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Mediterranean Diet and Magnetic Resonance Imaging-Assessed Brain Atrophy in Cognitively Normal Individuals at Risk for Alzheimer's Disease

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Abstract

OBJECTIVES—Epidemiological evidence linking diet, one of the most important modifiable environmental factors, and risk of Alzheimer's disease (AD) is rapidly increasing. Several studies have shown that higher adherence to a Mediterranean diet (MeDi) is associated with reduced risk of AD. This study examines the associations between high vs. lower adherence to a MeDi and structural MRI-based brain atrophy in key regions for AD in cognitively normal (NL) individuals with and without risk factors for AD.

DESIGN—Cross-sectional study.

SETTING—Manhattan (broader area).

PARTICIPANTS—Fifty-two NL individuals (age 54+12 y, 70% women) with complete dietary information and cross-sectional, 3D T1-weighted MRI scans were examined.

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MEASUREMENTS—Subjects were dichotomized into those showing higher vs. lower adherences to the MeDi using published protocols. Estimates of cortical thickness for entorhinal cortex (EC), inferior parietal lobe, middle temporal gyrus, orbitofrontal cortex (OFC) and posterior cingulate cortex (PCC) were obtained by use of automated segmentation tools (FreeSurfer). Multivariate general linear models and linear regressions assessed the associations of MeDi with MRI measures.

RESULTS—Of the 52 participants, 20 (39%) showed higher MeDi adherence (MeDi+) and 32 (61%) showed lower adherence (MeDi-). Groups were comparable for clinical, neuropsychological measures, presence of a family history of AD (FH), and frequency of Apolipoprotein E (APOE) ε4 genotype. With and without controlling for age and total intracranial volume, MeDi+ subjects showed greater thickness of AD-vulnerable ROIs as compared to MeDi- subjects (Wilk's Lambda p=0.026). Group differences were most pronounced in OFC (p=0.001), EC (p=0.03) and PCC (p=0.04) of the left hemisphere. Adjusting for gender, education, FH, APOE status, BMI, insulin resistance scores and presence of hypertension did not attenuate the relationship.

CONCLUSION—NL individuals showing lower adherence to the MeDi had cortical thinning in the same brain regions as clinical AD patients compared to those showing higher adherence. These data indicate that the MeDi may have a protective effect against tissue loss, and suggest that dietary interventions may play a role in the prevention of AD.

Keywords

Alzheimer's disease; diet; Mediterranean diet; magnetic resonance imaging (MRI); early detection; brain imaging

INTRODUCTION

Epidemiological evidence linking diet, one of the most important modifiable environmental factors, and risk of Alzheimer's disease (AD), the most common cause of dementia, is rapidly increasing. Given the current lack of disease-modifying treatments, as well as increasing awareness that symptoms develop over many years or even decades, there has been growing interest in identifying effective strategies for prevention (1, 2). Delaying symptoms onset by as little as one year could potentially lower AD prevalence by more than 9 million cases over the next 40 years (1).

Several studies have provided evidence for dietary patterns that are protective against AD (3-8). Among possible dietary patterns (DPs), there is consensus that higher adherence to a Mediterranean diet (MeDi) is associated with reduced risk of AD (3, 4, 8-12). While regional differences may subsist, the MeDi is characterized by high intake of plant foods (i.e., fruits, nuts, legumes, and cereals); moderate consumption of dairy products, fish, poultry; with olive oil as the primary source of monounsaturated fats; low to moderate intake of wine, low intake of red meat and poultry, and very low intake of processed foods (12). This diet is known to be one of the healthiest dietary patterns in the world, and it has been associated with reduced risk of cardiovascular disease, cancer, and overall mortality rates (10, 13-15).

While there is growing interest in implementing dietary recommendations prior to the onset of symptoms of AD, the overall picture remains equivocal as clinical trials failed to show consistent relationships between the hypothesized protective nutrients and clinical outcome (16). These studies would greatly benefit from biological markers of disease as surrogate endpoints of clinical change (16), especially during the recently re-conceptualized preclinical stages of AD (2). In vivo biomarkers are needed to clarify how diet promotes healthy brain aging, and can therefore be protective against AD.

Pathologically, AD is characterized by presence of amyloid-beta (Aβ) plaques, neurofibrillary tangles and neuronal loss in selectively vulnerable brain regions (17). Neuronal loss in AD originates in the medial temporal lobes during the normal stages of cognition and spreads to cortical regions, especially posterior cingulate and parieto-temporal cortices, along with clinical progression (18). These changes can be visualized in vivo by means of Magnetic Resonance Imaging (MRI). Several studies have shown that brain atrophy can be detected on MRI several years prior to dementia onset and correlates with AD progression (17, 19-21).

MRI studies have shown that higher adherence to the MeDi is associated with reduced cerebrovascular disease burden (i.e., white matter lesions) in the elderly (22, 23). However, to the best of our knowledge, there are no MRI studies that examined the MeDi in relation to brain atrophy in cognitively normal (NL) individuals with and without risk factors of AD. Here, we investigated whether structural MRI-based measures of cortical thickness (i.e., brain atrophy) in key AD-regions differ among young to late middle-aged NL individuals as a function of higher vs. lower adherence to the MeDi.

METHODS

Participants

Among a larger pool of clinically and cognitively normal (NL) individuals participating in longitudinal brain MRI imaging studies at NYU School of Medicine, this study included a sub-set of 52 NL participants who completed clinical, laboratory, MRI exams and dietary questionnaires within 4 months of each other between 2013-2014. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. Informed consent was obtained from all subjects for participation in this NYU institutional review board-approved study.

