group. Linear mixed regression tested the main effect of CSF CCK on neuropsychological performance, modulated GM maps, FDG maps, and CSF biomarkers including $A\beta_{1\text{-}42}$, total tau, and p-tau-181. Covariates included age at baseline and sex in all models. Years of education was also covaried when analyzing memory and cognitive performance. For voxel-wise analysis, 2nd-level linear mixed models tested the main effect of CCK on regional GM volume and FDG, controlling for age, sex, education, and baseline diagnosis. Based on the literature, contrasts tested if higher CCK was related to more regional GM or FDG. Statistical thresholds were set at $p < .005$ (uncorrected) and $p < .05$ (corrected) for voxels and clusters respectively. Results were considered significant at the cluster level. As described previously (Willette et al. 2015a), in order to reduce Type 1 error, we utilized a GM threshold of 0.2 to ensure that voxels with <20% likelihood of being GM were not analyzed. For GM, Monte Carlo simulations in ClusterSim (http://afni.nimh.nih.gov/afni/doc/manual/3dClustSim) were used to estimate that 462 contiguous voxels were needed for such a cluster to occur at $p < 0.05$ family-wise error corrected. For FDG voxel-wise analyses, Monte Carlo simulations in ClusterSim were used

to estimate that 224 contiguous voxels were needed for such a cluster to occur at $p < 0.05$ family-wise error corrected.

Results

Data Summary

Clinical, demographic, and CSF data for subjects with CSF CCK are presented in **Table 1**. Years of education, percent of APOE4 carriers, and age were not significantly different between participants diagnosed as CN, MCI or AD. As anticipated for this ADNI sub-population, cognitive function, observed utilizing global cognitive tests, was significantly different across CN, MCI, and AD groups (all $p < 0.05$). CSF CCK levels were significantly lower for persons with AD ($p<.001$) versus participants with MCI or AD.

Table 2.1 Demographic Data for Subjects with CSF CCK

	CN (N=86)	MCI (N=135)	AD (N=66)
Age (years)	75.70 ± 5.54	74.69 ± 7.35	74.98 ± 7.57
Education (years)	15.64 ± 2.97	16.00 ± 2.96	15.11 ± 2.96
Sex (% Female)	48.8%	32.59%	43.9%
APOE Status (% E4 carriers)	24.4%	52.6%	71.2%
Cholecystokinin (ng/mL)	13.48 ± 0.56	13.47 ± 0.53	13.23 ± 0.56
CSF Total Tau (pg/mL)	70.33 ± 27.64	102.99 ± 51.68	126.17 ± 60.69
Ptau-181 (pg/mL)	24.12 ± 11.97	35.25 ± 15.13	41.95 ± 20.60
Abeta 1-42 (pg/mL)	208.20 ± 56.05	161.21 ± 52.72	141.12 ± 37.39
CDR-sob	0.02 ± 0.11	1.56 ± 0.88	4.34 ± 1.56
MMSE	29.05 ± 1.02	26.91 ± 1.74	23.52 ± 1.85
ADAS-COG11	6.05 ± 2.90	11.72 ± 4.33	18.88 ± 6.71
Memory Factor (Z-score)	0.98 ± 0.50	-0.15 ± 0.57	-0.90 ± 0.55

Values are mean ± SD. Chi-square analyses were conducted to examine differences between gender and APOE4 status. The ADNI memory factor values are Z-scored with mean 0 and a standard deviation of 1, based on 810 ADNI subjects with baseline memory data (Crane et al.

2012). AD-Alzheimer's disease; AD Assessment Schedule - Cognition (ADAS-Cog); Clinical Dementia Rating sum of boxes (CDR-sob); CN-cognitively normal; MCI-mild cognitive impairment; Mini-Mental Status Exam (MMSE).

Clinical Characteristics and AD Risk

Logistic regression was used to examine if higher CSF CCK expression predicted a decreased likelihood of being MCI or AD. The reference group was CN. The likelihood ratio statistic [X^2=27.563, p<.001] indicated that higher CSF CCK levels predicted a lower Odds Ratio for being MCI or AD [Wald=13.437, β=-1.039, Exp(B)=0.354, p < 0.001]. These results suggest that a per ng/mL increase in CSF CCK corresponded to a roughly 65% less likelihood of being diagnosed with AD versus CN or MCI. Higher levels of CSF CCK were not related to increased risk when comparing CN vs. MCI, CN vs. AD, or MCI vs. AD individually.

AD CSF Biomarkers

To examine the relationship between CSF CCK and AD CSF biomarkers $A\beta_{1-42}$, ptau-181, and total tau regression model analyses were performed with age, sex, BMI, baseline diagnosis, APOE4 status as covariates. A significant association with $A\beta_{1-42}$ was not observed. However, as seen in **Figure 1.2**, higher levels of CSF CCK were significantly associated with higher levels of CSF total tau (β±SE = 37.857±4.799, F=62.237, p< 0.001) and CSF ptau-181 (β±SE = 10.046±1.630, F=37.992, p< 0.001).

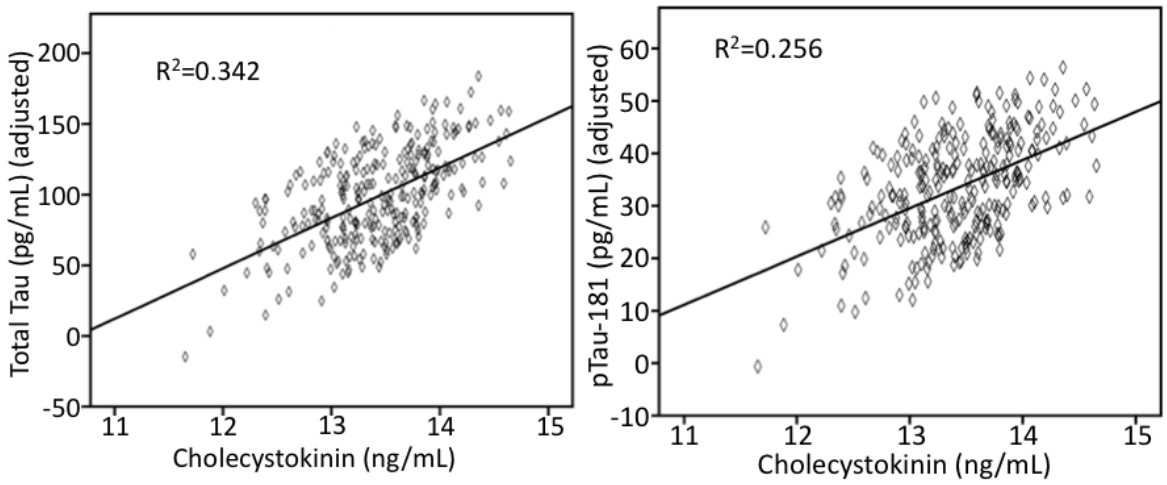


Figure 2.2 Significant Associations between CSF CCK and CSF AD Biomarkers

Global Cognition, Memory, and Executive Function

As illustrated in **Figure 1.3**, regression models showed that higher CSF CCK was related to better global cognition scores for CDR-sob, ADAS-cog11, and MMSE. Similarly, higher CCK was associated with better memory factor and executive function factor ($\beta \pm SE = 0.156 \pm 0.077$, $p < .05$) scores.

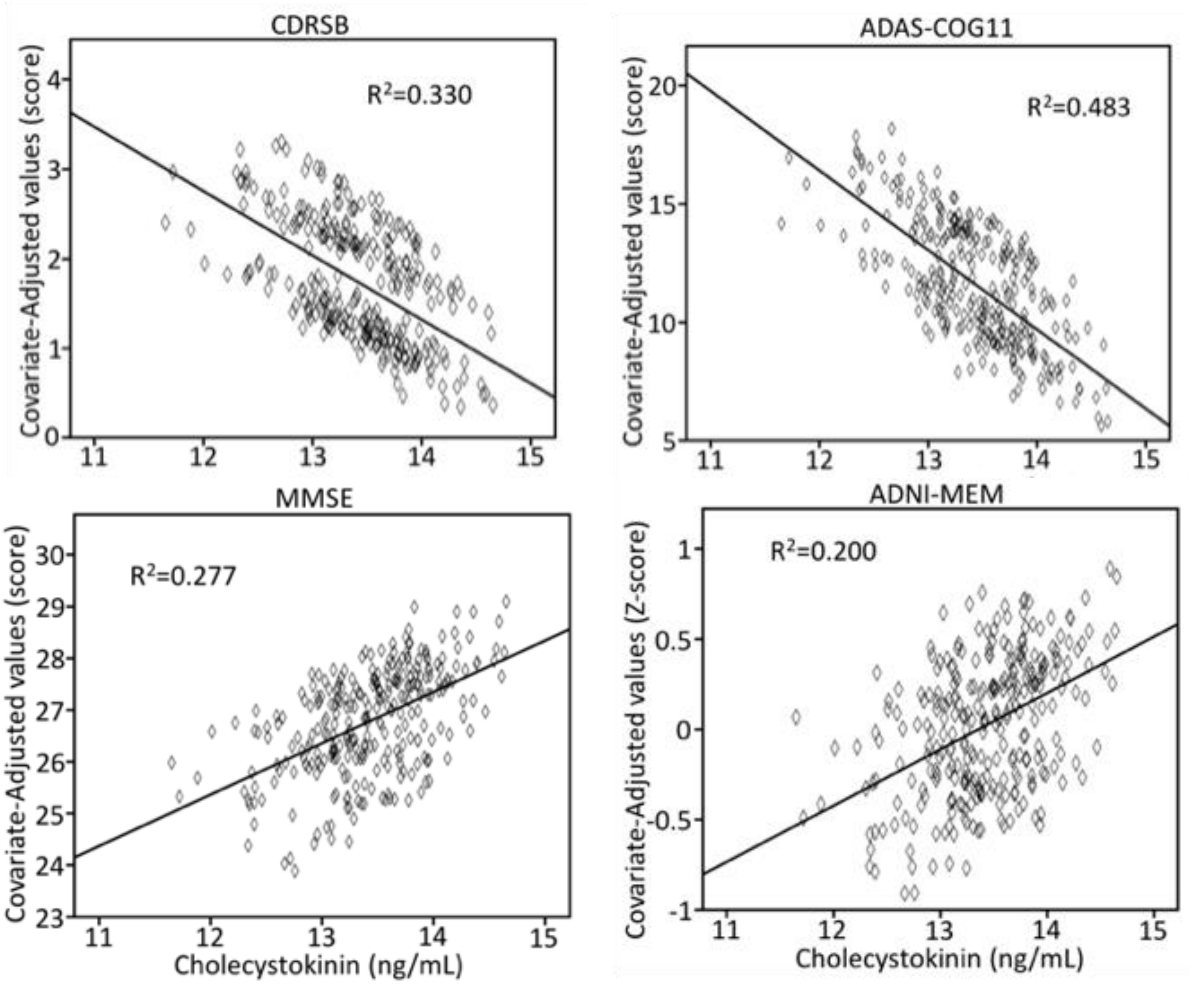


Figure 2.3 Cerebral Spinal Fluid Cholecystokinin and Cognitive Outcomes

Preacher-Hayes Mediation of CCK and Cognition Outcomes

We also explored if CSF AD biomarkers modified associations between CCK and cognitive outcomes. For CDR-sob, no CSF markers mediated associations with CCK.

For ADAS-cog11 and CCK (direct effect β±SE= -3.110±0.585, p<.001), higher total tau acted as a partial mediator, reducing the influence of CCK by 24% (indirect effect β±SE=0.735±0.063, p<.05). For MMSE and CCK (direct effect β±SE=0.631±0.190, p<.001),

ptau-181 acted as a partial mediator, reducing the influence of CCK by 26% (indirect effect β±SE= -0.164±0.095, p<.05).

For the memory factor and CCK, both total tau and p-tau181 acted as partial mediators. Specifically, as indicated in **Figure 1.4,** total tau reduced the influence of CCK on the memory factor by nearly half. In a separate model, p-tau181 reduced the influence of CCK on the memory factor (direct effect β±SE=0.186±0.064) by 36% (indirect effect β±SE=-0.067±.0263). $A\beta_{1\text{-}42}$ was not a significant mediator for any cognitive measure.

Finally, for the executive function factor and CCK, both total tau and p-tau181 acted as partial mediators. Specifically, total tau reduced the influence of CCK on the memory factor (direct effect β±SE=0.355±0.087, p<.001) by 50% (indirect effect β±SE= -0.178±0.041, p<.001). In a separate model, p-tau181 reduced the influence of CCK on the memory factor (direct effect β±SE=0.315±0.082, p<.001) by 47% (indirect effect β±SE= -0.148±0.036, p<.001).

Regional Gray Matter Volume

To determine the relationship between CSF CCK and regional gray matter volume, a voxel-wise analysis was performed using SPM 12 among a subset of 303 participants. Higher CSF CCK was significantly associated with greater GM volume in a large cluster of voxels (k=11,962) primarily spanning cingulate cortex and parahippocampal gyrus, as well as thalamus, superior temporal sulcus, and medial prefrontal cortex (**Figure 1.5** and **Supplementary Table 1.1**).

