Mediterranean diet, Alzheimer's disease biomarkers and brain atrophy in old age

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Abstract

Objective: To determine if following a Mediterranean-like diet (MeDi) relates to cognitive functions and *in vivo* biomarkers for Alzheimer's disease (AD), we analyzed cross-sectional data from the German Longitudinal Cognitive Impairment and Dementia Study

Method: The sample (n=512, mean age: 69.5±5.9 years) included 169 cognitively normal participants and subjects at higher AD risk (53 AD relatives, 209 SCD and 81 MCI). We defined MeDi adherence based on the Food Frequency Questionnaire. Brain volume outcomes were generated via voxel-based morphometry on T1-MRI and cognitive performance with an extensive neuropsychological battery. AD-related biomarkers (Aβ42/40 ratio, pTau181) in cerebrospinal fluid were assessed in n=226 individuals. We analyzed the associations between MeDi and the outcomes with linear regression models controlling for several covariates. Additionally, we applied hypothesis-driven mediation and moderation analysis.

Results: Higher MeDi adherence related to larger mediotemporal gray matter volume ($p<0.05$ FWE corrected), better memory ($\beta \pm SE = 0.03 \pm 0.02$; $p=0.038$), and less amyloid (Aβ42/40 ratio, $\beta \pm SE = 0.003 \pm 0.001$; $p=0.008$) and pTau181 pathology ($\beta \pm SE = -1.96 \pm 0.68$; $p=0.004$). Mediotemporal volume mediated the association between MeDi and memory (40% indirect mediation). Finally, MeDi favorably moderated the associations between Aβ42/40 ratio, pTau181 and mediotemporal atrophy. Results were consistent correcting for ApoE-ε4 status.

Conclusion: Our findings corroborate the view of MeDi as a protective factor against memory decline and mediotemporal atrophy. Importantly, they suggest that these associations might be explained by a decrease of amyloidosis and tau-pathology. Longitudinal and dietary intervention studies should further examine this conjecture and its treatment implications.

Introduction

Healthy dietary patterns, such as the Mediterranean diet (MeDi), might reduce the risk of dementia and cognitive decline [1–4]. Although contrasting findings have been reported as well [5,6], encouraging results were provided by the PREDIMED study, a randomized clinical trial in which a MeDi intervention was associated with both improved cognitive functioning [7] and reduced incident mild cognitive impairment [8]. Likewise, adherence to MeDi could diminish the conversion rate from mild cognitive impairment to dementia [9,10].

At the biomarker level, MeDi has been associated with preserved cortical thickness and brain volume in middle-aged [11,12] and old individuals [13–15], especially in brain regions associated with aging and Alzheimer's disease (AD). Moreover, adherence to MeDi has been related to lower amyloid load studied with ^{11}C-Pittsburgh compound B[PiB]-PET in cognitively unimpaired individuals [11,16,17], while another study could not find such an association using ^{18}F-Florbetaben-PET [18]. Furthermore, one study found an association in both volunteers with subjective or mild cognitive impairment (SCD and MCI, respectively) between MeDi and lower FDDNP-PET, a compound measure of amyloid and tau pathology [19]. Two longitudinal studies reported better MeDi adherence to be associated with less amyloid accumulation over time [17,20].

This initial evidence suggests that MeDi might reduce amyloid deposition since midlife with a probable downstream effect on neurodegeneration and cognition. We additionally hypothesized that MeDi is associated with Tau levels and moderates the associations between amyloid, tau and brain atrophy. Here, we examined these questions by leveraging a large cohort of old individuals at increased risk for AD.