All subjects underwent a thorough physical examination and a detailed medical history was recorded. Individuals with medical conditions or history of conditions that may affect brain structure or function, i.e. stroke, diabetes, head trauma, any neurodegenerative diseases, depression, hydrocephalus, intracranial mass, and infarcts on MRI, and those taking psychoactive medications were excluded. Subjects were 25-72 y of age, with education>12 y, Clinical Dementia Rating (CDR)=0, Global Deterioration Scale (GDS)<2, Mini Mental State Examination (MMSE)>28, Hamilton depression scale<16, Modified Hachinski Ischemia Scale<4 and normal cognitive test performance for age and education (24). None of the participants were diabetics, smokers, or met criteria for obesity as defined by a Body-Mass index (BMI)>30 kg/m2. While all subjects were normoglycemic young adults, the

Homeostasis Model Assessment (HOMA) (25) for insulin sensitivity was calculated, as there is evidence for an association between increased insulin resistance (IR) and reduced brain volumes in AD-regions (26). Presence of hypertension (HTN) was determined based on current antihypertensive treatment or blood pressure assessments performed in a sitting position after 5 min rest (systolic blood pressure≥ 140 mmHg or diastolic blood pressure≥ 90 mmHg) (27, 28). Subjects were divided into 2 groups based on presence (HTN+) or absence of HTN (HTN-). A family history (FH) of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires (24). DNA was obtained from venous blood samples to determine APOE genotypes using standard polymerase chain reaction (PCR) techniques (29, 30).

Dietary intake of nutrients

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Harvard/Willett's semi-quantitative food frequency questionnaire (SFFQ) (31). The SFFQ has been used and validated for the determination of nutrient intake in the elderly as well as in young adults, yielding high reliability (31-35). The 61 food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (grams per day) of each food group was then calculated by summing the intakes of member food items. The daily intake of nutrients was computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion(31).

Published methods were followed for the construction of the MeDi (3, 4, 8-12). Briefly, we first regressed caloric intake (in kilocalories) and calculated the derived residuals of daily gram intake for each of the following seven food categories: dairy, meat, fruits, vegetables, legumes, cereals, and fish. The median value was determined for each caloric intake-residual food category. Categories were divided into beneficial (fruits, vegetables, legumes, cereals and fish) or detrimental (meat and dairy products). A value of 0 or 1 was assigned to each subject based on their scores on each of the seven above categories, using sex-specific medians as cut-offs, following standardized scoring procedures. Specifically, (a) subjects whose consumption of beneficial components was below the median were assigned a value of 0, while those whose consumption was at or above the median were assigned a value of 1, for each of the 5 categories.

(b) Subjects whose consumption of detrimental components was at or above the median were assigned a value of 0, while those whose consumption was below the median were assigned a value of 1, for each of the 2 categories. (c) For fat intake (8th food category), we used the ratio of daily consumption of monounsaturated to saturated fats (in grams) (12) using sex-specific median cutoffs for assignment of values of 0 for low monounsaturated/ saturated fats ratio (reflecting higher intake of saturated vs. monounsaturated fats) and 1 for high monounsaturated/saturated fats ratio (reflecting higher intake of monounsaturated vs. saturated fats). (d) For alcohol intake (9th food category), alcohol consumption was dichotomized into mild to moderate alcohol consumption (>0 drinks per week but <2 drinks per day in the previous year) and no (0 g/day) or more than moderate (>2 drinks per day) consumption (3, 4, 8-12). Subjects showing mild to moderate consumption were assigned a

value of 1, other subjects a value of 0, as a moderate amount of alcohol consumption with meals is a characteristic component of the MeDi. The MeDi score was generated for each participant as the sum of the scores in the food categories (range 0-9), with a greater score indicating higher adherence to the MeDi. The MeDi score was analyzed as a dichotomous variable (<5: low vs. >5: high adherence) and as a continuous variable to facilitate comparison with other studies of the MeDi score.

Data Acquisition and Preparation

All subjects received a diagnostic and a research MRI study on a 1.5 T GE Signa imager (General Electric, Milwaukee, USA). The diagnostic study was performed using contiguous 3 mm axial T2-weighted images. The research scan was a 124 slice T1-weighted Fast-Gradient-Echo acquired in a sagittal orientation as 1.2 mm thick sections (field of view=25 cm, NEX=1, matrix=256x128, repetition time= 35 ms, echo time= 9 ms and flip angle=60 0, no interslice gaps). Clinical scans were used to rule out MRI evidence of hydrocephalus, intracranial mass, strokes, subcortical gray matter lacunes, non-specific white matter disease, and to identify focal white matter hyperintensities.