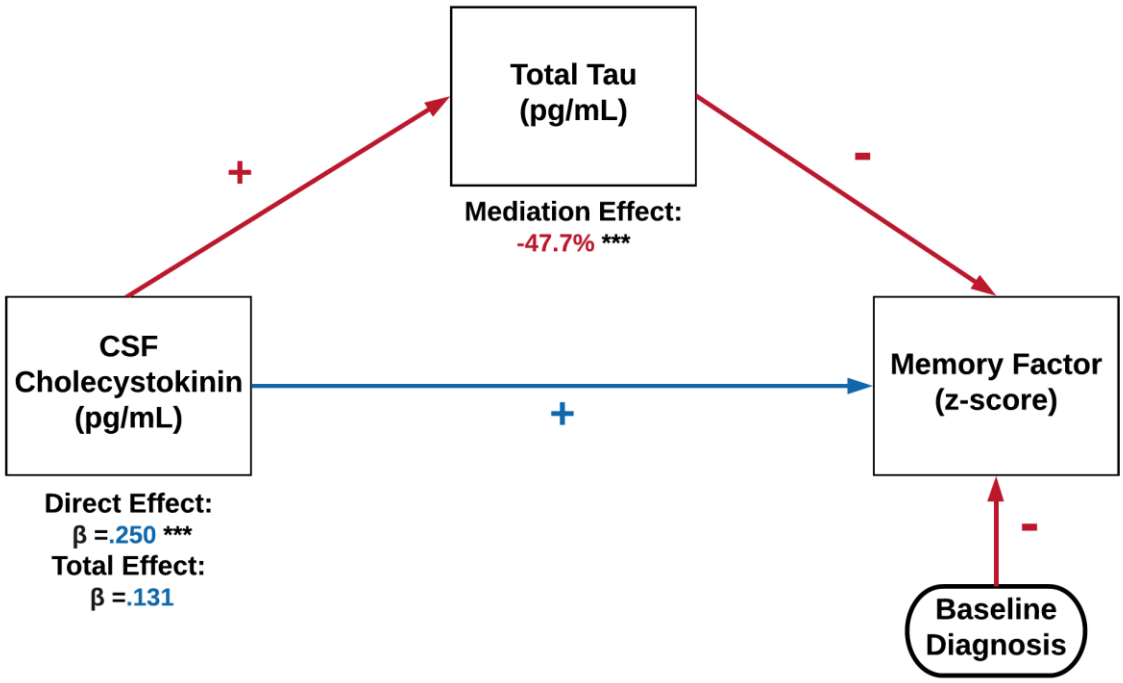


Figure 2.4 Preacher-Hayes mediation of CSF CCK, total tau, and a composite memory score at baseline.

Regional ^{18}F-Fluorodeoxyglucose Positron Emission Tomography

Among 138 participants with FDG data, higher CSF CCK was not significantly associated with an increase in ^{18}F-fluorodeoxyglucose Positron Emission Tomography (FDG PET) glucose uptake.

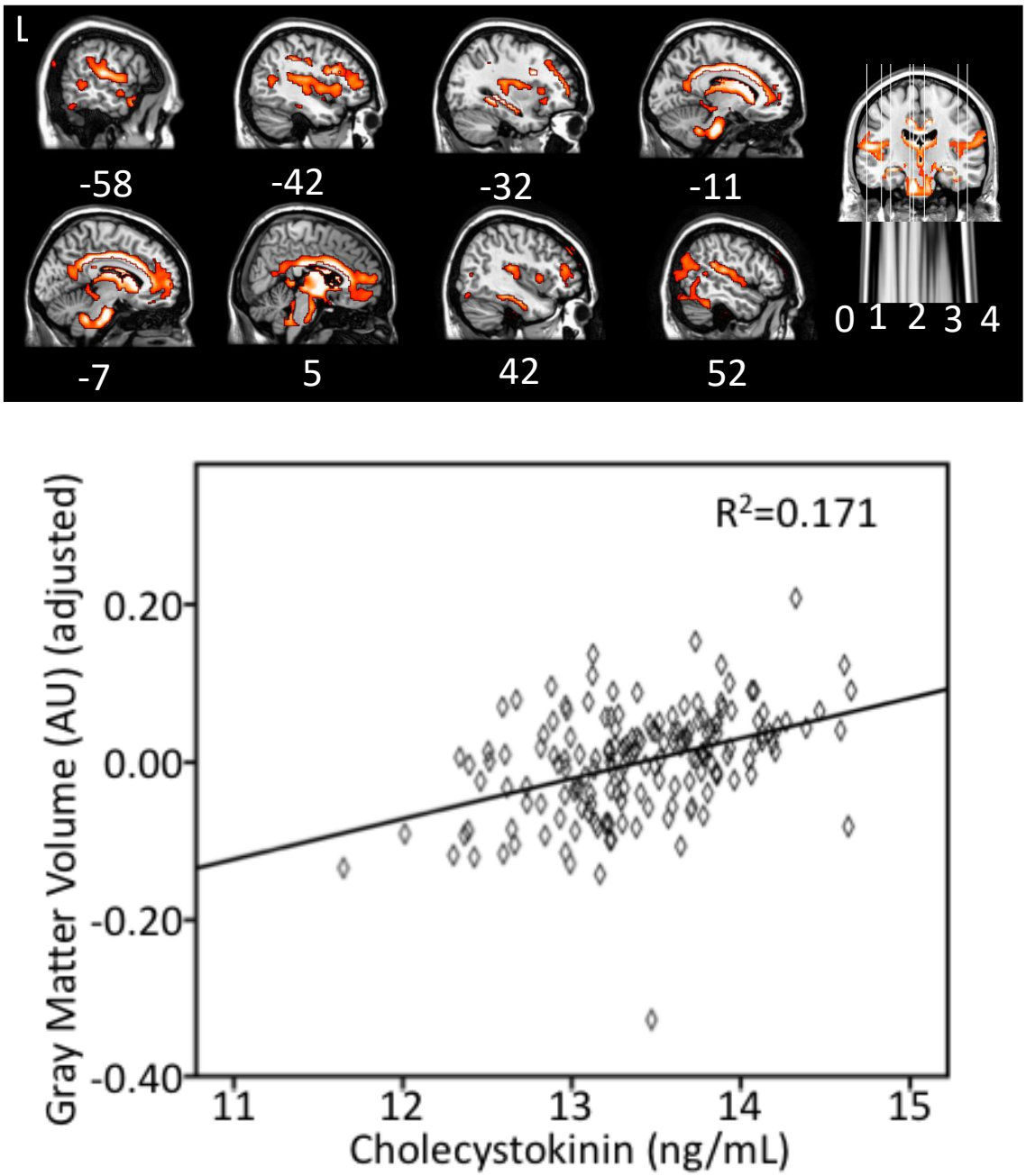


Figure 2.5 Higher CSF CCK and More Regional Gray Matter Volume

Discussion

In this study, we hypothesized that CCK may serve as a useful metabolic biomarker for predicting AD outcomes, due to previous research looking at CCK-B and its role in maintaining or enhancing memory (Liu and Kato 1996) (Sebret et al. 1999) (Wen et al. 2014). We found that individuals with AD had modestly lower CCK than CN or MCI. Post-mortem tissue analysis has been mixed, with some groups noting no change (Perry et al. 1981) (Ferrier et al. 1983) or decreased expression (Mazurek and Beal 1991). Per ng/mL increase in CCK, there was a roughly 65% decrease in likelihood of being diagnosed with MCI or AD. Similarly, Higher CCK was associated with better performance in memory, executive function, and global cognitive tests, which via mediation was partly mitigated by levels of CSF tau species but not $A\beta_{1-42}$. CCK has consistently been implicated as a protective or enhancing factor for memory formation. For example, in a rodent study that included CCK knockout mice (CCK-KO), the mice without CCK performed worse on the Morris water-maze test compared to wild type mice while evincing similar locomotion and food intake, indicating that CCK was a factor in learning and memory (Lo et al. 2008). CCK administration is directly able to induce or curb long-term potentiation (Sebret et al. 1999) (Wen et al. 2014), which is a well-established molecular process thought to underlie learning and memory.

We further observed that higher CSF CCK levels were also correlated with more regional GM volume in areas such as parahippocampal gyrus, hippocampus, posterior cingulate cortex, and superior and medial prefrontal gyri. The parahippocampal gyrus is part of the limbic system, which plays a crucial role in memory and is affected in AD with atrophy in GM (Köhler et al. 1998). Atrophy in the hippocampus and posterior cingulate cortex strongly track disease progression and underlie memory decline (Pengas et al. 2010).

Medial prefrontal cortex is not only integral for memory retrieval, but also executive function as well (West 1996). These results suggest that as CCK levels increase, cognitive functions such as memory may improve due to the protection of GM in memory-intensive regions of the brain.

CCK may exercise these effects by acting against the opiate system. Wen and colleagues found that CCK binding to CCK-B receptors contributed to opiate dependence, and that morphine withdrawal symptoms worsened after administration of a CCK receptor agonist (2014). The authors concluded that CCK works anti-parallel to the opiate system and accelerated opiate-dependence by inhibiting GABA receptors. Opiates have been shown to cause pathological memory formations, forming plasticity in neurons that have been shown to create addictions (Kauer and Malenka 2007). Thus, CCK works against the opiate system in attempts to regain functionality of brain regions especially in regards to memory formation and retrieval.

In our study, we found no correlation between CSF CCK and Aβ, however, strong relationships between higher CCK and higher tau levels were observed. While no existing work ties CCK to amyloid or tau to our knowledge, other studies have tested the relationship between AD markers and other satiety hormones. In a study conducted by Guo et al. (2016), Aβ was added to PC12 cells to reaffirm the fact that Aβ causes apoptosis due to cytotoxicity. However, when leptin, a satiety hormone released from adipose tissue, was added to the PC12 cells along with the Aβ, significantly less cell death was observed. This protective phenomenon of leptin may be due to increased activation of JAK2, used in the regulation of the phosphorylation of the tau protein. When JAK2 was inhibited in the presence of Aβ, there was an increase in phosphorylated tau regardless of whether leptin was present.

Similarly, with leptin administration, there was more JAK2 activation which caused decreased GSK-3 activation and less damage caused by the presence of Aβ (Guo et al. 2016). GSK-3 is found in the brains of many persons with AD (Asuni et al. 2006) and is involved with the hyper phosphorylation of the tau protein. Thus, CCK may serve as a protectant against AD by suppressing expression of GSK-3 and increasing JAK2 activation. With increased CCK levels in individuals with more severe pathology, it may be possible that CCK is, acting in a similar way to leptin, trying to protect the brain from neuronal cell death. At a certain point, GSK-3 levels may increase such that the compensatory function of CCK is overridden, leading to an increase in accumulation and phosphorylation of tau. Indeed, total tau and ptau-181 levels partly mediate CCK and cognitive scores and strongly decrease such associations.

Limitations of this study should be addressed. Using data from ADNI, we were unable to obtain dietary data, or other measures of body composition besides BMI. We were also unable to track changes in CCK over time as this was only measured at baseline. In conclusion, higher levels of CCK predicted better cognitive outcomes and more gray matter in memory-specific regions. Higher CCK was also related to more CSF total tau and ptau-181. CCK may act as a protectant against AD by activated JAK2, and thus reducing the GSK-3 activation. We propose that as AD progression occurs, CCK levels increase in efforts to protect against further damage potentially induced by tau. Additional research would need to be done to further examine the relationship between CCK and tau over time. CCK levels may be a useful marker of cognitive and volumetric loss due in part to increased accumulation of tau, which may be useful for AD prognosis or a potential target to maintain memory in the face of AD pathology.

