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Measures of MRI brain imaging biomarkers in middle age according to average Mediterranean diet score over the previous 25 years

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Epidemiology

by

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ABSTRACT OF THE THESIS

Measures of MRI brain imaging biomarkers in middle age according to average Mediterranean diet score over the previous 25 years

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Master of Science in Epidemiology

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Associate Professor Andrew Odegaard, Chair

The Mediterranean diet (MedDiet) has been linked with preservation of brain structures. Brain structural changes associated with cognitive impairment may occur during midlife, and timing of exposure may be critical. This study aims to examine the association of cumulative average MedDiet score from early through middle adulthood, and MedDiet scores at individual timepoints in relation to the midlife MRI brain measures. This study will also assess statistical mediation of brain measures on the association between cumulative average MedDiet score adherence and four cognitive domains: executive function, global cognition, processing speed, and verbal memory. 515 participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study were included. Cumulative average MedDiet scores were calculated by averaging scores from baseline, year 7, and year 20 (mean age 25, 32, and 45, respectively). MRI brain scans were obtained at year 25 (mean age 50). General linear models adjusted for demographic variables, lifestyle factors, and vascular and metabolic comorbidities were used to examine all exposure-outcome relationships. Cumulative average MedDiet scores measured in tertiles were not associated with brain

volumes. MedDiet scores examined separately at years 0, 7, and 20 were not associated with midlife brain measures. Higher cumulative average MedDiet scores were associated with better executive function (β= -0.38, p_{trend} <0.001). Brain volumes did not statistically mediate this association. This study's findings suggest that diet characterized by MedDiet scores were not associated with midlife brain volumes. Midlife brain volumes did not statistically mediate the association between the MedDiet and cognition.

INTRODUCTION

Given that available pharmacological treatments are ineffective in halting the progression of cognitive impairment[1], targeting modifiable risk factors remains the most effective means for preserving cognitive function. Procuring an understanding of how modifiable risk factors relate to underlying brain pathologies through neuroimaging studies is critical in order to pave the way for novel preventive measures. Furthermore, it is important to investigate life-course trajectories of risk factors in relation to biomarkers of cognitive impairment in order to identify the optimal time point for prevention.[2] Brain structural changes that underly cognitive impairment begin decades before manifestation of symptoms.[3] In fact, evidence suggests that structural brain deficits can manifest as soon as early adulthood.[4]

The extant literature supports that the Mediterranean diet (MedDiet), which is characterized by consumption of fish, unsaturated fats, whole grains, fruits and vegetables, nuts and legumes[5], may have neuroprotective properties.[6-8] Magnetic resonance imaging (MRI) studies have elucidated several brain regions that may be associated with the MedDiet. MRI studies done in older adults have found that higher MedDiet scores were associated with preserved white matter integrity[9,10], lower white matter hyperintensities[11], larger mediotemporal gray matter volume[12], and larger cortical thickness[13]. Since brain structural changes occur well before old age, investigations of MedDiet in relation to brain MRI measures in midlife are important for identifying critical exposure and outcome windows. The few studies that have investigated the relationship between the MEDdiet and brain measures in midlife have produced conflicting results. Two studies reported no association between the MedDiet and midlife brain structures[14,15], whereas two other studies

demonstrated links between higher MedDiet scores and larger midlife gray matter volumes in AD brain regions[16] and cortical thickness.[17] However, these studies only included diet scores from one time point, and therefore did not capture long-term dietary patterns. Furthermore, previous midlife brain MRI studies have not included dietary intake from early adulthood. This warrants further investigation in light of studies that have demonstrated detrimenal effects of vascular risk factors during early adulthood on future brain structural integrity[18,19].

To that end, the present study aims to investigate the association between cumulative average MedDiet scores form early through middle adulthood and volumetric brain MRI measures at 25 years of follow up in participants of the Coronary Artery Risk Development in Young Adults (CARDIA) brain MRI sub study. This study also aims to explore the assocation between MedDiet scores at individual timepoints and brain volumes at year 25. A prior CARDIA investigation[20] along with other previous studies have shown associations between higher cumulative average MedDiet scores and better cognitive function.[21-25] Therefore, this study's third aim is to assess statistical mediation of brain volumes on the relationship between cumulative average MedDiet scores and cognition.

METHODS

Study Design

The CARDIA study is a prospective cohort study of cardiovascular health in 5,115 Black and White adults from four US metropolitan areas: Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota, and Oakland, California. Participants were healthy young adults aged 18 to 30 years old at baseline (year 0) in 1985-1986.[26] Participants were followed up at eight time points over the course of 30 years: 1987 to 1988 (year 2), 1990 to 1991 (year 5), 1992 to 1993 (year 7), 1995 to 1996 (year 10), 2000 to 2001 (year 15), 2005 to 2006 (year 20), 2010 to 2011 (year 25), and 2015 to 2016 (year 30).[26] A study timeline of the visit years included in the present study along with corresponding variables is presented in Figure 1.