Volumetric segmentation, cortical surface reconstruction and parcellation of the research scans were performed using a data analysis pipeline based on the FreeSurfer software package (20, 36-38). The automated whole-brain segmentation procedure for volumetric measures of the different brain structures uses a probabilistic atlas and applies a Bayesian classification rule to assign a neuroanatomic label to each voxel (38). A label is automatically assigned to each voxel in the MRI volume based on probabilistic information automatically estimated from a manually labeled atlas. The atlas consists of a manually derived training set created by the Center for Morphometric Analysis (Massachusetts General Hospital, Harvard Medical School) from 40 individuals across the adult age range. The classification technique uses a registration procedure that is robust to anatomical variability, including the ventricular enlargement typically associated with aging, and which has been shown to be comparable in accuracy to manual labeling (38). Automated volumetric segmentation required only qualitative review to ensure that there was no technical failure of the application. The cortical surface was then reconstructed to measure thickness at each surface location, or vertex, to allow visualization of group differences at each vertex. Cortical thickness was obtained by reconstructing representations of the gray/white matter boundary and the cortical surface, and the distance between these surfaces at each point across the cortical mantle was calculated (20, 36-38). This method uses both intensity and continuity information from the entire 3D MR volume in segmentation and deformation procedures to construct representations of cortical thickness. The maps were created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The maps are not restricted to the voxel resolution of the original data and are thus capable of detecting sub-millimeter differences between groups (37). Maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full width at half maximum of 15 mm and averaged across participants using a non-rigid high-dimensional spherical averaging method to align cortical folding patterns (39). This procedure provides accurate matching of morphologically homologous cortical locations among participants on the basis of each individual's anatomy while minimizing

metric distortion, resulting in a measure of cortical thickness for each person at each point on the reconstructed surface. The surface was parceled into distinct regions of interest (ROIs). The cortical-surface model was manually reviewed and edited for accuracy. Minimal editing was performed according to standard, objective rules, including correction of errors in removal of non-brain areas and inclusion of white-matter areas of hypointensity adjacent to the cortical ribbon. Qualitative review and editing were performed, with blinding to the diagnostic status, by an expert neuroanatomist with more than 10 years of experience (Y.L.).

Thickness measures were calculated for 5 a priori selected ROIs which are known to show early atrophic changes in AD, including: entorhinal cortex, orbito-frontal cortex, inferior parietal lobule, inferior and middle temporal cortex and posterior cingulate cortex (17, 19-21). These ROIs were sampled separately for each hemisphere (Figure 1). The total intracranial volume (TIV) was used as the reference to account for possible differences in brain size.

Statistics

SPSS v.21 (SPPS Inc., 2013) was used for data analysis. Differences in clinical and demographical measures between groups were examined with $\chi 2$ tests and the general linear model (GLM), as appropriate. All regression models were tested for violations of the model assumptions. All dependent variables were normally distributed.

Multivariate GLMs with follow-up univariate post-hoc comparisons performed using F statistics were used to test for differences in ROI thickness between groups. Multivariate GLMs were used to test for main effects of MedDi group, with brain structure (5x2 levels) as the within-subjects factor (i.e., dependent variables), and MedDi group as the between-subjects factor (i.e., independent variable). The multivariate GLM is a statistical test procedure for comparing multivariate (population) means of two or more dependent variables of several groups. Unlike univariate analysis, it uses the variance-covariance between variables in testing the statistical significance of the mean differences, and in testing for interactions among the dependent variables. Age, gender, education, TIV, FH status (positive vs. negative FH), APOE genotype (APOE $\varepsilon 4$ carriers, APOE4+ vs. non carriers, APOE4-) [Model 1], BMI, HOMA-IR and HTN group [Model 2] were examined as confounders using two regression models so as to avoid over-fitting. Only covariates showing significant effects were retained in the models. Confounding variables which showed significant effects on the association between MRI measures and MeDi group were examined for interaction effects in adjusted models that included a 2-way interaction term. For example, to test main effects of MeDi group and MeDi × APOE interactions, GLMs were used, with brain structure (10 levels) as the within-subjects factor, and MeDi and APOE as between-subjects factors. Only significant interaction terms were retained in the model.

Results were considered significant at $p<0.05$. The multivariate approach controls for the multiple chances to find group differences, and it does so without assuming independence of the dependent variables, yielding corrected p values. We first examined all ROIs together (left and right hemisphere, i.e. 10 levels), and then each hemisphere separately (i.e., 5

levels). For the latter analysis, multivariate results were considered significant at a Bonferroni corrected p=0.05/2=0.025.

Linear regressions were used to evaluate the associations between MRI measures, neuropsychological measures (dependent variables), MeDi scores (independent variable), and the same confounds as above. MRI measures were regressed by age and TIV to generate age- and TIV-adjusted residuals. Neuropsychological measures were regressed by age and education to generate age- and education-adjusted residuals. Results were considered significant at $p<0.05$.

RESULTS

Subjects

Subjects' characteristics are found in Table 1. Of the 52 subjects, 20 (39%) showed higher adherence to a Mediterranean diet (MeDi+) and 32 (61%) showed lower adherence (MeDi-). There were no differences between MeDi groups for clinical, demographical and neuropsychological measures, frequency of APOE4 genotype and presence of a FH of AD. The MeDi- group showed a trend towards a higher frequency of HTN+ subjects than the MeDi+ group (p=0.06, Table 1).

MeDi group differences on MRI

All regions of interest. A multivariate GLM with 10 ROI measures (i.e., dependent variables), MeDi group (i.e., independent variable), age, gender, education, APOE, FH and TIV (i.e., covariates) showed significant effects of MeDi group on MRI measures (Wilk's Lambda p=0.015). In this fully corrected model, MeDi+ subjects had overall greater thickness of AD-vulnerable ROIs as compared to MeDi- subjects. Post-hoc analysis for each structure is presented in Table 2. On post-hoc examination, group differences were most pronounced in orbitofrontal cortex (OFC, 9%, p=0.004), entorhinal cortex (EC, 6%, p=0.028) and posterior cingulate cortex (PCC, 4%, p=0.05) of the left hemisphere, and there was a non-significant linear trend for PCC of the right hemisphere (3%, p=0.11).