Supplementary Table 2.1 Associations between higher CCK and more gray matter

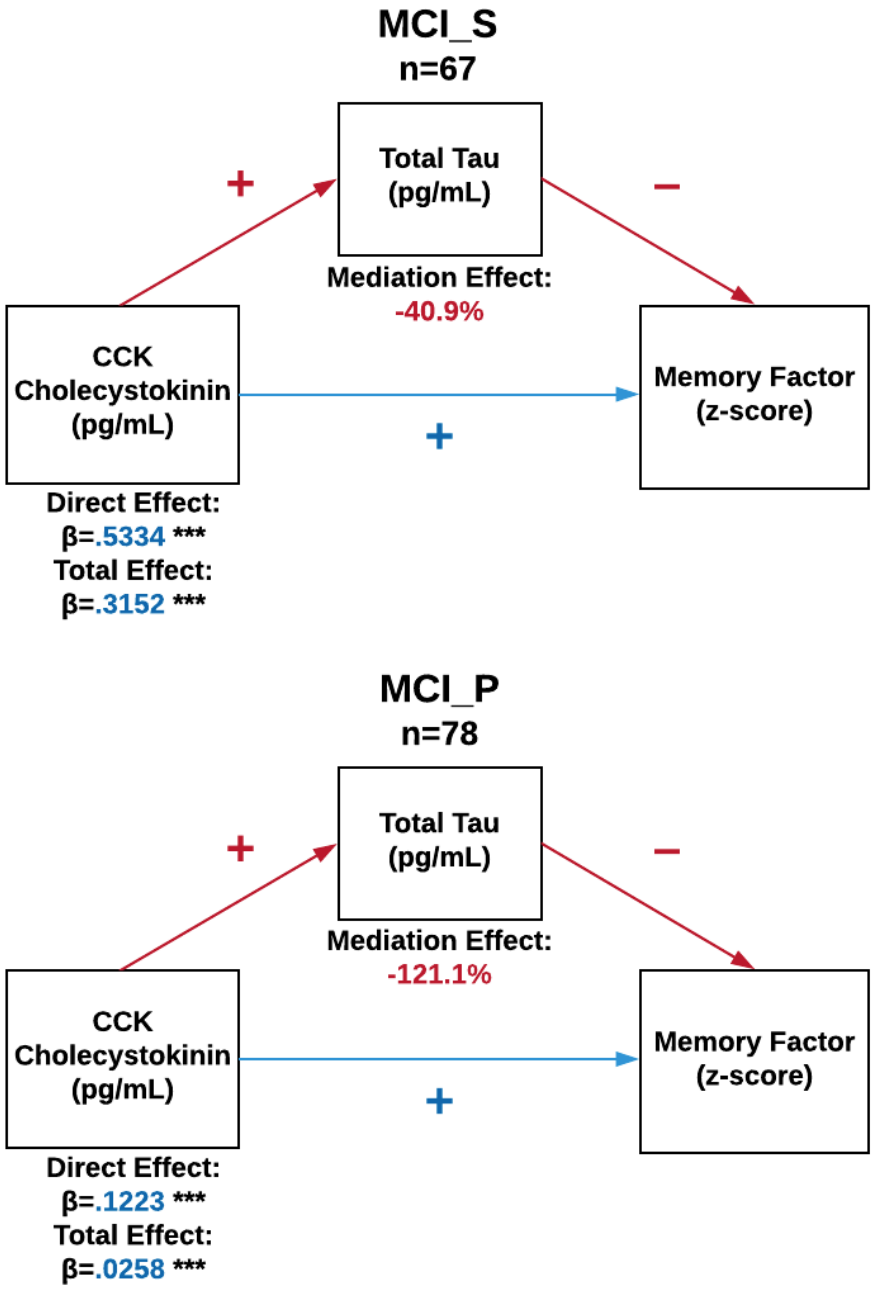
Location	T value	X, Y, Z	Cluster size (voxels)
Mid Cingulum L	6.17	-9, -28, 38	11,962
Mid Cingulum R	6.01	10, -27, 38	
Mid Cingulum L	5.95	"9, "'4, 38	
Parahippocampal Gyrus L	5.79	-33, -39, -3	
Pars Traingularis L	5.23	-36, 15, 26	9,501
Superior Temporal Gyrus L	5.12	-54, -20,12	
Middle Frontal Gyrus L	4.53	-30, 45, 14	
Inferior Parietal Gyrus L	4.67	"46, "34, 38	463
Postcentral Gyrus L	3.96	"45, "16, 33	
Middle Frontal Gyrus R	4.65	30, 42, 21	2022
Superior Frontal Gyrus R	4.04	20, 44, 32	
Middle Frontal Gyrus R	3.98	30, 33, 30	
Superior Temporal Gyrus R	4.24	52, -24, 15	2873
Superior Temporal Gyrus R	4.15	52, -36, 21	
Rolandic Operculum R	3.88	63, -18, 18	
Middle Temporal Gyrus R	3.68	56, -60, 18	1742
Middle Occipital Gyrus R	3.39	51, -72, 26	
Superior Temporal Gyrus R	3.31	60, -56, 24	
Inferior Temporal Gyrus R	3.55	50, "45, "27	1766
Middle Temporal Gyrus R	3.43	62, "52, "6	
Inferior Temporal Gyrus R	3.35	57, "54, "20	
Middle Temporal Gyrus L	3.54	-42, -62, 9	1003
Middle Temporal Gyrus L	3.53	-52, -48, 2	
Middle Temporal Gyrus L	3.29	-51, -72, 22	
Inferior Temportal Gyrus L	3.45	-52, -56, -22	475
Inferior Occipital Gyrus L	2.80	-50, -64, -18	

Supplementary Table 2.2 Associations between higher CCK and less gray matter

Location	T value	X, Y, Z	Cluster size (voxels)
Paracentral Lobule L	4.82	"10, "12, 76	3944
Superior Motor Area R	3.68	4, "9, 72	
Superior Frontal Gyrus L	3.68	"14, 2, 75	

Supplementary Table 2.3 Associations between higher CCK and less glucose uptake

Location	T value	X, Y, Z	Cluster size (voxels)
Mid Cingulate Gyrus R	5.69	6,2, 44	14,295
Superior Motor Area R	5.03	6, "20, 52	
Paracentral Lobule L	4.67	"14,"14, 68	
Cerebellum R	3.86	14, "54, "16	1337
Cerebellum L	3.78	"8, "64, "24	
Cerebellum R	2.98	10, "40, "14	
Rolandic Operculum L	3.77	"40, "22, 16	362
Putamen L	3.18	"24, "6, 12	
Putamen L	3.13	"30, "2, 6	
Putamen R	3.18	28, 4, 12	274
Insula R	3.08	34, "2, 10	
Putamen R	2.88	28, "10, 12	



Supplementary Figure 2.1 Preacher Hayes Mediation Effect with stable_MCI and MCI_progressors

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CHAPTER 3. FASTING GLUCOMSE, GENETIC RISK FOR ALZHEIMER'S DISEASE AND NEURAL MYELINATION PATTERNS

Modified from a manuscript to be submitted to Diabetes

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Introduction

Normal aging and particularly Alzheimer's disease (AD) are characterized by neural white matter demyelination and axonal degradation, which are strongly related to global decline as well as memory and executive function (Charlton et al. 2006, Bozoki et al. 2012, Charlton et al. 2010, Huang and Auchus 2007, Vernooij et al. 2009). Diffusion tensor imaging (DTI) can determine the myelin, axonal, and overall integrity of white matter tracts, depending on the proportion of perpendicular versus parallel or mean diffusion along tract fibers (Denis et al. 2001, Soares et al. 2013). Loss of tract integrity may be a very early marker of AD (Sperling et al. 2011) that precedes gray matter atrophy (Shim et al. 2017, Bailly et al. 2015), as well as progressive glucose hypometabolism such as with cingulum bundle and posterior cingulate cortex glucose uptake (Bozoki et al. 2012). Indeed, oligodendrocytes in at least rodents normally rely more on glycolysis-derived lactate for energy than aerobic respiration of glucose (Funfschilling et al. 2012, Tekkok et al. 2005, Morland et al. 2007). Consequently, mature oligodendrocytes are robust to low glucose levels in mutant mice with deficient mitochondrial respiration, due to increased glycolytic lactate production (Funfschilling et al. 2012).

Conversely, hyperglycemia caused by metabolic dysfunction, such as pre- and type 2 diabetes, can degrade white matter through increased neuroinflammation and oxidative stress (Marseglia et al. 2018). Pre-diabetes and type 2 diabetes in middle-aged and non-impaired aged adults is consistently related to less tract integrity in frontal, parietal, and temporal white matter(Hoogenboom et al. 2014, Reijmer et al. 2013b, Hsu et al. 2012, Yau et al. 2009)[,(2014),(2017)] (van Bloemendaal et al. 2016, Sun et al. 2018, Tan et al. 2019). In turn, these associations predict deficits in executive function, performance speed, and to a lesser extent acuity in memory(Reijmer et al. 2013a, Zhang et al. 2014, Xiong et al. 2016). Tracts have typically included superior longitudinal fasciculus, uncinate fasciculus, cingulum bundle proximal to cingulate gyrus or hippocampus, and corpus callosum.

It is of interest, then, that dysmetabolism contributes to AD risk, and that its pattern of white matter damage is similar to that of Mild Cognitive Impairment (MCI) due to AD and early AD (citation). Pre- and type 2 diabetes increase AD risk by roughly 1.7-3 fold (Cheng et al. 2012, Walker and Harrison 2015, Ott et al. 1999) and are related to poorer memory and executive function performance (Callisaya et al. 2018, Spauwen et al. 2013, Rawlings et al. 2014). In animal models and humans, hyperglycemia appears to induce AD-like neuropathology such as amyloid-beta (Aβ) deposition, tau hyperphosphorylation, and regional hypometabolism in posteromedial areas related to memory storage and retrieval (Oskarsson et al. 2015, Bharadwaj et al. 2017). In genetically at-risk, asymptomatic middle-aged adults, amyloid positivity (citation), AD parental family history, or Apolipoprotein E ε4 (APOE4) carriage was related to variation in tract density within cingulum bundle proximal to cingulate gyrus and hippocampus, fornix, superior longitudinal fasciculus, corpus callosum, and superior corona radiata (Racine et al. 2014, Adluru et al. 2014). In aged adults,

Douaud et al. found similar associations comparing controls to AD (Douaud et al. 2011). Of interest was that amnestic MCI vs. cognitively unimpaired (CU) participants had less integrity in the superior corona radiata, with greater degradation in the corpus callosum and cingulum bundle specific to AD vs. MCI.

Comparatively few studies have used DTI to examine AD-related longitudinal white matter changes, if metabolic function is associated with these changes, and if stronger associations are seen in adults with AD genetic risk factors, MCI, or AD. Nowrangi et al. found subtle but significant tract density decreases across 4 visits spanning 1 year in hippocampal cingulum for MCI and AD participants and fornix for MCI compared to CU participants (Nowrangi et al. 2013). Other reports have noted less integrity in tracts such as uncinate fasciculus over 1.5 years in AD vs. CU participants (Kitamura et al. 2013). Utilizing Alzheimer's Disease Neuroimaging Initiative 2 (ADNI2) data, as we do in this report, Mayo and colleagues (2017) found greater tract integrity loss.

To date, research has focused on normal aging and pre- or type 2 diabetes when examining neural activity and tract integrity36. Qi et al. and Xiong et al. found that MCI subjects with vs. without diabetes had less uncinate fasciculus and cingulate bundle integrity (Qi et al. 2017, Xiong et al. 2019). Yet, it is unknown how dysmetabolism predicts longitudinal changes in white matter DTI measures across the AD spectrum, and to what degree these associations mediate cognitive decline.

The current study tested the relationship between serum glucose levels and white matter integrity, cognition, and AD risk. We hypothesized that greater serum glucose would lead to a decrease in myelination integrity, decrease in cognitive performance, and increase the risk of developing AD. We also hypothesized that higher serum glucose in participants

with AD FH or APOE4 carriage, as well as MCI or AD, would be more strongly related to less integrity in frontal and temporal tracts that distinguish MCI and AD from CU aged adults.

Research Design and Methods

Participants

All data were downloaded from the ADNI2 database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Written informed consent was obtained from all ADNI participants at their respective ADNI sites. The ADNI protocol was approved by site-specific institutional review boards. For more information, please see www.adni-info.org.

Participants with MCI had the following diagnostic criteria: 1) memory complaint identified by the participant or their study partner; 2) abnormal memory as assessed by the Logical Memory II subscale from the Wechsler Memory Scale- Revised, with varying criteria based on years of education; 3) Mini-Mental State Exam (MMSE) score between 24 and 30; 4) Clinical dementia rating of 0.5; 5) Deficits not severe enough for the participant to be diagnosed with Alzheimer's disease by the physician on site at screening. Participants with AD met similar criteria. However, they were required to have an MMSE score between 20 and 26, a clinical dementia rating of 0.5 or 1.0, and NINCDS/ADRDA criteria for probable AD.

In this study, among 191 participants (53 CU, 97 MCI, and 41 AD), the following data were downloaded: 1) basic demographics, clinical diagnosis at baseline, and MCI

conversion to AD by 36 months; 2) baseline serum data for fasting glucose; 3) baseline AD CSF biomarkers for p-tau-181, total tau, and $A\beta_{1-42}$; 4) DTI scans acquired at baseline and 6, 12, 24, and 36 months; 5) global cognition indices, as well as memory and executive function factor scores; and 6) AD parental family history and APOE4 status.

Fasting Glucose

Serum glucose levels were assayed as part of a standard lab panel. Values were in ng/dL and log10 transformed to achieve a normal distribution.

Amyloid and Tau CSF Biomarkers

CSF sample collection, processing, and quality control of p-tau-181, total tau, and $A\beta_{1-42}$ are described (Shaw et al. 2011).

Brain Volumetry

Transformational matrices were derived from T1-weighted MRI scans and applied to DTI scans to bring them into Montreal Neurological Institute (MNI) atlas space. The 3D magnetization prepared rapid gradient echo (MP-RAGE) scanning protocol is described elsewhere [22].