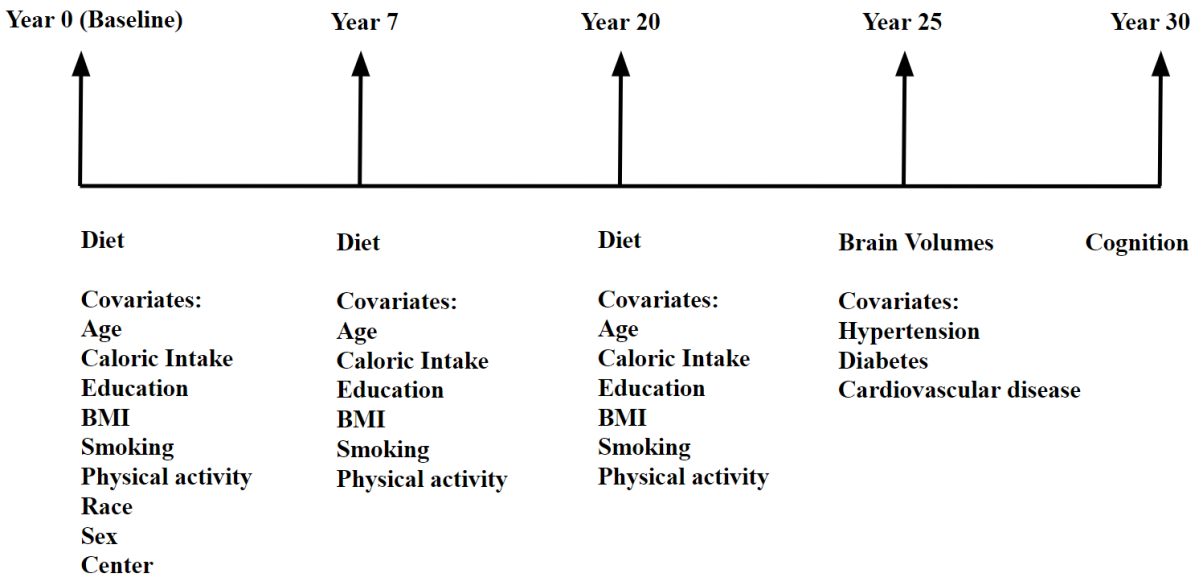

Figure 1: Study Timeline

Dietary intake was assessed at years 0, 7, and 20, and MRI brain scans were obtained at Year 25. During the Year 25 exam, 719 individuals partook in the CARDIA Brain MRI sub study, which aimed to characterize brain morphology, pathology, physiology and function.

Out of the 719 participants that underwent brain MRI at Year 25, 2 had missing data for certain brain regions. 577 out of the 717 participants with imaging data at Year 25 had complete dietary information from Year 20 and at least one other time point. Among these participants, 62 had implausible caloric intake (<600 or >8,000 kcal) at either Year 0, 7, and 20, and were therefore excluded from this analysis (n=62). The final analytic sample consisted of 515 participants (See Figure 2). The 515 participants included in the final analytic sample had dietary information from all three time points.