Gender, education and FH were not significantly associated with MRI measures and did not show interactions with MeDi group. Removing these variables from the model left results unchanged (Wilk's Lambda p=0.026), with group differences being most pronounced in left OFC (8%, p=0.001), EC (7%, p=0.034) and PCC (4%, p=0.041), and with a trend for right PCC (4%, p=0.10).

While APOE was borderline associated with MRI measures (p=0.12), there was a significant interaction between MeDi and APOE group (Wilk's Lambda p=0.013). Post-hoc examination showed that the interaction was driven by the fact that APOE4- subjects showing higher MeDi adherence had the greatest ROI thickness of all other subgroups (Figure 2).

A multivariate GLM with 10 ROI measures (i.e., dependent variables), MeDi group (i.e., independent variable), age, TIV, BMI, HOMA-IR, and HTN group (i.e., covariates) left results substantially unchanged (Wilk's Lambda p=0.029), with MeDi+ subjects showing

overall greater ROI thickness than MeDi- subjects, with most pronounced group differences in left OFC (p=0.004) and EC (p=0.028). BMI, HOMA-IR and HTN were not significantly associated with MRI measures and did not show interactions with MeDi group. Removing these variables resulted in the same results as with Model 1 (age and TIV-adjusted data).

Left hemisphere. A multivariate GLM with 5 ROI measures, MeDi group, and covariates confirmed results from the entire ROI data set (Wilk's Lambda p=0.003; Table 2). Gender, education, FH, BMI, HOMA-IR and HTN were not significantly associated with MRI measures of the left hemisphere. Removing these variables from the model left results unchanged (Wilk's Lambda p=0.002), with more pronounced group differences in OFC (p=0.002), EC (p=0.02) and PCC (p=0.04). The interaction between MeDi and APOE group remained significant (Wilk's Lambda p=0.02; Figure 2).

Right hemisphere. A multivariate GLM with 5 ROI measures, MeDi group, and covariates showed no significant effects of MeDi group on MRI measures (Wilk's Lambda p=0.56, n.s., Table 2). None of the covariates, except for TIV, were significantly associated with MRI measures of the right hemisphere. Results remained unchanged after removing non-significant confounds from the model (Wilk's Lambda p=0.55, n.s.).

Correlations between MeDi scores and MRI measures

MeDi scores were significantly associated with OFC, EC and PCC of the left hemisphere, with and without correcting for covariates (Figure 3). For every unit increase in MeDi scores, thickness of OFC increased by β=0.51 units (R2=0.28, p<0.001), EC by β=0.25 units (R2=0.07, p=0.05), and PCC by β=0.28 units (R2=0.08, p=0.04; Figure 3). Given the semi-categorical nature of the MeDi scores, non-parametric tests were used to confirm these associations (Spearman's rho: OFC σ=0.47, p<0.001, EC σ=0.26, p=0.03, PCC σ=0.29, p=0.02).

Correlations between MeDi scores, clinical and cognitive measures

Controlling for age, higher MeDi scores were associated with a smaller hip-to-waist ratio (β=−0.25, p=0.03) and were borderline associated with lower plasma insulin and triglycerides levels (β=−0.18 and β=−0.13, p<0.09). There were no significant associations between MeDi score and neuropsychological measures, with or without controlling for covariates. The MRI scans of two representative cases showing higher vs. lower adherence to the MeDi are shown in Figure 4.

Discussion

Among young to late middle aged NL individuals, lower adherence to a MeDiet was associated with structural MRI-based cortical thinning (i.e., atrophy) in key AD-regions as compared to a higher adherence. These effects were restricted to brain areas of the left hemisphere, and were most pronounced in OFC, EC and PCC. These results were independent of possible risk factors for LOAD such as age, gender, education, APOE genotype, FH, as well as of BMI, insulin resistance and hypertension.

Prospective studies have provided evidence for a favorable relation of a MeDi-type diet with slower cognitive decline, reduced risk of progression from mild cognitive impairment (MCI) to AD, lower risk of AD, and reduced mortality in AD patients (3, 4, 8-12). These effects were independent of physical activity (40) and were not mediated by vascular comorbidity (10).

Various nutrients have been associated with the MeDi pattern, including B-complex vitamins, antioxidants, vitamin D, and polyunsaturated fatty acids (PUFA), which are all known to have neuro-protective effects ranging from anti-oxidant, anti-inflammatory and Aβ anti-oligomerization properties, to vasculo-protective and synaptic plasticity-enhancing effects, and to modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products (41-47). Conversely, higher intake of saturated fats is known to have negative effects on cardiovascular function (5, 7).

Our findings of increased cortical thinning in NL showing lower adherence to the MeDi are consistent with epidemiological findings, and provide a possible pathophysiological substrate to the clinical data. Moreover, while all our subjects had lab values within normal limits and MeDi groups were comparable for clinical measures, lower MeDi scores were associated with larger hip-to-waist ratios and, to a lesser extent, with higher plasma insulin and triglycerides levels, which lends further support to prior observations of less favorable medical profiles.