DTI

DTI is typically used to examine the microstructural integrity of white matter tracts, by examining how constrained (i.e., anisotropic) water movement is by myelin or axonal density. The most common DTI indices are mean diffusivity (MD) and fractional anisotropy (FA), as well as axial diffusivity or AxD ($\lambda 1$) and radial diffusivity or RD ($[\lambda 2+\lambda 3]/2$) to respectively distinguish myelin vs. axonal damage. More MD, RD, or AxD reflect *less* microstructural integrity, whereas more FA reflects *more* microstructural integrity. DTI is quantitative in nature and is more sensitive to tissue damage than brain volume. To restrict

type 1 error, 7 tracts were chosen a priori that have shown degradation in pre- or type 2 diabetes and AD disease progression: cingulate gyrus cingulum, superior longitudinal fasciculus, uncinate fasciculus, fornix, parahippocampal cingulum, corpus callosum, and anterior corona radiata.

As described, mean FA, AxD, and RD were derived from DTI tracts of interest using the Johns Hopkins white matter template(Xiong et al. 2016, Nir et al. 2013). Briefly, using FSL, raw diffusion-weighted volumes were aligned to the mean b_0 image, motion and eddy current corrected, skull-stripped, and rigidly aligned to Montreal Neurological Institute MNI space via transformation matrices from rigid realignment of a given subject's T1-weighted volume image to an MNI template. At each voxel, a diffusion ellipsoid, or tensor, was calculated based on diffusion eigenvalues (λ1, λ2, λ3). Scalar anisotropic and diffusion maps were then created for FA using the standard equation:

$$FA = \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{2[\lambda_1^2 + \lambda_2^2 + \lambda_3^2]}}$$

AxD was estimated using the primary eigenvector of diffusion parallel to the axon (i.e., λ1 eigenvalue). RD was estimated by perpendicular eigenvectors using the standard equation:

$$\mathrm{RD} = \frac{\lambda_2 + \lambda_3}{2}$$

Due to the lack of specificity MD maps have for understanding microstructural pathology, and concerns about type 1 error, these maps were not utilized for analyses.

AD Family History

AD parental history was defined as presence of maternal or paternal history, as described by the participant, informant, or both.

Apolipoprotein E ε4 genotype

The ADNI Biomarker Core at the University of Pennsylvania conducted APOE genotyping. We characterized participants as being "non-APOE4" (i.e., zero APOE ε4 alleles) or "APOE4" (i.e., one to two APOE ε4 alleles).

Neuropsychological Assessment

ADNI utilizes an extensive battery of assessments to examine cognitive functioning with particular emphasis on domains relevant to AD. A full description is available at http://www.adni-info.org/Scientists/CognitiveTesting.aspx. All subjects underwent clinical and neuropsychological assessment at the time of scan acquisition. For this report, neuropsychological assessments considered included: The Clinical Dementia Rating sum of boxes (CDR-sob), Mini-Mental Status Exam (MMSE), and AD Assessment Schedule - Cognition (ADAS-Cog). Composite scores were used in formal analyses to represent global memory and executive function among subjects. The composite memory score encompassed the Rey Auditory Verbal Learning Test, ADAS-COG, MMSE, and Logical Memory assessments [20]. The composite executive function score comprising Category Fluency—animals, Category Fluency—vegetables, Trails A and B, Digit span backwards, Digit Symbol Substitution, Number Cancellation and 5 Clock Drawing items was used [21].

Statistical Analysis

All analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY). Linear mixed regression tested the main effect of serum glucose on neuropsychological test performance, white matter integrity using DTI scans, and CSF biomarkers including $A\beta_{1\text{-}42}$, total tau, and p-tau-181. Covariates included age and sex in all models. Years of education was also covaried when analyzing memory, executive function, and global cognitive performance. Interactions were tested for FH of AD and APOE4 status. ANOVAs, Chi

square, and AD risk analyses were completed to show significant differences across diagnosis groups.

Results

Data Summary

Clinical, demographic, and CSF data for participants with serum glucose are presented in **Table 2.1**. Gender, serum glucose, and AD biomarkers $A\beta_{1\text{-}42}$, ptau-181, and total tau did not show significant differences across diagnosis groups (*p*s>.05). Years of education and percent of APOE4 carriers were significantly different between diagnostic groups (*p*s<0.01). CN participants obtained more education more than AD participants (*p*<0.05), but those classified as MCI did not differ from either group. Age was also significantly different between participants diagnosed as CN, MCI or AD (*p*<0.05). Participants with AD were older than both their CN and MCI counterparts by about 3 years (*p*s< 0.05). As anticipated for this ADNI sub-population, cognitive function, observed utilizing global cognitive tests, was significantly different across CN, MCI, and AD groups (*p*s< 0.001).

Clinical Characteristics and AD Risk

Bivariate correlations were used to examine if higher serum glucose expression predicted an increased likelihood of converting from MCI to AD. The likelihood ratio statistic [X^2=0.127, p<.01] indicated that higher serum glucose levels predicted a higher Odds Ratio for being a MCI progressor versus remaining stable. These results suggest that a per ng/dL increase in serum glucose corresponded to a roughly 37% greater likelihood of converting from MCI to AD compared to staying stable MCI [Wald=37.857, β=-0.553, Exp(B)=0.575, p < 0.001].

Table 3.1 Demographic Data for Subjects with Serum Glucose

	CN (N=53)	MCI (N=97)	AD (N=41)
Age (years)*	72.87 ± 5.79	72.99 ± 7.31	74.84 ± 8.65
Education (years)**	16.40 ± 2.69	15.89 ± 2.72	15.24 ± 2.90
Sex (% Female)	52.8%	35.1%	36.6%
APOE Status (% E4 carriers)**	32.1%	54.6%	65.9%
Serum Glucose (ng/dL)	2.00 ± 0.06	2.00 ± 0.10	2.00 ± 0.08
CSF Total Tau (pg/mL)	77.24 ± 53.35	88.02 ± 55.85	84.89 ± 43.99
Ptau-181 (pg/mL)	33.09 ± 17.08	36.69 ± 19.04	36.83 ± 20.99
Abeta 1-42 (pg/mL)	191.59± 52.33	179.60 ± 52.89	190.00 ± 48.73
MMSE***	29.90 ± 1.32	27.93 ± 1.73	23.56 ± 1.76
Executive Function (Z-score)***	0.88 ± 0.73	0.22 ± 0.80	-0.86 ± 0.87
Memory Factor (Z-score)***	1.06 ± 0.61	0.21 ± 0.70	-0.95 ± 0.50

Values are mean ± SD. Chi-square analyses were conducted to examine differences between gender and APOE4 status. Serum glucose was log10 transformed to better achieve normality. The ADNI memory factor values are Z-scored with mean 0 and a standard deviation of 1, based on 810 ADNI subjects with baseline memory data (Crane et al. 2012). AD-Alzheimer's disease; AD Assessment Schedule - Cognition (ADAS-Cog); Clinical Dementia Rating sum of boxes (CDR-sob); CN-cognitively normal; MCI-mild cognitive impairment; Mini-Mental Status Exam (MMSE).

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

White Matter Integrity

Linear mixed models were performed with age and sex as covariates to test the association between serum glucose levels and white matter integrity. Results of the significant main effects and 2-way interaction analyses are displayed in **Supplementary Table 2.1.** In general, significant main effects were observed with serum glucose in the

anterior corona radiata, cingulum near the cingulate gyrus, and superior longitudinal fasciculus (*p*s <.05). Main effects were also found in the cingulum near the hippocampus and the fornix (*p*s <.001). Interactions with at-risk groups were found significant in the cingulum near the hippocampus, fornix, superior longitudinal fasciculus, and the uncinate fasciculus for individuals with a FH, the fornix for APOE4 carriers, and the corpus callosum and fornix for MCI and AD persons. Additionally, FH, APOE4, and diagnosis interactions were examined across time and results of the 3-way interactions are presented in **Supplementary Table 2.2**. Three-way interactions between time, serum glucose, and risk factors were significant for the fornix, cingulum near the hippocampus, and superior longitudinal fasciculus (*p*s <.05). **Supplementary Table 2.3** displays the association between DTI WM and cognitive functioning stratified for each at-risk group and each group by DTI interaction effects.

Differences by FH and APOE4 status best predicted the relationship between serum glucose and FA of the fornix, in particular, thus these results are shown in **Figure 2.1** and **Figure 2.2** respectively. In **Figure 2.1** relationships with family history are examined; participants with a family history of the disease displayed improved myelin integrity as glucose levels increase. Clinical diagnosis predicted the relationship between serum glucose and the RD of the fornix, as shown in **Figure 2.2.** For participants with AD, higher glucose levels were associated with more demyelination, while CN and MCI participants followed the opposite relationship.

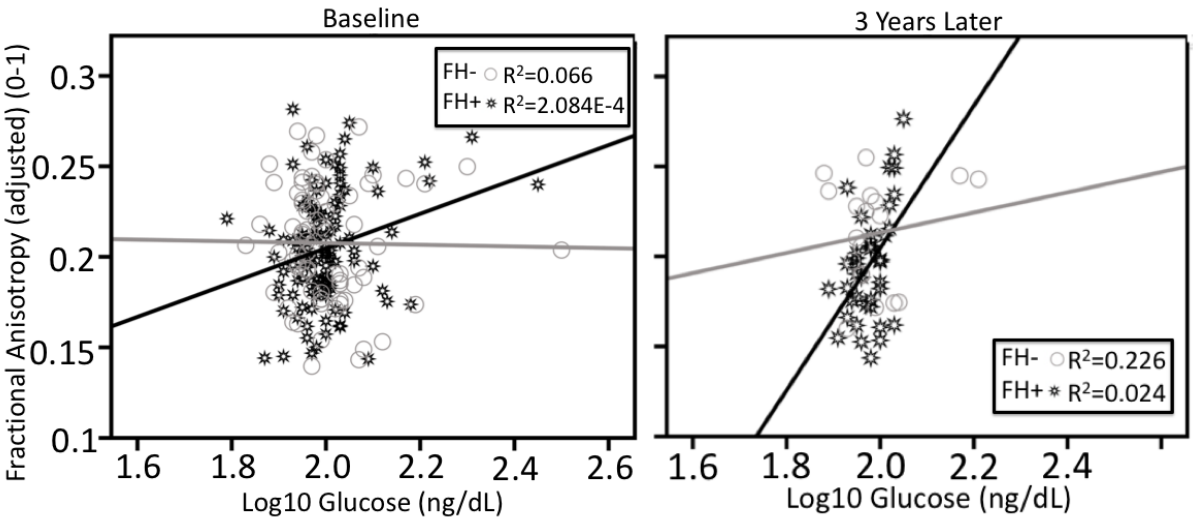

Figure 3.1: Serum Glucose and DTI Fractional Anisotropy of Fornix by Family History

Global Cognition, Memory, and Executive Function

Regression model analyses were performed with age, sex, and education level as covariates to test the serum glucose by white matter integrity interactions on cognitive function (MMSE, memory scores, and executive function). These analyses were also performed with FH, APOE4, and diagnosis to analyze the interaction of these three predictors of AD across time. The results from analyses are shown in **Supplementary Table 2.4.** Significant effects were observed between FH, APOE4, and diagnosis. There were no significant interactions for glucose and cognition.