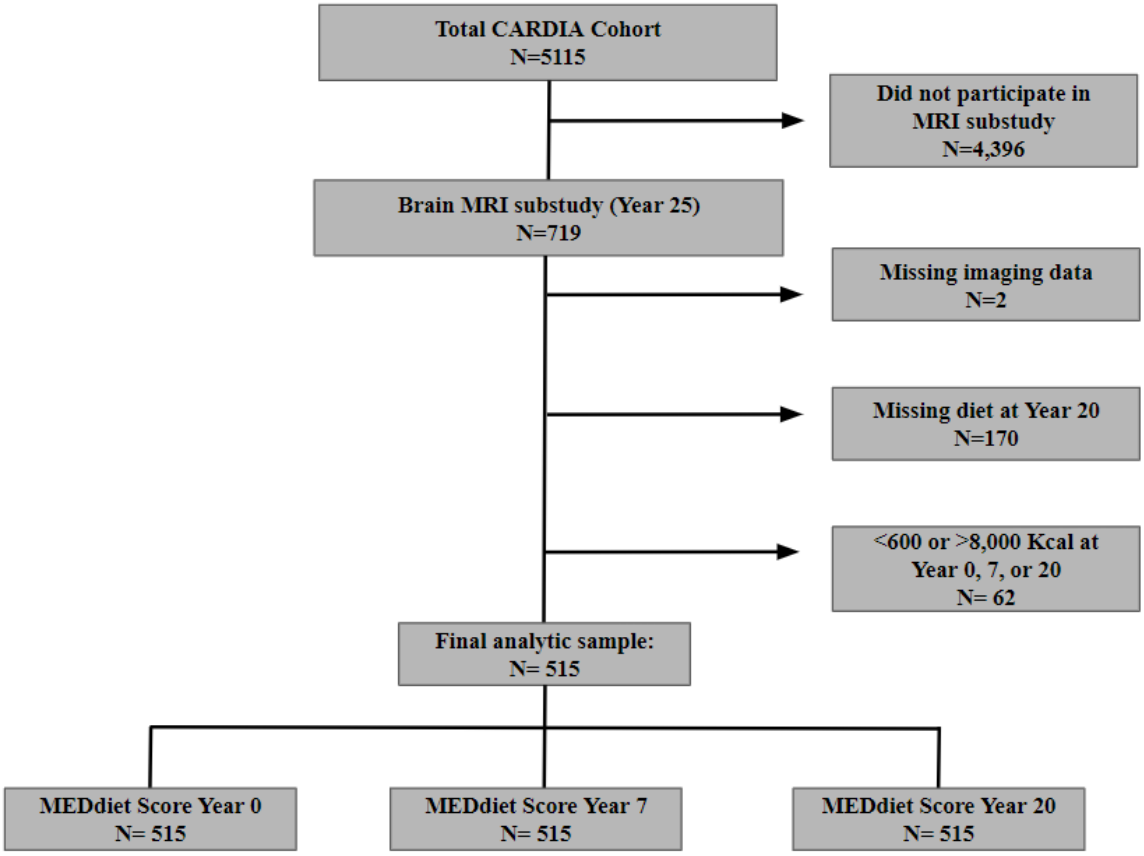

Figure 2: Exclusion Criteria

Standard protocol approvals, registrations, and patient consents

The study was approved by institutional review boards for the protection of human participants for the CARDIA study sites, and written informed consent was obtained from all participants at each examination.

Dietary assessment and dietary pattern scores

Dietary intake was assessed at years 0, 7, and 20 through an interviewer-administered, open-ended diet history.[27] Participants were asked open-ended questions about their food intake over the past month across 100 food categories.[27] Food and beverages were categorized into 166 groups by a food grouping system developed by the University of Minnesota Nutrition Coordinating Center (NCC).[27] Reported servings for each item were converted to standard serving in accordance with the US Department of Agriculture recommendations. Individual food group intake was calculated as the total number of standard servings reported per day of each food within a given food group.[27]

The MedDiet score (range 0-55) was calculated from 11 food items as previously done.[20] Non-refined grains, fruits, vegetables, potatoes, legumes, fish, alcohol, and the ratio of monounsaturated to saturated fatty acids were scored on a scale from 0-5, with a higher score indicating higher adherence to the Mediterranean diet. Red meat, poultry, and full-fat dairy were scored on a reverse scale. A ratio ≥2 of monounsaturated to saturated fatty acids was assigned a score of 5. Alcohol was scored as 0 for non-consumption or for consumption greater 10-50 grams for men and 5-25 grams for women.[28] A score of 5 was assigned for alcohol consumption of 10-50 grams for men and 5-25 grams for women.[28]

Brain MRI Methods

The following brain measures were studied: total brain, grey matter, normal white

matter, abnormal white matter, entorhinal area, amygdala, hippocampus, posterior cingulate gyrus, and precuneus volumes. MRI acquisition and processing have been described previously [29]. Exclusion criteria for sample selection included contraindication to MRI, possible pregnancy, or a body size that was too large for the MRI tube bore. 3T magnetic resonance scanners were used to acquire structural imaging, standardized across machines using a common machine head phantom (Oakland: Siemens [Munich, Germany] 3T Tim Trio/VB 15 platform; Minneapolis: Siemens 3T Tim Trio/VB 15 platform; and Birmingham: Philips [Best, the Netherlands] 3T Achieva/2.6.3.6 platform).[29] Using sagittal 3D T1 sequence, total intracranial volume (TICV) was estimated as the sum of gray matter (GM), white matter (WM), and CSF volumes, and total brain volume (TBV) as the sum of GM and WM volumes. Structural image processing was based on an automated multispectral computer algorithm that classified all supratentorial brain tissue into GM, WM, and CSF. WM was further characterized as normal (NWM) and abnormal (AWM) and into specific regions of interest.[29] AWM tissue was estimated from the sagittal 3D fluid-attenuated inversion recovery, T1, and T2 sequences. AWM included tissue damage due to ischemia, demyelination and inflammation, as well as the damaged penumbra tissue surrounding focal infarcts.[30] Volumes of abnormal GM and other regional brain structures were small and therefore not assessed. All image processing was done at the Section of Biomedical Image Analysis, Department of Radiology, University of Pennsylvania. Quality assurance protocols, developed for the Functional Bioinformatics Research Network, and the Alzheimer's Disease Neuroimaging Initiative, included the evaluation of scanner stability and image distortion prior to each site's acceptance and subsequent quarterly quality control evaluations including additional checks for motion artifacts or other quality issues before image

processing. Brain volumes were expressed as the percent of TICV. AWM was log transformed to normalize skewness.