MeDi effects on MRI biomarkers were significant in the left, but not in the right hemisphere, and were most pronounced in OFC, EC and PCC. Previous MRI studies have shown that atrophic changes in these AD-vulnerable regions, especially of the left hemisphere, are associated with increased risk for developing memory impairments and dementia (48-51). Given the known relationship between brain atrophy and onset of clinical symptoms in AD (17), our data suggests that the pathological AD process leading to neuronal loss may be influenced by modifiable lifestyle practices, such as a healthy diet, during the normal stages of cognition. Additionally, a novel association between MeDi and APOE status was observed, as APOE4- showing higher adherence to the MeDi diet had the largest ROI thickness of all other subgroups. The APOE ε4 genotype is a well-established risk factor for late-onset AD and has been associated with increased brain atrophy in NL elderly (52). To our knowledge, there are no prior investigations of interactive effects of APOE status and MeDi diet on MRI biomarkers in NL individuals. Within the MeDi+ group, APOE4- showed greater cortical thickness than APOE4+, whereas no APOE group differences were observed within the MeDi- group. These findings suggest that diet may have greater impact on APOE4-, as APOE4+ subjects seem to develop brain atrophy regardless of diet, while APOE4- may put themselves at greater risk for AD-related brain changes by not following a healthy diet. These findings are consistent with previous reports of more beneficial effects of physical activity for APOE4-than APOE4+ individuals (53), although results are not always consistent (54, 55). More studies with larger samples and longitudinal follow-ups are warranted to replicate our preliminary research studies and to specifically examine the effects of APOE status on dietary patterns in AD, and whether the relationship varies with age and disease.

The biological mechanisms for the observed associations between MeDi and cortical thickness remain to be clarified. In the adjusted models, the association between MeDi and MRI features was essentially unchanged by including age, gender, education, presence of family history, APOE status, BMI, insulin resistance scores and presence of hypertension as possible confounds. These data suggest that MeDi is a protective factor independent of traditional AD risk factors. Other studies are needed to assess whether the observed association would change depending on additional factors such as vascular structure/ function or markers of inflammation (56).

Most, if not all participants reported stability of their dietary patterns over the past 2-5 years. Examination of our records showed that approximately 90% of the surveyed participants have been living the lifestyle reported in the surveys for 5 years or more, with a very conscientious focus on their diet and food choices. Approximately 8% of those surveyed reported their nutritional intake to be a lifestyle span of about 2-5 years. Only 1 participant in the MeDi- group reported their nutritional behavior starting within the last 1-2 years. Overall, our MeDi+ cohort included people for whom the MeDi was their normal dietary pattern, and most of the MeDi+ participants reported following the MeDi since childhood. Previous longitudinal studies of the MeDi with repeated dietary assessments over up to 13 years, demonstrated that adherence to the MeDi is remarkably stable over time, especially in healthy individuals (10, 57, 58). However, while we consider it more likely that the MeDi adherence reported reflects our population's longstanding dietary habits, because of the synchronous timing of dietary and MRI assessments and the cross-sectional nature of our study, we cannot exclude that adherence the MeDi may be a more recent lifestyle choice in our cohort. Since this is the first study demonstrating an association between the MeDi and MRI biomarkers of AD in a relatively young NL population, future studies are needed to replicate our preliminary research findings, to test whether cortical thickness changes only after long-term exposure to certain ingredients of MeDi (e.g., vitamin B, antioxidants, etc.) or whether short-term exposure is sufficient to preserve brain volumes, and whether adherence to the MeDi from a very young age would be particularly beneficial to healthy brain aging. These biomarker findings are valuable for future research studies as well as possible randomized clinical trials in which participants are assigned to a standard low-fat diet vs. MeDi, with change in ROI thickness being a primary endpoint or outcome measure.

MeDi scores were not associated with neuropsychological measures, most likely because our subjects were cognitively normal and all high-school graduates, which resulted in a “ceiling-effect”. As such, present cross-sectional findings do not offer information on risk of future AD in our NL cohort. Longitudinal studies with larger samples are warranted to determine whether reduced brain thickness in MeDi- vs. MeDi+ subjects is predictive of cognitive decline, and whether the relationship between MeDi, AD-biomarkers and cognitive performance varies with age and disease. Our preliminary results provide the rationale for performing a larger, longitudinal study to assess how diet, biomarkers and risk factors of AD modulate AD-risk years in advance of possible clinical symptoms. We caution that present results were found in small numbers of carefully screened subjects under controlled clinical conditions. Most participants belonged to middle-class; none were smokers, diabetics, met criteria for obesity or had significant cardiovascular disease. Replication of these preliminary findings in community-based populations with more

diversified socio-economic and medical status, as well as with other biomarkers of AD, particularly of AD pathology (i.e., amyloid beta and neurofibrillary tangles), is warranted and clinical application is not justified.

Conclusions

Our biomarker findings provide biological evidence in support of epidemiological studies showing that the MeDi diet may be protective against AD. In our study, lower adherence to the MeDi was associated with increased atrophy of key brain regions for AD among NL individuals, which provides support for further exploration of dietary behavior as a possible AD prevention strategy.