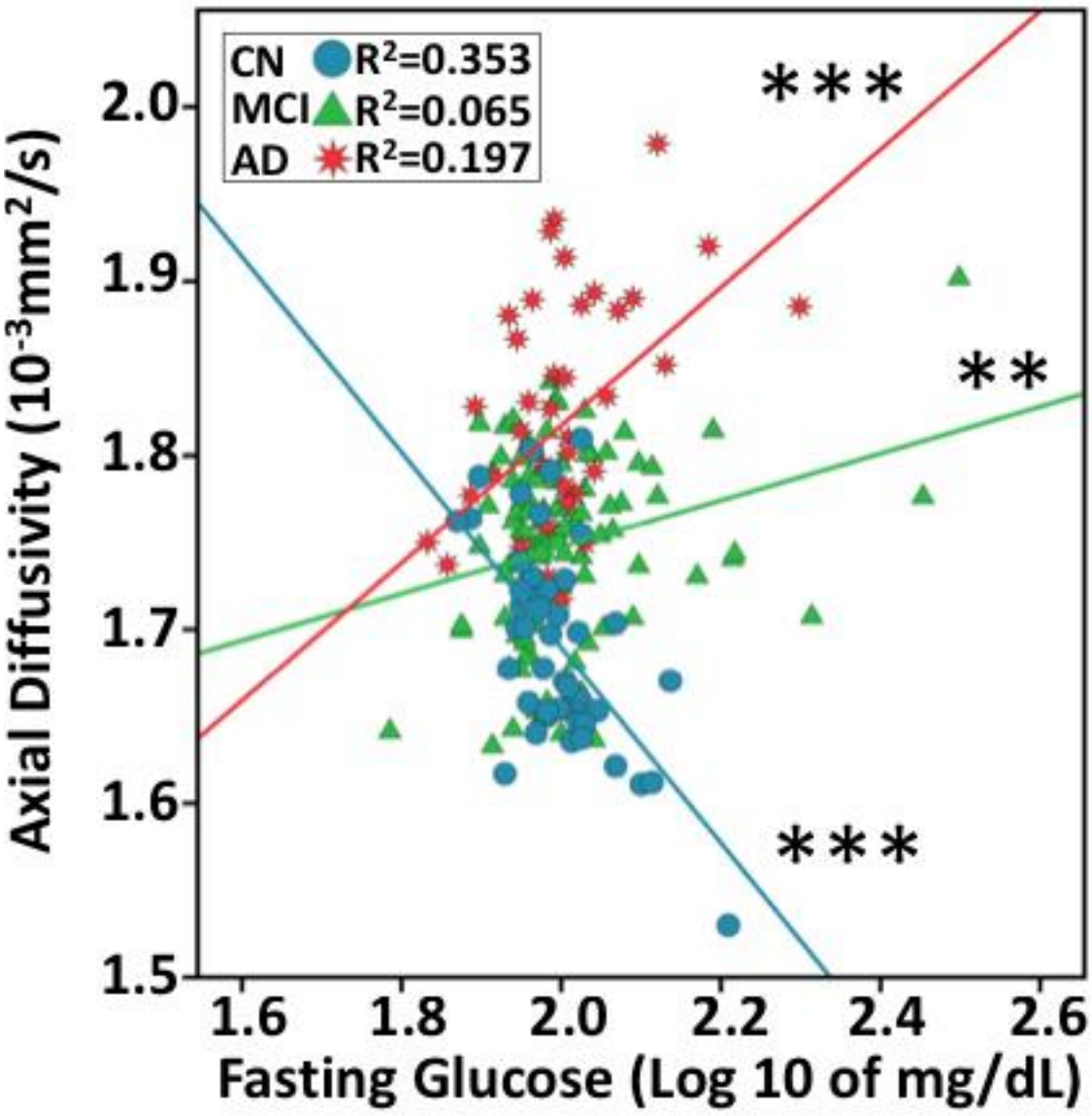

Figure 3.2: Serum Glucose and DTI Axial Diffusivity in Corpus Callosum by Clinical Diagnosis

AD CSF Biomarkers

To examine the relationship between the interaction of serum glucose and white matter integrity and AD CSF biomarkers ($A\beta_{1\text{-}42}$, ptau-181, and total tau), regression model

analyses were performed which individually examined diagnosis, APOE4 status, and FH as covariates. A significant association with $A\beta_{1-42}$, ptau-181, or total tau was not observed.

Discussion

In this study, we hypothesized that with higher serum glucose levels, myelin integrity would decrease causing a decline in cognitive function. We also hypothesized that participants with a family history of AD and AD diagnosis, compared to MCI or CN, would show faster rates of demyelination with increased glucose levels. These hypotheses are based on previous research examining family history of AD, memory decline, and cortical thinning (Haussmann et al. 2018). Many studies have looked at the increased risk of developing AD when APOE4 allele is present; this allele has been correlated with increased levels of the tau protein (Jones and Rebeck 2018) and impaired insulin resistance (Zhao et al. 2017). Further, ApoE aids in the transport of cholesterol, including the reformation of cholesterol-rich myelin (Cantuti-Castelvetri et al. 2018, Westlye et al. 2012). The correlations between type II diabetes and AD connected with increased peripheral glucose levels and insulin resistance have also been shown (Wijesekara et al. 2018), leading this study to examine associations between serum glucose, white matter integrity, and factors such as FH of AD and APOE4.

One interesting finding of our study was the relationship between serum glucose and fornix myelin integrity specific to FH and diagnostic status. The fornix is a part of the brain that aids as the major output from the hippocampus, and thus has been shown to be important in memory formation, and maintaining cognitive function and memory in AD (Hescham et al. 2017). We found greater myelination in the fornix with increased glucose for participants with a FH of AD. There has been conflicting evidence for FH of AD regarding myelin integrity. One study found that individuals with a FH of AD showed increased FA (Adluru et al. 2014) whereas many other studies that have found the opposite effect, decreased FA(Pitel

et al. 2010, Di Paola et al. 2010). Metabolic dysfunction has also been associated with FH of AD. For instance, cognitively normal individuals with maternal FH have shown hypometabolism of glucose in the brain compared to age-matched controls (Mosconi et al. 2007b).

Although results with FH were not as hypothesized, we explain these findings as a compensatory mechanism used to fight the progression of the disease. Previous research has found that higher insulin resistance is related to more glucose uptake in the medial temporal lobe of MCI converters explained by a similar temporary compensatory trend (Willette et al. 2015c). AD is characterized by hypometabolism of glucose in the brain (Mosconi et al. 2007a). Therefore, individuals with a family history may show a premature or accelerated accumulation of either amyloid beta protein or tau protein deposition. This would explain the hypermetabolism of glucose; a mechanism used to delay onset or decrease damage caused by the pathology of the disease. Recent work suggesting that with increased amyloid beta deposition, there is increased glucose metabolism in CN individuals further substantiate our results (2016). When examining regions more metabolically active among healthy younger adults, Oh and colleagues (2016) found greater amyloid beta deposition in regions of interest with high metabolism for aged CN Aβ+ participants but not those classified as aged CN Aβ- or AD. However, there were no significant findings of CSF AD biomarkers such as tau, ptau, or Aβ with increased serum glucose. This could be explained by increased glucose compensating for greater deposition in the brain, and, in turn, decreased CSF amyloid observed with AD progression A recent meta-analysis did not find differences between individuals with prediabetes and controls when examining CSF AB1-42, t-tau, and p-tau

levels, however, AD clinical characteristics may modify the observed relationship (Lu et al. 2018).

Both strengths and limitations of this study should be addressed. Using data from ADNI, we were unable to obtain dietary data, or other measures of body composition besides BMI. Specific diets could not be used to study correlations. Further, serum glucose levels were only measured at one time point preventing the investigation of metabolic activity over time. However, we were able to test across the spectrum of AD. We also examined the relationship between metabolism and markers related to LOAD such as genetic risk factors. By doing so, additional mechanisms tying metabolic dysfunction and cognitive impairment were able to be explored. The longitudinal design of the current study is another strength as changes in myelin integrity across time were able to be measured.

In conclusion, we found that demyelination occurs more readily with increased glucose levels in participants with AD versus MCI or CN individuals. This result reinstates the idea that in CN and MCI individuals, glucose metabolism is normal, or in the case of pathology, predicted by family history status, hypermetabolism occurs to offset the progression of the disease. When AD is diagnosed, the damage has exceeded past the point where excess glucose metabolism is beneficially; here we see an increase in demyelination with increased glucose levels. With more knowledge on the pathology of the disease related to white matter, targeted treatments may postpone the age of onset and decrease severity of the disease.

Supplementary Table 3.1 Effects for significant 2-way interactions with glucose

ROI	Modality	F-value	Glucose		Glucose x FH		Glucose x APOE4 Status		Glucose x Baseline Dx		Glucose x Conversion	
			β	SD	β	SD	β	SD	β	SD	β	SD
ACR	FA											
	AD	8.568	-0.0343*	0.0117								
	RD	17.934	-0.0412**	0.0097								
CC	FA											
	AD								-0.8338@	0.3332		
	RD											
CCG	FA	3.939	0.006*	0.003								
	AD											
	RD											
Cing Hipp	FA											
	AD	11.411	-0.0668***	0.0198	0.0984**	0.0367						
	RD	16.72	-0.07***	0.0171	0.0969*	0.0339						
Fornix	FA	18.923	0.0175***	0.004	0.053*	0.0175	0.0274*	0.0086				
	AD								0.5054*	0.1516		
	RD	8.125	-0.1006**	0.0353					0.348**	0.1188		
SLF	FA											
	AD		0.1044@@	0.0367	0.0429*	0.0149						
	RD	4.686	-0.0132*	0.0061								
UNC	FA				-0.1884@@	0.0622						
	AD											
	RD											

p<0.05@, No Time Effect p<0.05* p<0.01** p<0.-001***

Supplementary Table 3.2 Effects for significant 3-way interactions with glucose

ROI	Modality	F-value	lg10Glucose*Time	lg10 Glucose*FH*time			lg10 Glucose*APOE4*time			lg10 Glucose*BIDx*time			
				Overall Interaction	FH+	FH-	Overall Interaction	APOE4+	APOE4-	Overall Interaction	CN	MCI	AD
	FA												
	AD	11.411	-0.0668**										
Cing Hipp	RD	16.72	-0.07***	0.0969*	-0.15*	0.11							
	FA												
	AD			0.0429*	0.03	0.14***							
SLF	RD												
	FA	18.923	0.0175***	0.053*	0.14***	0.0085	0.0274*	0.05*	0.14***				
	AD									0.5054*	-0.44	-0.42*	-0.04
Fornix	RD	8.125	-0.1006**							0.348*	-0.81*	-0.53*	0.2
	FA												
	AD	8.568	-0.0343**										
ACR	RD	17.934	-0.0412***							0.0871*	-0.25**	0.03	0.08
	FA	8.034	0.0090**	-0.0158*	0.07*	-0.08*							
	AD												
CC	RD												
	FA												
	AD			0.058**	0.00706	0.11***							
CCG	RD												
	FA												
	AD												
UNC	RD												

p<0.05* p<0.01** p<0.-001***

Supplementary Table 3.3 Association between DTI WM and cognitive functioning stratified for group and group*DTI Interaction effects

ROI	FH+ Estimate	FH- Estimate	Estimate for FH+*DTI interaction effect	Estimate for FH-*DTI interaction effect
MMSE				
FA Fornix	-203.0427**	148.1783*	113.1243***	-65.4271
AD SLF	153.4046*	-66.3014	-82.8953**	29.2228
ADNI_MEM				
FA Fornix	10.1783	78.7735**	-1.2349	-36.6415***
FA UNC	-84.5693*	-28.5875	42.1994*	14.5415
AD SLF	51.0404*	-39.5662	-27.8661**	17.8526
ADNI_EF				
FA Fornix	-38.2356	121.0802***	23.8627*	-57.902***
AD SLF	40.0962	-53.3919*	-22.5239*	23.4846*

ROI	APOE4+. Estimate	APOE4- estimate	estimate for APOE4+*DTI interaction effect	estimate for APOE4-*DTI interaction effect
ADNI_MEM				
FA Fornix	73.3719**	12.617	-33.0766**	-4.1867
ADNI_EF				
FA Fornix	74.3258**	-2.5954	-31.326*	3.636

Supplementary Table 3.4 (continued)

ROI	CN estimate	MCI estimate	AD estimate	estimate for CN* DTI interaction effect	estimate for MCI*DTI interaction effect	estimate for AD*DTI interaction effect
MMSE						
FA Fornix	-192.4109***	-30.6631	-44.7376	97.498**	19.277	30.3784
ADNI_MEM						
FA Fornix	1.2396	64.2151**	37.8519	-1.2396	-31.3471**	-17.0052
ADNI_EF						
FA Fornix	-22.169	63.5173**	7.3094	12.8058	-30.376**	0.4625

p<0.05* p<0.01 p<0.001*****

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CHAPTER 4. KETONE BODIES, GENETIC RISK FOR ALZHEIMER'S DISEASE AND NEURAL MYELINATION PATTERNS

Modified from a manuscript to be submitted to Advances in Nutrition

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Introduction

Ketone bodies are an alternative fuel source when the brain's primary source of energy, glucose, is insufficient and result from catabolizing lipids. The brain utilitzes water-solute ketone bodies, such as acetoacetate and beta-hydroxybutyrate, during periods of fasting or starvation via ketosis or ketogenesis (Sokoloff 1973).Due to the anti-inflammatory properties of ketones, many diseases have been treated with a ketogenic diet such as epilepsy and dermatologic disease, (Fomin, McDaniel and Crane 2017, Elia et al. 2017) while other properties such as alternative fuel source have made ketones a possible treatments for cancer (Fokidis et al. 2015). Ketogenic diets, intermittent fasting, and other dietary methods that increase ketone bodies are implemented in individuals with cognitive decline to ameliorate some of the cognitive changes observed with aging and Alzheimer's disease (Xin et al. 2018, Mattson et al. 2018).

Throughout the normal aging process poorer performance in tasks related to memory, executive function, and reaction time are observed. When declines in memory and cognitive function surpass normal aging, individuals may be diagnosed with mild cognitive impairment (MCI) or Alzheimer's disease (AD). MCI, preclinical diagnosis to AD, is characterized by decreased memory scores, executive function, and other cognitive functions, however, deficits are not severe enough for an AD diagnosis. Persons with MCI have more than one cognitive decline that affects their daily activities; a decrease in gray matter as well as

decreased myelin integrity is also observed (Kavroulakis et al. 2018, Knopman and Petersen 2014). AD is a neurodegenerative disorder characterized by a decline in cognitive function, especially memory, atrophy of gray matter in memory specific regions, and decreased myelin integrity. Accumulation of a misfolded protein referred to as amyloid beta, tangles of tau protein, and phosphorylated tau are commonly found in the brains of persons with AD.