Cognitive Measures

Cognitive function was assessed at year 30 using four cognitive tests. (1) Rey Auditory Verbal Learning Test (RAVLT) assessed verbal learning and memory; the number of words correctly recalled after a 10-minute delay was used in the current analyses (range 0–15), with higher scores indicating better performance.[31] (2) Digit Symbol Substitution Test (DSST) assessed processing speed and executive function (range 0–133), with higher scores for digits correctly substituted indicating better performance.[32] 3) The Stroop Test evaluated executive function by assessing the ability to view complex visual stimuli and to respond to one dimension while suppressing the response to another dimension.[33] The test was scored by the time to correctly state ink color (e.g., yellow) of color words (e.g., the word blue) plus number of errors; thus, a higher score (seconds plus errors) indicated worse performance. (4) The Montreal Cognitive Assessment (MoCA) to assess global cognitive function with components of attention, executive function, memory, language, visuospatial skills, calculations, and orientation.[34] Scores range from 0 to 30, with higher scores indicating better global cognitive function.

Covariates

Demographic variables, including age, race (Black or White), sex (male or female), education attainment (high school or less and greater than high school), caloric intake (Kcal), and CARDIA center, were obtained by self and interview administered questionnaires. Lifestyle factors included physical activity intensity and smoking status (current, former, never). Physical activity intensity, expressed in exercise units, was derived from 13

moderate and vigorous intensity exercises reported as part of the CARDIA physical activity questionnaire.[35] The algorithm used to calculate physical activity intensity was described previously.[36] Smoking status was self-reported. Vascular and metabolic comorbidities included body mass index (kg/m^2), binary classifications of diabetes, hypertension, and cardiovascular disease history. BMI was derived from weight and height measured in the clinic during visits. Diabetes diagnosis was defined as fasting glucose level ≥126, mg/dL which was assessed from blood samples, or self-reported intake of antidiabetic medication.[37] Hypertension was defined as systolic blood pressure ≥130 mm Hg or diastolic pressure ≥ 80 mm Hg, in accordance with AHA/ACC guidelines.[38] Blood pressure measurements were obtained by a trained technician using a standard automated BP measurement monitor (model HEM907XL; Omron, Bannockburn, Illinois) after a 5-minute seated rest.[37] Cardiovascular disease was ascertained by interviews, and included fatal or nonfatal coronary heart disease (myocardial infarction or non–myocardial infarction acute coronary syndrome), congestive heart failure, and stroke.[39]

Statistical Analysis

Cumulative average MedDiet scores were calculated by taking the mean of scores from baseline, year 7, and year 20 for the study's first and third aims. Differences between cumulative average MedDiet tertiles were assessed by ANOVA for continuous variables and chi-square for categorical variables. MedDiet scores were analyzed as tertiles in relation to brain volumes in order to assess a dose response relationship, and were presented as least square means and standard errors. General linear models were used to examine the association between MedDiet scores and brain volumes. Three models were constructed for this analysis. Model 1was adjusted for age, sex, race, education, caloric intake (Kcal), and

center. Model 2 was further adjusted for lifestyle factors: physical activity intensity and smoking status. Vascular and metabolic factors were included into Model 3: cardiovascular disease history, diabetes, BMI, and hypertension. Age, race, sex, and center were used from baseline (Year 0). The last non-missing values across years 0, 7, and 20 were used for education attainment and smoking status. BMI, physical activity intensity, and caloric intake were averaged from years 0, 7, and 20. Diabetes, hypertension, and cardiovascular disease history were obtained from Year 25.

MedDiet scores from individual timepoints were analyzed separately in relation to brain volumes using a fully adjusted (Model 3) general linear model. The goal of this analysis was to determine if the association between MedDiet and brain volumes differed depending on the time of exposure. This analysis was restricted to individuals with complete covariate information from years 0, 7, and 20. The following covariates were used from the timepoint corresponding with MEDdiet scores: age, education, caloric intake, smoking status, physical activity, and BMI. For example, the aforementioned covariates were taken from year 7 when examining MEDdiet scores from year 7 in relation to brain volumes. Beta coefficients (**β**) with 2-3 leading zeros were multiplied by 100, and **β** with >3 leading zeros were multiplied by 1000.

The association between cumulative average MedDiet scores as a continuous variable and cognitive test scores was examined using the three general linear models previously described. If cumulative average MedDiet scores were associated with cognitive test scores in the fully adjusted model, statistical mediation of brain volumes on the the relationship between MedDiet and cognition was assessed.

All statistical analyses were performed using SAS software version 9.4 for Windows (SAS Institute Inc., Cary, NC).