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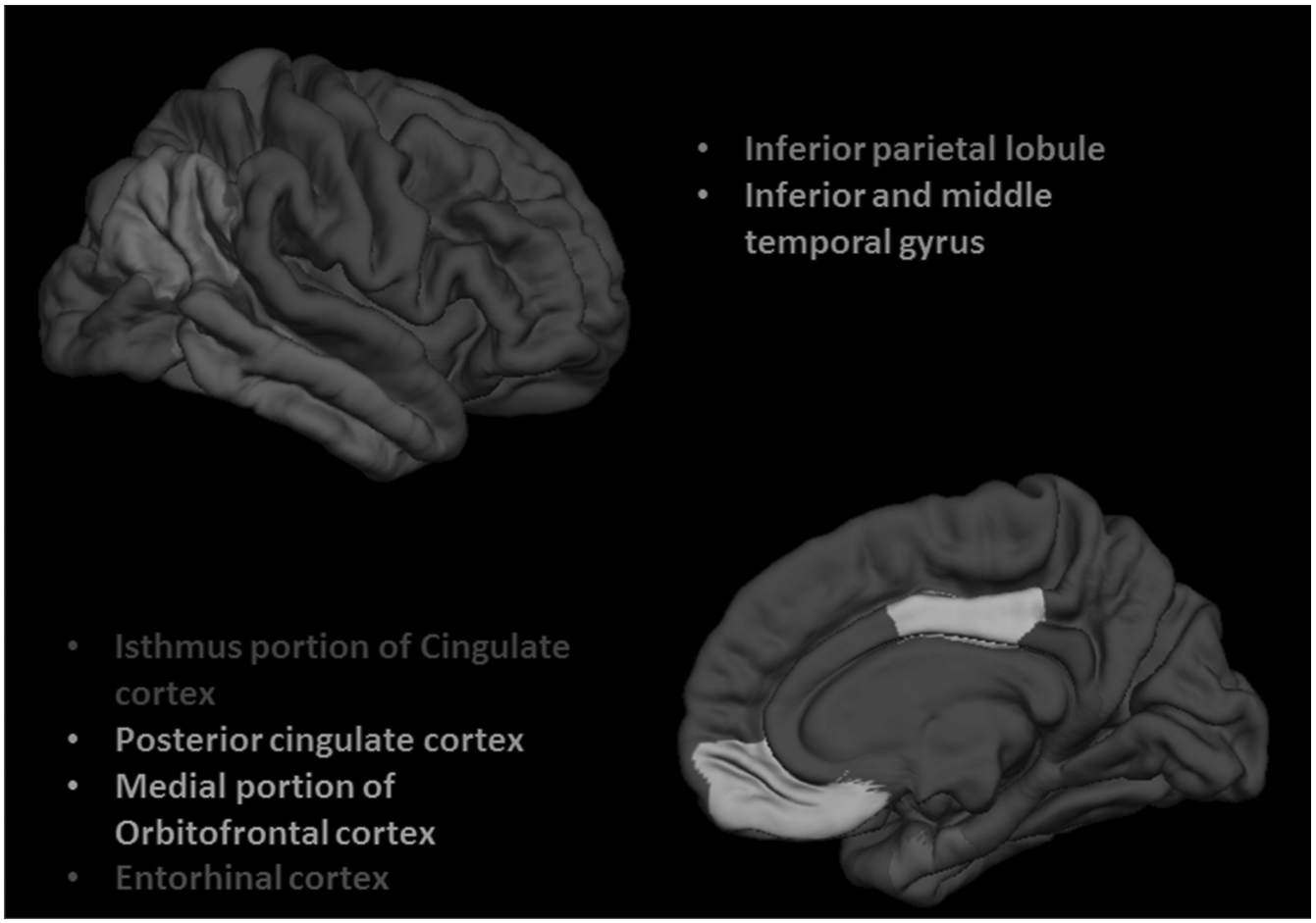

Figure 1.

Three-dimensional representations of the 5 ROIs examined in the current study (only right hemisphere is shown). All of the ROIs are visible in the lateral (top) and medial (bottom) views of the gray matter surface

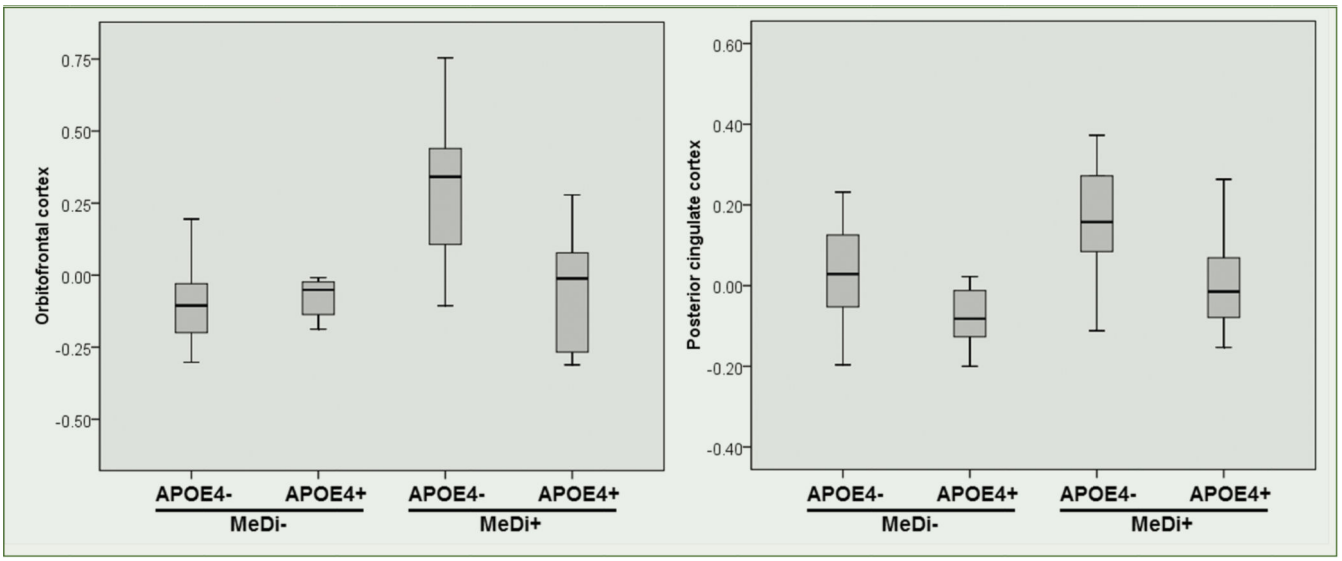

Figure 2.