There are numerous lifestyle and genetic factors that infer greater risk of developing AD, including family history (FH) and apolipoprotein E4 (APOE4) carriers. For example, FH positive individuals have cortical thinning which leads to an increased risk of developing the disease (Ganske et al. 2016, Haussmann et al. 2018). APOE4 has been linked to developing AD when either one or two alleles are present (Christensen and Pike 2018). Carriers of APOE4 show cognitive decline in addition to changes in brain metabolism such as decreased glucose uptake and utilization, which is in contrast to more efficient ketone body metabolism (Wu, Zhang and Zhao 2018).

Decreased glucose uptake and metabolism is found in individuals with AD, which contributes to cognitive decline (Szablewski 2017). Insulin resistance, and in turn Type II Diabetes (T2D), are predictors of AD (Hao et al. 2015) and has been shown to cause hypometabolism of glucose in the brain (Willette et al. 2015b). Further, hypometabolism in various regions of the brain has been found among individuals with dementia using 18-F-fluorodeoxyglucose (FDG) PET imaging (Kato et al. 2016). To study the white matter integrity of the brain, the tracts in the brain that allow signals to be sent and nutrients to be transported between gray matter regions, an imaging technique called diffusion tensor imaging, DTI, is used. DTI has been shown to be a more sensitive technique than FDG-PET in measuring early signs of deterioration in individuals with MCI (Szablewski 2017).

Demyelination, or the breakdown of white matter, starts in the prefrontal areas of the brain and progresses posteriorly throughout the normal aging process (Bartzokis et al., 2003). White matter integrity has been shown to predict declines in memory that may lead to AD (Chapman et al. 2016). In order to study the myelination, or white matter tracts, in the brain DTI is used as a noninvasive way to visualize the microstructural composition by studying the movement of water molecules to map out the white matter tracts. During the progression of AD, areas of the brain such as the telencephalon, entorhinal cortex, hippocampus, and amygdala, have an increased risk of demyelination as these areas were myelinated later in life (Bender, Völkle and Raz 2016). According to Bartzokis et al., demyelination of these areas are associated with accumulation of amyloid beta proteins, or plaques, that suggests the demyelination process leads to deposition of the plaques (2007). While the relationship between AD and white matter integrity has been extensively studied, less is known about the relationship between ketone bodies and myelination across the AD spectrum.

Acetoacetate and beta-hydroxybutyrate have been shown to enhance cognitive function while showing resistance to hormonal responses associated with hypoglycemia (Amiel et al. 1991, Veneman et al. 1994). Beta hydroxybutyrate, in particular, has been seen to protect neuronal integrity and prevent beta-amyloid accumulation (Kashiwaya et al. 2000). Triple transgenic AD mice had improved cognition and memory on various task-based tests, as well as decreases in amyloid beta deposition and phosphorylation of tau, after being fed a diet rich with ketone esters (Kashiwaya et al. 2013). These studies suggest a metabolic defect in the brain of individuals with AD. In individuals with a GLUT1 mutation, glucose cannot effectively be taken up in the brain, and leads to a delay in myelination. A ketogenic diet, consisting of fats and proteins, resulted in an increase in white matter in these peoples' brains

(Klepper et al. 2007). Ketone uptake in the brain is not compromised by the pathology of AD like glucose is, thus ketones provide the brain with an alternate energy source when glucose cannot be utilized in AD (Cunnane et al. 2016, Castellano et al. 2015).

Using data from the Alzheimer's Disease Neuroimaging Initiative, we hypothesized that more ketone bodies would lead to greater myelin integrity, better cognitive performance, and lower risk of developing AD. However, we anticipated APOE4 carriers and individuals with a FH of AD to have decreased myelination due to previous research with other at-risk populations suggesting these trends (Willette et al. 2015c, Haier et al. 2003). With more knowledge about disease-related white matter integrity, targeted treatments may postpone the age of onset and decrease severity of the disease.

Materials and Methods

Participants

Data from late middle-aged to aged adults were obtained from the ADNI database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org. Written informed consent was obtained from all ADNI participants at their respective ADNI sites. The ADNI protocol was approved by site-specific institutional review boards. All analyses used in this report only included baseline data, however measures were taken periodically for the database spanning a time of 24 months. Baseline serum data for all three ketone bodies was available for 692 subjects: 175 CN, 386 MCI, and 131 AD.

Participants with MCI had the following diagnostic criteria: 1) memory complaint identified by the participant or their study partner; 2) abnormal memory as assessed by the Logical Memory II subscale from the Wechsler Memory Scale- Revised, with varying criteria based on years of education; 3) Mini-Mental State Exam (MMSE) score between 24 and 30; 4) Clinical dementia rating of 0.5; 5) Deficits not severe enough for the participant to be diagnosed with Alzheimer's disease by the physician on site at screening. Participants with AD met similar criteria. However, they were required to have an MMSE score between 20 and 26, a clinical dementia rating of 0.5 or 1.0, and NINCDS/ADRDA criteria for probable AD.

Mass Spectrometry and Ketone Bodies

Data were downloaded from the ADNI Nightingale and DTI ROI dataset. Analyses for this report focused on serum ketone bodies, acetate, acetoacetate, and beta hydroxybutyrate, levels, which were assayed in the Nightingale dataset.

Amyloid and Tau CSF Biomarkers

CSF sample collection, processing, and quality control of p-tau-181, total tau, and $A\beta_{1-42}$ are described in the ADNI1 protocol manual (http://adni.loni.usc.edu/) and (Shaw et al. 2011).

Apolipoprotein E ε4 genotype

The ADNI Biomarker Core at the University of Pennsylvania conducted APOE genotyping. We characterized participants as being "non-APOE4" (i.e., zero APOE ε4 alleles) or "APOE4" (i.e., one to two APOE ε4 alleles).

Magnetic Resonance Imaging (MRI) Acquisition and Pre-Processing

T1-weighted MRI scans were acquired within 10-14 days of the screening visit following a back-to-back 3D magnetization prepared rapid gradient echo (MP-RAGE)

scanning protocol described elsewhere (Jagust et al. 2010). Images were pre-processed using techniques previously described (Willette et al. 2013). Briefly, the SPM12 "New Segmentation" tool was used to extract modulated gray matter (GM) volume maps. Maps were smoothed with a 8mm Gaussian kernel and then used for voxel-wise analyses.

Diffusion Tensor Imaging

DTI preprocessing has been described (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3792746/). Briefly, using FSL, raw diffusion-weighted volumes were aligned to the mean b_0 image, motion and eddy current corrected, skull-stripped, and rigidly aligned to Montreal Neurological Institute (MNI) space via transformation matrices from rigid realignment of a given subject's T1-weighted volume image to an MNI template. At each voxel, a diffusion ellipsoid, or tensor, was calculated based on diffusion eigenvalues ($\lambda 1$, $\lambda 2$, $\lambda 3$). Scalar anisotropic and diffusion maps were then created for Fractional Anisotropy, or FA, using the standard equation:

$$FA = \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{2[\lambda_1^2 + \lambda_2^2 + \lambda_3^2]}}$$

Axial Diffusivity, or AxD, was estimated using the $\lambda 1$ eigenvalue. Radial Diffusivity, or RD, was estimated using the standard equation:

$$\mathrm{RD} = \frac{\lambda_2 + \lambda_3}{2}$$

Due to concerns about type 1 error, Mean Diffusivity maps were not utilized for analyses.

Statistical Analysis

All analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY). Linear mixed regression tested the main effect of serum ketone bodies on neuropsychological

performance, white matter integrity using DTI scans, and CSF biomarkers including $A\beta_{1-42}$, total tau, and p-tau-181. Covariates included age and sex in all models. Years of education was also covaried when analyzing memory and cognitive performance.

Results

Data Summary

Clinical, demographic, and CSF data for subjects with serum ketone bodies are presented in **Table 1**. Serum acetoacetate and serum 3-hydroxybutyrate levels, and AD biomarkers: $A\beta_{1-42}$, ptau-181, and total tau did not show significant differences across diagnosis groups. Years of education and age were significantly different between groups: CN, MCI, and AD (p-value<0.01). Serum acetate levels and percent of APOE4 carriers and were significantly different between participants diagnosed as CN, MCI or AD (p-value <0.01 and p-value <0.001 respectively). As anticipated for this ADNI sub-population, cognitive function, observed utilizing global cognitive tests, was significantly different across CN, MCI, and AD groups (all $p < 0.001$).

Table 4.1 Demographic Data for Subjects with Serum Ketone Bodies

	CN (N=175)	MCI (N=386)	AD (N=131)
Age (years)*	72.83 ± 5.85	72.76 ± 7.13	74.79 ± 8.62
Education (years)*	16.41 ± 2.62	16.10 ± 2.70	15.52 ± 2.76
Sex (% Female)**	50.0%	37.3%	35.5%
APOE Status (% E4 carriers)***	32.4%	54.0%	70.2%
Serum Acetate (mmol/l)**	0.0368 ± 0.00846	0.0389 ± 0.00952	0.0408 ± 0.01542
Serum Acetoacetate (mmol/l)	0.0392 ± 0.01663	0.0421 ± 0.02307	0.0424 ± 0.03603
Serum 3-hydroxybutyrate (mmol/l)	0.1467 ± 0.08659	0.1580 ± 0.10549	0.1698 ± 0.20090
CSF Total Tau (pg/mL)	75.97 ± 45.87	81.21 ± 51.67	84.69 ± 59.80
Ptau-181 (pg/mL)	33.16 ± 16.86	34.93 ± 18.83	35.92 ± 20.74
Abeta 1-42 (pg/mL)	195.48± 48.86	185.85 ± 52.67	191.57± 54.17
MMSE***	28.88 ± 1.31	27.95 ± 1.70	23.58± 1.75
Executive Function (Z-score)***	0.87 ± 0.73	0.24 ± 0.80	-0.86 ± 0.88
Memory Factor (Z-score)***	1.04 ± 0.59	0.22 ± 0.71	-0.96 ± 0.51

Values are mean ± SD. Chi-square analyses were conducted to examine differences between gender and APOE4 status. The ADNI memory factor values are Z-scored with mean 0 and a standard deviation of 1, based on 810 ADNI subjects with baseline memory data (Crane et al.

2012). *, p<0.05; **, p<0.01; ***, p<0.001. CN-cognitively normal; MCI-mild cognitive impairment.

White Matter Integrity

Regression model analyses were performed with age and sex to show the association between ketone body levels and white matter integrity using DTI scans. These analyses were shown over time displayed in **Supplementary Table 3.1.** These analyses were performed with family history (FH), APOE4, and diagnosis to analyze the interaction of these three predictors of AD across time displayed in **Supplementary Table 3.2.** A summary of the results based on areas of the brain are displayed in **Supplementary Table 3.3.**

The main effect of acetoacetate levels on white mater integrity using fractional anisotropy. This type of DTI is on a scale 0 to 1 with a higher value indicating more myelin integrity. In the uncinate fasciculus we found that with an increase in acetoacetate, there was a correlation with increase myelin integrity show in **Figure 1a.** In the splenius of the corpus callosum, a DTI using radial diffusivity is used to show as acetoacetate levels increase, the amount of demyelination decreases shown in **Figure 1b.**

Figure 1c displays the at-risk groups; the individuals with a FH were examined in the cingulum around the cingulate gyrus where we found no correlation with myelin integrity as acetoacetate levels increased compared to the FH negative group where we saw a correlation with increased myelin integrity as the level of ketone body raised.

Using AD, the uncinate fasciculus was examined the amount of axonal degeneration occurring as acetoacetate levels increased comparing another at risk group, the APOE4 carriers. Shown in **Figure 2a**, as acetoacetate levels increased there was a correlation with an increase in the amount of axonal degeneration occurring in APOE4 carriers while no correlation was found with non-carriers.

This trend was seen using beta hydroxybutyrate in the tapetum using RD DTI. In **Figure 2b,** as beta hydroxybutyrate levels increased, there was an increase in the amount of demyelination in APOE4 carriers and no significant trend for non-carriers.

Figure 3a depicts the levels of demyelination across the AD spectrum as acetoacetate levels increase shown in the tapetum using RD. For CN participants, as acetoacetate levels increased, the amount of myelin breakdown decreased; for MCI participants there was less of an association, but related to higher demyelination, and individuals with AD were shown to have the highest amount of demyelination with increase ketone body levels.

Figure 3b shows the demyelination of the cingulum around the hippocampus using RD. With increased levels of acetoacetate at baseline, there was an increase in the amount of demyelination in MCI progressors and no association with stable MCI. However, two years later, there is less demyelination occurring with an increase in acetoacetate in both groups.