RESULTS

Participants Characteristics

The study consisted of 515 individuals, with a mean age of 25.6 ± 3.5 at baseline (Year 0), 32.5 ± 3.5 at Year 7, 45.6 ± 3.5 at Year 20, and 50.5 ± 3.5 at the time of the MRI brain scan (Year 25) (Table 1). 55.5% of the participants were female, 64.1 % White, 16.9 % current smokers, and 65.6% had completed education beyond high school. 23.1% had hypertension at Year 25, 1.8 % had cardiovascular disease, and 10.7 % had diabetes.

As shown in Table 1, participants in the highest cumulative average MedDiet tertile were more likely to be older, White, physically active, and have completed more than high school degree (all $p_{trend} > 0.05$). Participants in the highest cumulative average MedDiet tertile were also less likely to be current smokers, have hypertension, and have lower BMIs (all $p_{trend} > 0.05$).

As Table 2 shows, fish, fruit, legume, monounsaturated fat, potato, vegetable, and whole grain consumption increased linearly across MedDiet tertiles (all $p_{trend} > 0.05$). Dairy, poultry, and red meat consumption decreased linearly across MedDiet tertiles (all $p_{trend} > 0.05$). Alcohol consumption was also most optimal in participants in the highest MedDiet tertile (all $p_{trend} > 0.05$).

Table 1: Cohort characteristics by tertile of cumulative average MedDiet scores (N=515)

	Low (N = 161)	*Middle (N = 182)*	*High (N = 172)*	*Total (N = 515)*	*P Value*
Age at Year 0					
Mean (SD)	24.52 (3.56)	25.68 (3.62)	26.44 (2.93)	25.57 (3.47)	<0.001
Age at Year 7					
Mean (SD)	31.48 (3.60)	32.65 (3.60)	33.33 (2.94)	32.51 (3.47)	<0.001
Age at Year 20					
Mean (SD)	44.56 (3.61)	45.69 (3.61)	46.47 (2.92)	45.60 (3.48)	<0.001
Age at Year 25					
Mean (SD)	49.47 (3.55)	50.68 (3.60)	51.38 (2.90)	50.54 (3.45)	<0.001
Age at Year 30					
Mean (SD)	54.39 (3.70)	55.73 (3.62)	56.48 (2.94)	55.58 (3.53)	<0.001
Sex (Baseline)					
Female, N (%)	87 (54.04%)	106 (58.24%)	93 (54.07%)	286 (55.53%)	0.66
Race (Baseline)					
White, N (%)	73 (45.34%)	115 (63.19%)	142 (82.56%)	330 (64.08%)	<0.001
Education (Cumulative)					
>High School, N (%)	83 (51.55%)	115 (63.19%)	140 (81.40%)	338 (65.63%)	<0.001
Smoking Status (Cumulative)					
Current Smoker, N (%)	38 (23.60%)	31 (17.03%)	18 (10.47%)	87 (16.89%)	0.002
Hypertension (Year 25)					
Yes, N (%)	45 (27.95%)	48 (26.37%)	26 (15.12%)	119 (23.11%)	0.009
Cardiovascular Disease (Year 25)					
Yes, N (%)	4 (2.48%)	4 (2.20%)	1 (0.58%)	9 (1.75%)	0.37
Diabetes (Year 25)					
Yes, N (%)	24 (14.91%)	19 (10.44%)	12 (6.98%)	55 (10.68%)	0.06
Physical Activity Intensity (Cumulative)					
Mean (SD)	304.77 (213.01)	345.34 (212.56)	454.43 (229.10)	369.09 (226.78)	<0.001
BMI (Cumulative)					
Mean (SD)	26.90 (5.11)	26.27 (4.82)	24.71 (4.04)	25.95 (4.75)	<0.001

Table 2: Dietary patterns by tertile of cumulative average MedDiet scores (N=515)