Mediterranean diet and APOE genotype interactions on regional MRI thickness

Abbreviations: MeDi = Mediterranean diet group (MeDi- = lower adherence vs MeDi+ = higher adherence), APOE4 = Apolipoprotein E ε4 allele (APOE4- = non carriers, APOE4+ = carriers). MRI measures are age and total intracranial volume-adjusted residuals

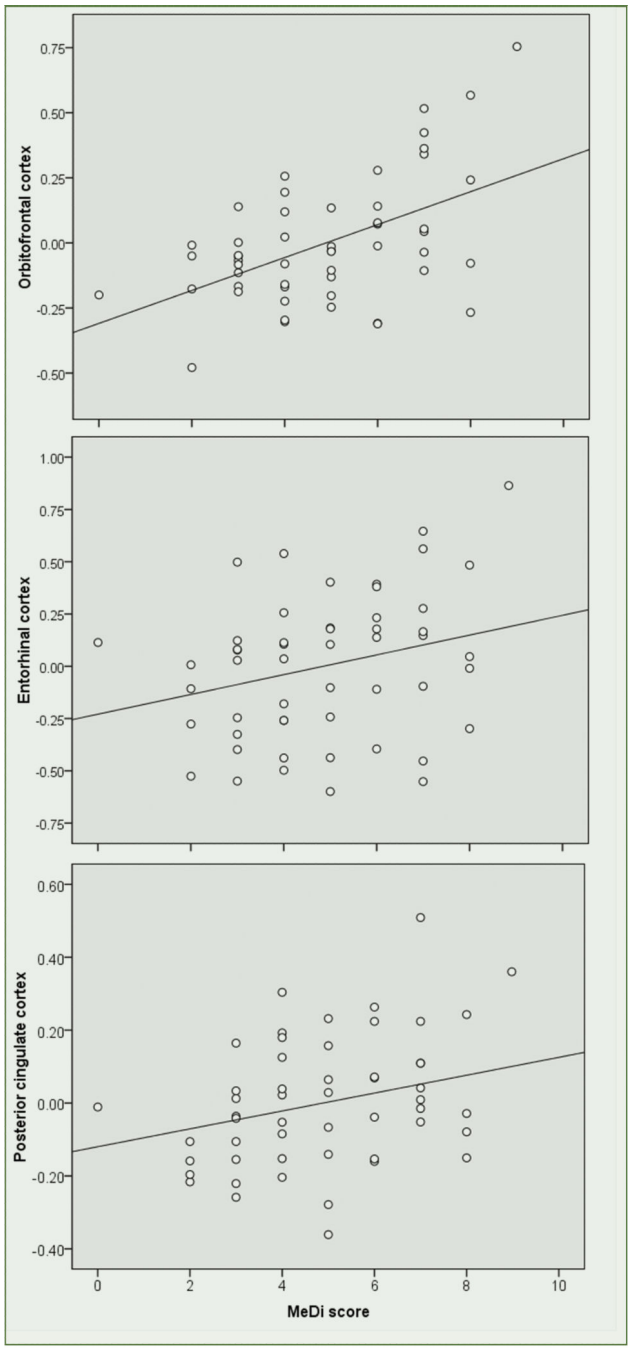

Figure 3.