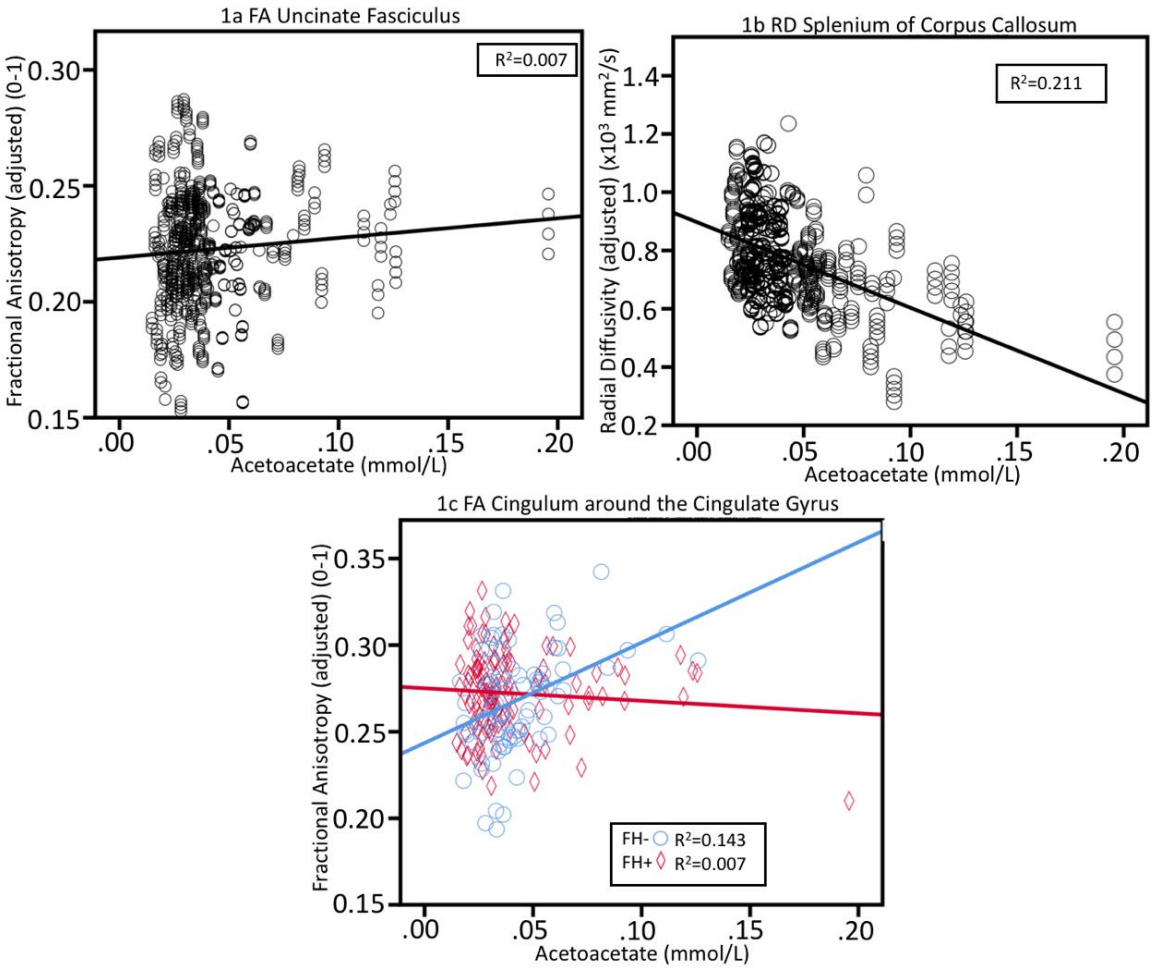

Figure 4.1 Beneficial Effects of Ketone Bodies on White Matter Integrity.

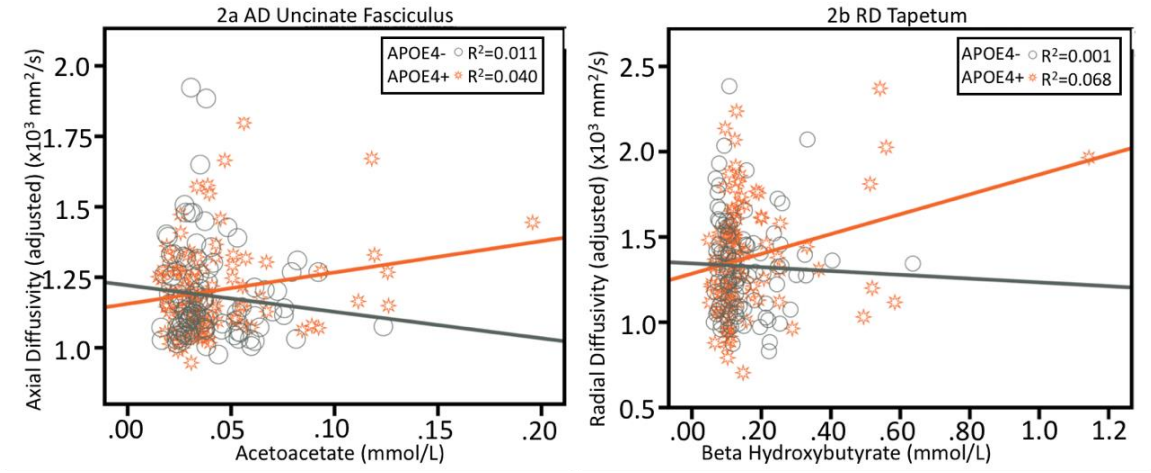

Figure 4.2 Effects on White Matter Integrity with Increases in Ketone Bodies in APOE4 Carriers.

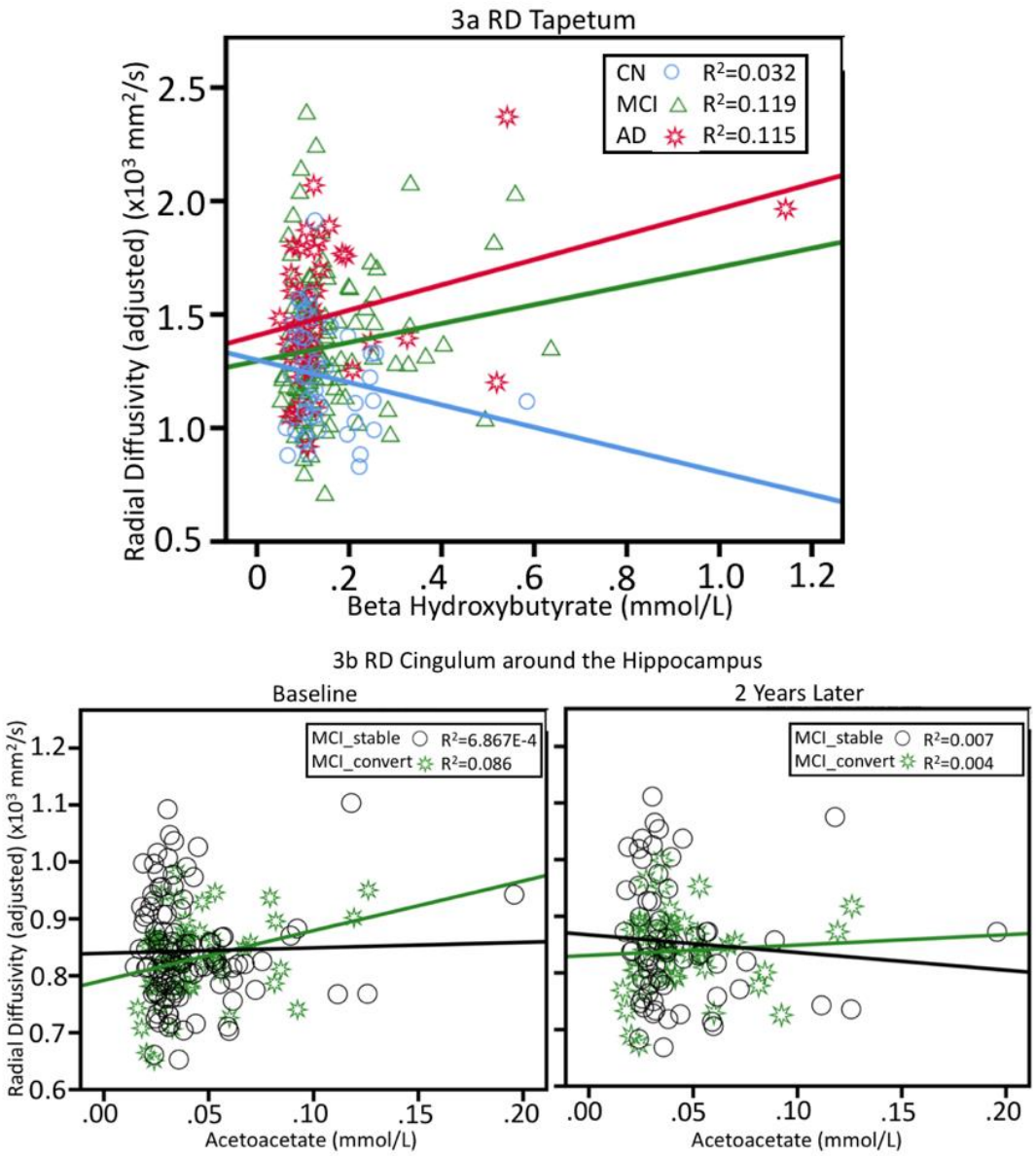

Figure 4.3 Effect on White Matter Integrity with Increases in Ketone Bodies on MCI and AD Participants.

Discussion

Hypometabolism in the brain is a distinct feature of Alzheimer's disease (Brown et al. 2014). For these individuals, the brain is starving due to a lack of glucose uptake in the brain. Ketone bodies, the brain's alternative fuel source, were examined to determine their

availability and use throughout the AD process. For this study, we hypothesized that with an increase in ketone body levels, there would be less demyelination and improved cognition over time. APOE4 carriers and individuals with FH of the disease were hypothesized to show accelerated disease progression due to previous research(Ganske et al. 2016, Haussmann et al. 2018, Cannon-Albright et al. 2019). In this study, we predicted that these individuals would show an increased amount of demyelination despite increased ketone body levels.

We found, using several DTI measures, a correlation between increased levels of ketone bodies and increased myelin integrity. As myelin is composed of lipids and ketone bodies are the functional and useable part of lipids that are metabolized, we propose that as more ketone bodies are present, there is an increase in building blocks to synthesize more myelin. The formation of myelin is preferred to be derived from ketone bodies as opposed to glucose substrates (Koper, Lopes-Cardozo and Van Golde 1981). A ketogenic diet, which increase serum ketone bodies, was shown to promote myelination and decrease axon degeneration in individuals with myelin disease (Stumpf et al. 2019).

At-risk groups of developing AD were examined to look at predispositions that may lead to changes in white matter integrity. Participants with a FH of the disease showed no correlation with increased in ketone bodies and white mater integrity while participants without a FH of the disease had a correlation of increased white matter integrity. Participants with the APOE4 allele were found to have no correlation or increases in demyelination compared to non-carriers. Other research found that people with a family history or APOE4 carriers have differences in the microstructure of the white matter before symptoms of the disease have started (Adluru et al. 2014, Bartzokis et al. 2007). White matter hyperintensities, or loss of white matter is found in CN individuals and is associated with risks of developing

AD (Salvadó et al. 2019). Reger and colleagues (year) found, with an ingestion of medium chain triglycerides to increase circulating ketone bodies, there was an improvement on memory scores for APOE4 non-carriers and a decline on memory scores for APOE4 carriers in memory-impaired participants. Even though the APOE4 carriers had greater increases in available ketone bodies, the ability to metabolize them was unfavorable (Reger et al. 2004). This may explain the reasons for increases in demyelination in these genetically at-risk groups.

Participants diagnosed with MCI or AD showed increases in demyelination as beta hydroxybutyrate levels increased while the opposite relationship was found among CN participants. The brain's rate of uptake for ketone bodies remains unchanged throughout the progression of MCI and AD (Croteau et al. 2018), however, the ability of the brain to uptake ketone bodies has not been studied. Individuals with MCI and AD have increased demyelination which leads to increases in ketone bodies in the CSF. We believe people with MCI and AD have an impaired ability to uptake and metabolize ketone bodies, which leads to the increases in ketone concentrations in the serum.

People at-risk for developing AD are predisposed to having poorer myelin integrity. Individuals with a FH of the disease, carriers of APOE4, and participants with MCI and AD show more demyelination; as ketone bodies increase, we see a detrimental effect of higher ketone bodies on white matter integrity initially. However, after two years, the correlation for these groups decreases or becomes non-existent. We predict, at the beginning, these at-risk individuals are breaking down myelin, which leads to the increase in ketone bodies in the CSF, but is eventually overcome by a protective effect of the increased ketone bodies concentration which slows the demyelination. Increases in ketone bodies for groups not at

risk for the disease show a neutral or increased effect on white matter integrity. Higher ketone bodies in healthy cognitively normal individuals is related to more white matter integrity which is supported by other researchers (Klosinski et al. 2015).