	Low (N = 161)	*Middle (N = 182)*	*High (N = 172)*	*Total (N = 515)*	*P Value*
Caloric Intake (Kcal)					
Mean (SD)	2545.33 (1014.38)	2521.37 (924.70)	2554.17 (863.29)	2539.81 (932.49)	0.94
Alcohol Consumption (0= None or >25 gm for females or >50 gm for males, 5= 5-25 gm for females, 10-50 gm for males)					
Mean (SD)	1.97 (1.29)	2.82 (1.40)	3.46 (1.32)	2.77 (1.46)	<0.001
Fish Consumption (0=None, 5=Highest)					
Mean (SD)	2.26 (1.17)	2.88 (1.08)	3.37 (1.11)	2.85 (1.20)	<0.001
Fruit Consumption (0=None, 5=Highest)					
Mean (SD)	2.23 (1.08)	3.00 (1.06)	3.74 (0.84)	3.00 (1.17)	<0.001
Legume Consumption (0=None, 5=Highest)					
Mean (SD)	1.88 (1.20)	2.60 (1.19)	3.12 (1.14)	2.55 (1.28)	<0.001
MUFA/SAFA (0=Lowest, 5= Highest)					
Mean (SD)	1.72 (0.96)	2.02 (0.99)	2.42 (0.89)	2.06 (0.99)	<0.001
Potato Consumption (0=None, 5=Highest)					
Mean (SD)	2.63 (1.19)	2.99 (1.00)	3.08 (1.04)	2.91 (1.09)	<0.001
Vegetable Consumption (0=None, 5=Highest)					
Mean (SD)	2.33 (0.90)	2.97 (0.95)	3.76 (0.87)	3.03 (1.07)	<0.001
Whole Grain Consumption (0=None, 5=Highest)					
Mean (SD)	2.28 (1.07)	2.93 (0.96)	3.67 (0.89)	2.97 (1.12)	<0.001
Dairy Consumption (0=Highest, 5= None)					
Mean (SD)	1.88 (1.04)	2.08 (1.01)	2.26 (1.04)	2.08 (1.04)	0.004
Poultry Consumption (0=Highest, 5= None)					
Mean (SD)	2.09 (1.04)	2.05 (1.05)	2.18 (1.13)	2.11 (1.08)	0.51
Red Meat Consumption (0=Highest, 5= None)					
Mean (SD)	1.77 (1.08)	1.96 (1.09)	2.48 (1.19)	2.07 (1.16)	<0.001

Cumulative average MedDiet scores and brain volumes in midlife

Cumulative average MedDiet scores were not associated with brain volumes during midlife in any of the three models (see Table 3).

Table 3: Association (Least Square Means (SE)) of cumulative average MedDiet scores in tertiles with brain volumes (MRI) at Year 25 (N=515)

Brain Volumes	Low Meddiet (N=161)	Mid Meddiet (N=182)	High Meddiet (N=172)	P-Value
Total Brain Volume				
Model 1	84.9 (0.22)	85.1 (0.20)	85.2 (0.22)	0.55
Model 2	84.9 (0.23)	85.1 (0.20)	85.2 (0.22)	0.53
Model 3	84.9 (0.23)	85.1 (0.20)	85.2 (0.22)	0.68
GM				
Model 1	46.7 (0.17)	46.8 (0.16)	46.9 (0.17)	0.50
Model 2	46.6 (0.18)	46.8 (0.16)	46.9 (0.17)	0.40
Model 3	46.7 (0.18)	46.8 (0.16)	46.9 (0.17)	0.55
WM				
Model 1	38.2 (0.13)	38.3 (0.12)	38.3 (0.13)	0.96
Model 2	38.3 (0.13)	38.3 (0.12)	38.3 (0.13)	0.99
Model 3	38.3 (0.13)	38.3 (0.12)	38.3 (0.13)	0.99
Abnormal WM				
Model 1	-3 (0.07)	-3 (0.06)	-3.1 (0.07)	0.30
Model 2	-3 (0.07)	-3 (0.06)	-3.1 (0.07)	0.31
Model 3	-3 (0.07)	-3 (0.06)	-3.1 (0.07)	0.32
Entorhinal Area				
Model 1	0.33 (0.003)	0.33 (0.003)	0.33 (0.003)	0.98
Model 2	0.33 (0.003)	0.33 (0.002)	0.33 (0.003)	0.81
Model 3	0.33 (0.003)	0.33 (0.002)	0.33 (0.003)	0.88
Amygdala				
Model 1	0.16 (0.001)	0.16 (0.001)	0.16 (0.001)	0.70
Model 2	0.16 (0.001)	0.16 (0.001)	0.16 (0.001)	0.77
Model 3	0.16 (0.001)	0.16 (0.001)	0.16 (0.001)	0.85
Hippocampus				
Model 1	0.56 (0.004)	0.56 (0.004)	0.56 (0.004)	0.55
Model 2	0.56 (0.004)	0.56 (0.004)	0.56 (0.004)	0.64
Model 3	0.56 (0.004)	0.56 (0.004)	0.56 (0.004)	0.69
Posterior Cingulate Gyrus				
Model 1	0.56 (0.004)	0.56 (0.004)	0.55 (0.004)	0.62
Model 2	0.56 (0.004)	0.56 (0.004)	0.55 (0.004)	0.73
Model 3	0.56 (0.004)	0.56 (0.004)	0.55 (0.004)	0.5
Precuneus				
Model 1	1.5 (0.01)	1.5 (0.01)	1.5 (0.01)	0.93
Model 2	1.5 (0.01)	1.5 (0.01)	1.5 (0.01)	0.95
Model 3	1.5 (0.01)	1.5(0.01)	1.5 (0.01)	0.95

Model 1: Adjusted for age, sex, race, education, caloric intake (Kcal), and center, Model 2: Model 1 + physical activity intensity and smoking status, Model 3: Model 2 + bmi, diabetes, hypertension, and cardiovascular disease

MedDiet scores at different time points and brain volumes in midlife

Higher MedDiet scores at Year 0 were associated with lower hippocampal (β= -0.10, p_{trend}= 0.028) and precuneus (β= -0.30, p_{trend}= 0.05) volumes. Higher MedDiet scores at Year 7 were modestly associated with lower abnormal white matter volume (β= -0.01, p_{trend}= 0.057). Year 20 diet scores were not associated with brain volumes. These results are summarized in Table 4.