Associations between Mediterranean diet scores and regional MRI thickness

MRI measures are age and total intracranial volume-adjusted residuals

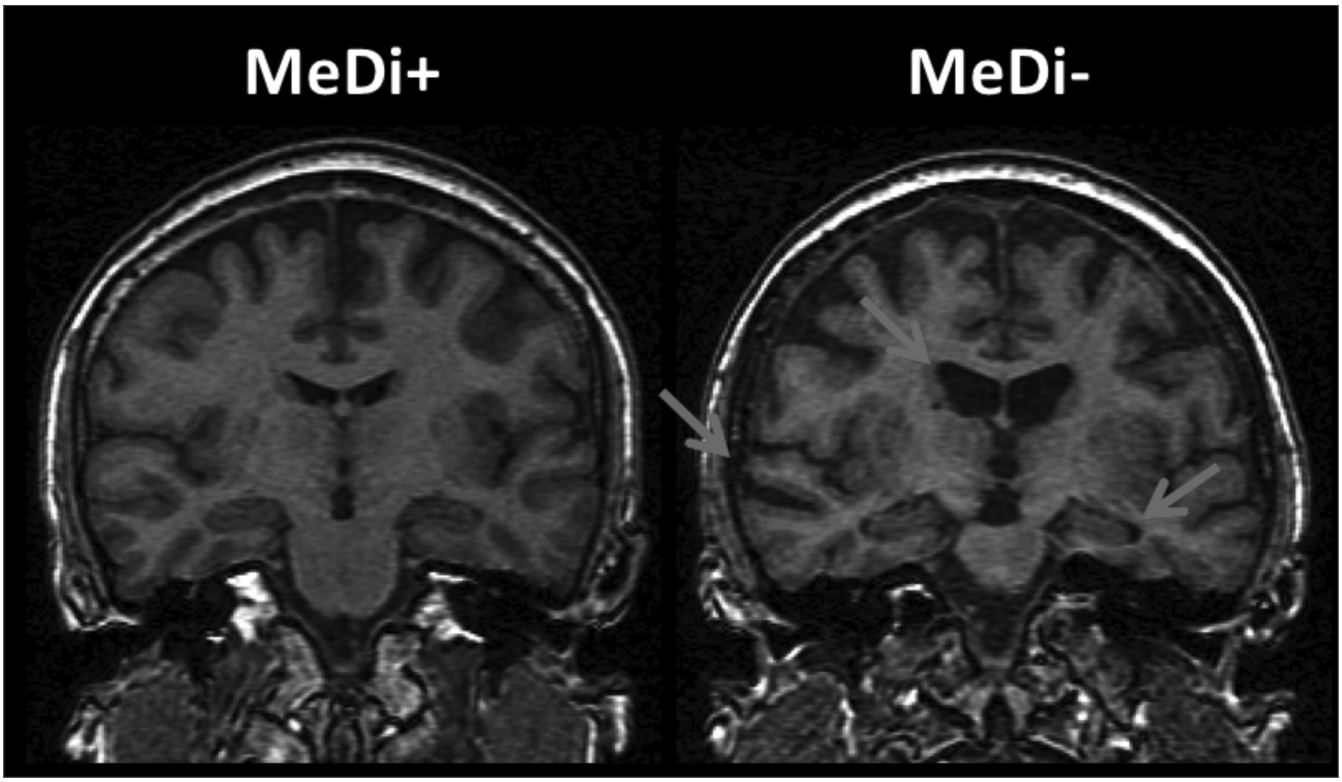

Figure 4.
MRI scans of two representative NL cases showing higher vs. lower adherence to the MeDi. Participants were 52 and 50 year old, respectively, with MMSE>28, education>12 y, normal cognitive test performance by age and education. The MeDi+ subject shows no ventricular enlargement or hippocampal atrophy by age. The MeDi- subject shows mild ventricular enlargement, hippocampal and temporal cortex atrophy by age (arrows)

Table 1

Demographic and clinical characteristics by MeDi group

	MeDi-	MeDi+
N	32	20
Age, y, mean (SD)	53(13)	55(12)
Gender, % female	63%	85%
Education, y, mean (SD)	16(2)	16(2)
Family history of LOAD, % positive	69%	60%
APOE ε4 status, % positive	39%	56%
Ethnicity		
White	83%	80%
Black	6%	3%
Hispanic	3%	6%
Other	8%	11%
MeDi score [unitless]	3.6(1.1) range 0-5	6.9(1.2) range 6-9
Hypertension, % positive	31%	10%
HOMA-IR score [unitless]	1.5(1.9)	1.7(2.0)
Hip to waist ratio [unitless]	1.18(0.12)	1.16(0.11)
Blood pressure (mm/Hg)		
Systolic	117(15)	122(12)
Diastolic	69(15)	69(18)
Glucose (mg/dl)	76(9)	81(15)
Cholesterol (mg/dl)	193(35)	206(41)
HDL: LDL ratio (unitless)	0.6(0.2)	0.5(0.2)
Triglycerids (mg/dl)	86(34)	96(49)
Homocysteine (micromol/l)	10(2)	10(3)
Neuropsychological tests		
Mini Mental State Exam	29(1)	29(1)
Digit symbol substitution	65(10)	63(11)
Paired associates delayed recall	7(2)	6(3)
Paragraph delayed recall	10(2)	10(3)
Designs	8(2)	8(2)
Object naming	55(12)	53(13)
WAIS-vocabulary	67(10)	63(18)

Values are mean (SD) unless otherwise specified; Abbreviations: MeDi = Mediterranean diet, lower (MeDi-) vs. higher (MeDi+) adherence

Table 2

Regional MRI thickness measures by MeDi group

		Uncorrected data				Age and TIV-adjusted data			
		MeDi-		MeDi+		MeDi-		MeDi+	
ROI thickness (cm)	Side	mean	SEM	mean	SEM	mean	SEM	mean	SEM
EC	Left	*3.17	0.06	3.38	0.08	*3.16	0.06	3.40	0.08
	Right	3.35	0.07	3.38	0.08	3.33	0.07	3.41	0.08
IPL	Left	2.52	0.03	2.54	0.04	2.52	0.03	2.55	0.04
	Right	2.54	0.03	2.52	0.03	2.55	0.03	2.52	0.03
MTG	Left	2.95	0.03	2.93	0.04	2.96	0.03	2.94	0.04
	Right	3.01	0.03	3.05	0.03	3.02	0.03	3.04	0.03
OFC	Left	**2.44	0.04	2.67	0.05	**2.45	0.04	2.67	0.05
	Right	2.39	0.03	2.43	0.04	2.39	0.03	2.43	0.04
PCC	Left	*2.56	0.03	2.66	0.04	*2.56	0.03	2.66	0.04
	Right	2.50	0.03	2.58	0.04	2.50	0.03	2.59	0.04
TIV (cm^3)	n.a.	1514	0.286	1577	0.399	n.a.			

Lower than MeDi+

Abbreviations: EC = entorhinal cortex, IPL = MeDi = inferior parietal lobule, Mediterranean diet, lower (MeDi-) vs. higher (MeDi+) adherence, MTG = middle temporal gyrus, n.a. = not applicable, OFC = orbitofrontal cortex, PCC = posterior cingulate cortex, TIV = total intracranial volume

*$p<0.05$

**$p<0.01$ on post-hoc univariate GLM analysis