Further, MCI-progressors had more demyelination with increasing acetoacetate levels. We believe that as the brain is starving due to the hypometabolism of glucose, myelin is being broken down resulting in higher levels of ketone bodies in the CSF as well as more demyelination. As time goes on, the brain is protected by the increase in ketone bodies leading to decrease inflammation and decreased demyelination, explaining the loss of correlation after two years.

Calorie restriction has also been shown to maintain and enhance white matter integrity (Guo, Bakshi and Lin 2015) which provides further evidence for our findings. Calorie restriction is a dietary protocol that reduces the number of calories, and has been shown to increase the amount of available ketone bodies by inducing ketosis in the body (Veech et al. 2017, Edwards, Copes and Bradshaw 2015, Shimazu et al. 2013). Calorie restriction has also been shown to protect against AD and other neurological diseases by decreasing neuronal cell death (Veech et al. 2001).

There are several mechanisms that could explain our findings. Glucose is the main and preferred source of fuel for the brain. However, oligodendrocytes, cells that form new myelin, have been shown to survive in vivo by aerobic glycolysis with the use of a byproduct, lactate. Lactate can be metabolized in the white mater tracts when energy is deprived (Fünfschilling et al. 2012). As individuals with AD struggle to metabolize glucose in the brain, an excess of ketone bodies may lead to an improvement in cognitive function by providing the brain with a source of fuel such as lactate.

Limitations of the study should be addressed. The data used from the cohort, ADNI, did not record any dietary measures or body composition aside from BMI. We were able to study participants across the AD spectrum and had access to genetic risk factors. This study was longitudinal, so changes in myelin integrity could also be tested across time. Future research should examine whether dietary changes such as fasting, calorie restricting, or increases in dietary fat leads to increases in ketone bodies in both the serum and CSF; if such changes occur, it would be beneficial to measure inflammatory markers of oxidative stress and changes in white matter integrity.

In conclusion, we found at-risk groups of developing AD, such as an APOE4 carriers, those with a FH of the disease, or being diagnosed with MCI or AD, had initially higher levels of demyelination associated with increases in ketone bodies. However, within two years, these association decreased or were non-significant. Thus, dietary changes, such as an increase in dietary fats or calorie restriction, during the progression of the disease would lead to an increase in ketone bodies in the CSF and protect against demyelination and slow the progression of the disease. If such dietary changes were implemented early in at-risk populations for AD, there is a greater chance of adaption the brain to utilizing ketone bodies and may reduce the effects of the disease.

Supplementary Table 4.1 Significant Main Effects and 2-way interactions with at-risk groups for Acetoacetate

ROI	Modality	F-value	ACACE		ACACE x FH		ACACE x APOE4 Status		ACACE x Baseline Dx		ACACE x Conversion	
			β	SD	β	SD	β	SD	β	SD	β	SD
ACR	FA						*					
	AD											
	RD											
CC	FA						*					
	AD						***					
	RD											
CCG	FA				***							
	AD								*			
	RD						*		*			
Cing Hipp	FA										*	
	AD											
	RD	3.036	0.6593***	0.3784	***		***					
Fornix	FA						*					
	AD				***							
	RD						*		*			
SLF	FA	4.444	0.1714*	0.0813	*							
	AD								**			
	RD								*			
UNC	FA	4.019	0.2274*	0.1134								
	AD	4.401	1.0464*	0.4988			*		**			
	RD								**			
Tap	FA						*					
	AD								*			
	RD											
SCC	FA						*					
	AD				***							
	RD	10.318	-3.4837***	1.0844			***					

p<0.05* p<0.01 p<0.001*****

Supplementary Table 4.2 Significant 3-way interactions with at-risk groups for Acetoacetate

ROI	Modality	F-value	ACACE*time		ACACE x FH x time		ACACE x APOE4 Status x time		ACACE x Baseline Dx x time		ACACE x Conversion x time	
			β		β		β		β		β	
ACR	FA											
	AD											
	RD				*							
CC	FA	9.143	-0.0437**	0.0145								
	AD	4.324	0.1399*	0.0673	**							
	RD				**							
CCG	FA											
	AD											
	RD				*							
Cing Hipp	FA											
	AD								*		*	
	RD	6.602	-0.1789***	0.0696	***		***				*	
Fornix	FA											
	AD				***				**			
	RD								***			
SLF	FA											
	AD											
	RD										*	
UNC	FA						*					
	AD	12.291	-0.3239**	0.0924								
	RD	9.514	-0.3009**	0.0975								
Tap	FA						*					
	AD											
	RD											
SCC	FA	38.811	-0.0696***	0.0112			**				*	
	AD				***							
	RD	2374.17	0.368***	0.0076								

p<0.05* *p<0.01 *p<0.001***

Supplementary Table 4.3 Significant Main Effects and 2-way interactions with at-risk groups for Beta-hydroxybutyrate

ROI	Modality	F-value	BOHBUT		BOHBUT x FH		BOHBUT x APOE4 Status		BOHBUT x Baseline Dx		BOHBUT x Conversion	
			β	SD	β	SD	β	SD	β	SD	β	SD
ACR	FA						**					
	AD											
	RD						*					
CC	FA						**					
	AD						*					
	RD						*					
CCG	FA						*		*			
	AD						*					
	RD						**		*			
Cing Hip	FA											
	AD											
	RD						*					
Fornix	FA											
	AD											
	RD						*					
SLF	FA	5.074	0.0356*	0.0158	*							
	AD								*			
	RD						*		*			
UNC	FA											
	AD	5.553	0.2267*	0.0975					**			
	RD	5.748	0.2287*	0.0954					**			
Tap	FA						*		**			
	AD						*					
	RD	3.95*	0.4027	0.2026			*		*			
SCC	FA						*					
	AD						*					
	RD						*					

p<0.05* p<0.01 p<0.001***

Supplementary Table 4.4 Significant 3-way interactions with at-risk groups for Beta Hydroxybutyrate

ROI	Modality	F-value	BOHBUT*time		BOHBUT x FH x time		BOHBUT x APOE4 Status x time		BOHBUT x Baseline Dx x time		BOHBUT x Conversion x time	
			β	SD	β		β		β		β	
ACR	FA											
	AD											
	RD				**							
CC	FA								**			
	AD				**						*	
	RD				**							
CCG	FA											
	AD											
	RD				*							
Cing Hipp	FA											
	AD								*		*	
	RD	4.708	-0.025*	0.0115							*	
Fornix	FA											
	AD								**		**	
	RD						*		***		***	
SLF	FA											
	AD											
	RD				*						**	
UNC	FA						*					
	AD	15.19	-0.0707***	0.0037					**			
	RD	13.634	-0.0684***	0.0185					*			
Tap	FA						*					
	AD											
	RD											
SCC	FA	11.117	-0.0102**	0.0031					***			
	AD				**							
	RD	6.263	0.0399*	0.0159	*				*			

p<0.05* p<0.01 p<0.001*****

Supplementary Table 4.5 Summary of Results split by brain region

Tap				
	FA	RD	AD	MD
Main Effect BHB		x		
BHB*Dx	x	x		
BHB*MCI_convert				
BHB*FH				
BHB*APOE4	x	x	x	x
BHB*Time				
BHB*Time*DX				
BHB*Time*MCI_convert				
BHB*Time*FH				
BHB*Time*APOE4	x			x
Main Effect of ACACE				x
ACACE*Dx	x		x	
ACACE*MCI_convert				
ACACE*FH				x
ACACE*APOE4	x	x		x
ACACE*Time				x
ACACE*Time*DX				
ACACE*Time*MCI_convert				
ACACE*Time*FH				x
ACACE*Time*APOE4	x			

Supplementary Table 4.6 (continued)

Cing Hipp				
	FA	RD	AD	MD
Main Effect BHB				
BHB*Dx				
BHB*MCI_convert				
BHB*FH				
BHB*APOE4		x		
BHB*Time		x		
BHB*Time*DX			x	
BHB*Time*MCI_convert		x	x	
BHB*Time*FH				
BHB*Time*APOE4				
Main Effect of ACACE		x		
ACACE*Dx				
ACACE*MCI_convert	x			
ACACE*FH		x		
ACACE*APOE4		x		
ACACE*Time		x		
ACACE*Time*DX			x	
ACACE*Time*MCI_convert		x	x	
ACACE*Time*FH		x		
ACACE*Time*APOE4		x		

Supplementary Table 4.7 (continued)

GCC				
	FA	RD	AD	MD
Main Effect BHB				
BHB*Dx				x
BHB*MCI_convert				
BHB*FH	x	x		
BHB*APOE4	x		x	x
BHB*Time	x			
BHB*Time*DX				x
BHB*Time*MCI_convert			x	x
BHB*Time*FH		x		
BHB*Time*APOE4				
Main Effect of ACACE				
ACACE*Dx			x	x
ACACE*MCI_convert				
ACACE*FH	x			x
ACACE*APOE4			x	x
ACACE*Time				
ACACE*Time*DX		x		x
ACACE*Time*MCI_convert	x	x	x	
ACACE*Time*FH				
ACACE*Time*APOE4				

Supplementary Table 4.8 (continued)

UNC				
	FA	RD	AD	MD
Main Effect BHB		x	x	
BHB*Dx		x	x	
BHB*MCI_convert				
BHB*FH				
BHB*APOE4				
BHB*Time		x	x	
BHB*Time*DX		x	x	
BHB*Time*MCI_convert				
BHB*Time*FH				
BHB*Time*APOE4	x			
Main Effect of ACACE	x		x	
ACACE*Dx		x	x	x
ACACE*MCI_convert				
ACACE*FH				
ACACE*APOE4			x	x
ACACE*Time		x	x	
ACACE*Time*DX				
ACACE*Time*MCI_convert				
ACACE*Time*FH				
ACACE*Time*APOE4	x			

Supplementary Table 4.9 (continued)

SLF				
	FA	RD	AD	MD
Main Effect BHB	x			
BHB*Dx		x	x	
BHB*MCI_convert				
BHB*FH	x			
BHB*APOE4		x		
BHB*Time				
BHB*Time*DX				
BHB*Time*MCI_convert		x		
BHB*Time*FH		x		
BHB*Time*APOE4				
Main Effect of ACACE	x			
ACACE*Dx		x	x	x
ACACE*MCI_convert				
ACACE*FH	x			
ACACE*APOE4				
ACACE*Time				
ACACE*Time*DX				
ACACE*Time*MCI_convert		x		
ACACE*Time*FH				x
ACACE*Time*APOE4				

Supplementary Table 4.10 (continued)

CCG				
	FA	RD	AD	MD
Main Effect BHB				
BHB*Dx	x	x		
BHB*MCI_convert				
BHB*FH		x		
BHB*APOE4	x	x	x	x
BHB*Time				x
BHB*Time*DX				
BHB*Time*MCI_convert				
BHB*Time*FH		x		
BHB*Time*APOE4				
Main Effect of ACACE				
ACACE*Dx		x	x	
ACACE*MCI_convert				
ACACE*FH	x			
ACACE*APOE4		x		
ACACE*Time				
ACACE*Time*DX				
ACACE*Time*MCI_convert				
ACACE*Time*FH		x		
ACACE*Time*APOE4				

Supplementary Table 4.11 (continued)

SCC				
	FA	RD	AD	MD
Main Effect BHB				
BHB*Dx				
BHB*MCI_convert				
BHB*FH				
BHB*APOE4	x	x	x	
BHB*Time	x	x		x
BHB*Time*DX	x	x		
BHB*Time*MCI_convert				
BHB*Time*FH		x	x	
BHB*Time*APOE4				
Main Effect of ACACE		x		
ACACE*Dx				
ACACE*MCI_convert				
ACACE*FH			x	
ACACE*APOE4	x	x		x
ACACE*Time	x	x		x
ACACE*Time*DX	x			x
ACACE*Time*MCI_convert	x			
ACACE*Time*FH			x	x
ACACE*Time*APOE4	x			

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CHAPTER 5. GENERAL CONCLUSION

Alzheimer's disease is a complex disease with genetic, environment, and lifestyle risks; this disease may also contain a metabolic component. With many similarities with type II diabetes, including insulin resistance and inflammation, the foods we are eating may affect the functionality and disease progression in our brains. Based on our research, due to hypometabolism of glucose in individuals with AD, increases in ketone bodies would increase energy availability for the brain to compensate for the glucose deficiencies especially in at-risk populations. We believe future research should look into dietary changes, such as an increase in dietary fat in efforts to increase CCK, where we should see improved cognition, increases in tau proteins, and increases in gray matter in memory-specific regions of the brain. With increases in dietary fat, there should also be increases in ketone bodies in the serum, which should improve myelin integrity and decrease the amount of demyelination over time. Another dietary intervention that should be studied is a decrease in dietary carbohydrates, where we expect to see increases in glucose. This increase in serum glucose should lead to more myelin integrity in individuals with a parental FH of AD, while those suffering with the disease should increase the amount of demyelination occurring compared to individuals with MCI and normal cognition. With both dietary changes, we expect to see a reduction in inflammation and oxidative stress as well as a reduction in the effects of the diseases by providing the brain with an alternative source of fuel early, especially in the individuals with genetic risks of developing AD.