Table 4: Association (β (SE)) of MedDiet scores at individual time points with brain volumes (MRI) at Year 25 (N=482)

Brain Volumes	Year 0	Year 7	Year 20
Total Brain Volume	-0.02 (0.02)	-0.01 (0.02)	0.02 (0.02)
GM	-0.03 (0.02)	-0.01 (0.02)	0.02 (0.02)
WM	0.20 (0.01)	0.20 (0.01)	-0.01 (0.01)
Abnormal WM[a]	-0.01 (0.01)	**-0.01 (0.01)+**	-0.01 (0.01)
Entorhinal Area	-0.01 (0.0003)	-0.05 (0.0003)	0.04 (0.0003)
Amygdala	-0.02 (0.0001)	-0.01 (0.0001)	0.003 (0.0001)
Hippocampus	**-0.10 (0.0004)***	**-0.10 (0.0004)+**	-0.03 (0.0004)
Posterior Cingulate Gyrus	0.01 (0.0004)	-0.02 (0.0004)	-0.03 (0.0004)
Precuneus	**-0.30 (0.001)***	-0.20 (0.001)	0.01 (0.001)

Adjusted for: Age, sex, race, education, caloric intake (Kcal), center, physical activity intensity, smoking status, bmi, diabetes, hypertension, and cardiovascular disease
+P values less than 0.10
** P value less than 0.05*
*** P value less than 0.01*
**** P value less than 0.001*

Mediation analysis: Brain volumes on the relationship between cumulative average MedDiet scores and cognition at Year 30

448 participants had complete data for all four cognitive tests at Year 30. The

association between cumulative average MedDiet scoresand cognitive test scores are shown in Table 5. Cumulative average MedDiet scores were not associated with RAVLT performance in any of the three models. Higher cumulative average MedDiet scores were associated with better MOCA scores in Model 1 (β= 0.08, p_{trend}= 0.018). Higher cumulative average MedDiet scores were associated with better performance on the DSST in all Models 1 and 2 (p_{trend} <0.05). Higher cumulative average MedDiet scores were associated with better performance on the Stroop test in all models (all p_{trend} <0.001). Therefore, mediation analyses were carried out for the Stroop test using the fully adjusted model. Brain volumes did not statistically mediate the relationship between cumulative average MedDiet scores and Stroop performance (see Table 6).

Table 5: Association (β (SE)) of cumulative average MedDiet scores and cognition at Year 30 (N=448)

Cognitive Test Scores	Model 1	Model 2	Model 3
DSST	0.36 (0.25)*	0.32 (0.16)*	0.28 (0.16)+
MOCA	0.08 (0.03)*	0.05 (0.03)	0.04 (0.03)
RAVLT	0.03 (0.02)+	0.03 (0.02)	0.02 (0.02)
Stroop	-0.44 (0.11)***	-0.42 (0.12)***	-0.38 (0.12)***

Model 1: Adjusted for age, sex, race, education, caloric intake (Kcal), and center, Model 2: Model 1 + physical activity intensity and smoking status, Model 3: Model 2 + bmi, diabetes, hypertension, and cardiovascular disease
+P values less than 0.10
** P value less than 0.05*
*** P value less than 0.01*
**** P value less than 0.001*

Table 6: Mediation analysis for brain volumes on the association between cumulative average MedDiet scores and the Stroop test (N=448)

	Stroop	
Brain Volumes	**% Mediated**	**P-value**
Total Brain Volume	0.36	0.76
GM	0.06	0.92
WM	1.8	0.52
Abnormal WM	1.1	0.63
Entorhinal Area	-0.61	0.67
Amygdala	-1.9	0.35
Hippocampus	2.8	0.36
Posterior Cingulate Gyrus	-0.38	0.85
Precuneus	-0.79	0.61

Adjusted for: Age, sex, race, education, caloric intake (Kcal), center, physical activity intensity, smoking status, bmi, diabetes, hypertension, and cardiovascular disease

DISCUSSION

This study investigated MedDiet scores from early through middle adulthood in relation to midlife brain MRI measures obtained at the 25th year of follow up. Overall, MedDiet scores were not associated with brain structural integrity at midlife. No association was found between cumulative average MEDdiet scores , which was calculated by averaging MedDiet scores from years 0, 7, and 20, and midlife brain volumes. MedDiet scores at individual timepoints were not associated with midlife brain volumes. Brain volumes from year 25 did not mediate the association between cumulative average MedDiet scores and cognition at year 30.

This study's findings are not in line with the majority of cross-sectional[9,11-13,40] and longitudinal[10,41] studies exploring late life MEDdiet scores and brain volumes done in elderly populations, which have reported that the MedDiet may confer protection against brain atrophy. A plausible explanation for this discrepancy is that the MedDiet is associated with age-related brain changes only in the short term. Life course studies with multiple assessments of diet are necessary in order to elucidate this matter. Another possibility is that the the association between the MedDiet and MRI brain measures may not be apparent during middle age[14], which would be in line with evidence suggesting that brain atrophy accelerates increasingly after the age of 60.[42] It would be of interest to acquire MRI data in the CARDIA study in the future closer to the time of dementia diagnosis to explore this possibility.

The few studies that have examined the association between the MedDiet and MRI-based brain volumetric measures in midlife have provided inconclusive evidence. Some of these studies have demonstrated positive associations between the MedDiet and brain

structural integrity, whereas some have reported no association. The few studies that have investigated this exposure-outcome relationship in middle-aged adults are not entirely comparable with our results since they investigated MedDiet scores strictly in midlife. A brain imaging study (mean age 50 ± 8 at baseline) done at NYU and Cornell with a three year follow up found no significant cross-sectional or longitudinal association between midlife MedDiet scores and midlife volumetric brain MRI measures[14],consistent with our findings regarding cumulative average and and midlife (Year 20) MedDiet scores. The UK Biobank study reported no association between a cross-sectional analysis of midlife (mean age 53.8 ± 6.9) MedDiet scores and MRI brain measures.[15] Contrastingly, a cross-sectional study of middle-aged adults (mean age 54 ± 11) reported an association between higher MedDiet scores and larger gray matter volumes in AD brain regions.[16] Similarly, another cross-sectional study of middle-aged adults (mean age 50 ± 11) found that higher MedDiet scores were associated with increased cortical thickness.[17] The differences in the findings of some of these studies and the present study may be attributed to the methods used to score MedDiet and differences in study design. The transcultural adaptability, applicability, and reliability of commonly used MedDiet scoring systems are not clearly understood.[43] This allows for the possibility that some scoring systems may not be appropriate for capturing MedDiet adherence across different populations. Establishing validated standard procedures and methodology to enhance generalizability would be essential for improving comparability of studies. Reverse causation may be present in the aforementioned studies that reported positive associations due to those study's cross-sectional design. Participants may have adopted healthier eating habits near the time of MRI scans to reverse or manage negative health outcomes related to brain volume.

The results generated from the analysis examining MedDiet scores at individual time points showed that higher MedDiet scores at Year 0 were associated with reduced hippocampal and precuneus volumes. These findings likely do not represent biologically relevant associations . To our knowledge, no prior study has reported a negative association between higher MedDiet scores and brain structural integrity. Artifacts within patients' bodies may reduce scanning quality or be confused with pathologies.[44] These paradoxical findings could also be due to noise present in the MRI scans. Noise present during the measurement process can negatively impact the accuracy and reliability of imaging data.[45]

This study's results do not support statistical mediation of brain volumes on the assocation between MedDiet and cognitive test scores. The brain regions analyzed in this study in relation to MedDiet have been linked with cognitive function in the literature.[46] There may be mechanisms other than brain volumes underlying the observed relationship between the MedDiet and cognitive test scores. However, the present study was not able to elucidate possible underlying pathways.

The current study has several strengths. First, this study included detailed repeated measures of dietary intake from early and middle adulthood over the course of 20 years. Second, bias due to reverse causality is less likely since dietary information was collected before the MRI visit. Reverse causation would occur in the event that participants change their diets close to the time of MRI due to health conditions related to brain pathology. Third, this study is among few to explore a wide range of brain measures related to cognitive impairment in the context of the MedDiet in a biracial sample. This study's limitation is the presence of selection bias into the CARDIA MRI substudy. The participants of the MRI sub study were more likely to be White, older, and more educated; less likely to be current

smokers, have hypertension, and have a history of cardiovascular disease; and had lower BMIs and higher MedDiet scores.

CONCLUSION

The current study does not support associations between dietary patterns characterized by the MedDiet from early through middle adulthood and midlife brain volumes. Additionally, brain volumes do not statistically medieate the association between the MedDiet and cognition. It is possible that the MRI scans were obtained too early into middle age (50 years old) to observe an exposure-outcome relationship. Future studies should investigate repeat MRI brain measures throughout middle age in order to identify time points in which the MedDiet may exert its benefits on brain structural integrity.

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