Materials and Methods

Participants

As of July 2020, the baseline of the German multicenter Longitudinal Cognitive Impairment and Dementia Study (DELCODE) includes 1079 individuals. A complete overview of the study design, group definitions and aims is provided in Jessen et al. (2018) [21]. Here, we selected 512 subjects (average age ± standard deviation (SD): 69.49±5.86, 270 female, self-reported sex) according to availability of both the detailed Food Frequency Questionnaire (FFQ) and T1-weighted MRI. The sample was enriched for risk of AD as it included individuals with SCD (n=209, 41%) or amnestic MCI (n=81, 16%) who were referrals to the participating memory clinics. SCD participants reported self-perceived cognitive decline with concerns, while showing a preserved performance in all tests of the Consortium to Establish a Registry for Alzheimer's Disease – CERAD – neuropsychological battery (above -1.5 standard deviations compared to age, sex and education adjusted norms). Conversely, amnestic MCI subjects performed below -1.5 standard deviations on the delayed-recall trial of the CERAD word-list episodic memory tests. The clinical diagnoses were part of the clinical work-up at each site (not of DELCODE itself) and conformed to published research criteria [22–24]. In addition, first-degree relatives of AD patients (n=53, 10%) and cognitively normal volunteers without increased risk for AD (n=169, 33%) were recruited with an advertisement campaign on the local newspaper. Both groups met the requirement for an unimpaired cognitive performance on the CERAD battery (as the SCD group).

Complete demographic information is reported in Table 1 and stratified by clinical group in Table e-2. A sub-sample of 226 participants additionally underwent lumbar puncture for assessment of AD-related neuropathological biomarkers in cerebrospinal fluid (CSF). Comparing the groups with and without CSF information we did not find

differences in age, sex distribution, prevalence of ApoE-ε4, body mass index (BMI), kcal/day, level of physical activity (as measured with the Physical Activity Scale for the Elderly) [25] or MeDi score. However, subjects with CSF data available had a lower educational attainment, a higher prevalence of MCI and, accordingly, a lower performance in the mini-mental scale examination (Table e-1).

Standard Protocol Approvals, Registrations, and Patient Consent

At each DELCODE site, the local institutional review boards approved the study protocol and the ethical committees issued local ethical approval. DELCODE is registered at the German Clinical Trials Register (DRKS00007966; 4/05/2015). The study protocol followed the ethical principles for human experimentation in accordance with the Declaration of Helsinki. All participants in the study provided written informed consent.

Magnetic Resonance Imaging acquisition

The acquisition of structural brain images was performed with 3 Tesla MRI scanners mounting 32-channel head array coils. A 3D T1-weighted Magnetization Prepared-RApid Gradient Echo – MPRAGE – sequence was used, with echo time of 4.37 ms, repetition time of 2500 ms, inversion time of 1100 ms and flip angle of 7°. All images had a 1 mm^3 isotropic nominal image resolution with a final image matrix of 256×256×192. Four different MRI scanners from SIEMENS manufacturer (Siemens Healthcare, Erlangen, Germany) were used across centers: MAGNETOM TrioTim (N=209), Verio (N=163), Skyra (N=110), and Prisma (N=30). Image quality assessment is described in the supplements (Dryad-link).

Cognitive assessment

All study participants underwent an in-depth neuropsychological assessment to cover a broad spectrum of cognitive functioning [21]. Our analysis focused on five factor scores derived from a confirmatory factor analysis and capturing the cognitive performance in different domains: memory, language, executive functions, working memory and visuospatial abilities. Rationale and methods for the definition of factor scores are described in Wolfsgruber et al. (2020) [26]. A list of the cognitive tests contributing to each cognitive domain is reported in Table e-3.

Dietary assessment and MeDi score definition

We administered the German adaptation of the semi-quantitative European Prospective Investigation of Cancer FFQ (EPIC-FFQ) [27] (more details in supplements). Our sample of 512 participants did not include subjects who reported abnormal daily energy intake defined as less of 500 kcal/day or more than 5000 kcal/day (n=4) and subjects who did not answer more than 20% of the FFQ questions (n=2).

We computed the *a priori* MeDi score based on sex-specific medians from this study population. Briefly, food items from the EPIC-FFQ were clustered into 9 food categories. A score of 1 was assigned when the food intake for one subject was equal or above the sex-specific median for six food categories typical of MeDi (fish, vegetables, fruits/nuts, legumes, cereals and higher ratio of monounsaturated/saturated fats) or below the cut-off for foods non-typical of MeDi (meat, dairy products). For alcohol, a moderate consumption (10-50 g/day in men and 5-25 g/day in women) was

considered beneficial and scored 1 point. The final MeDi score can span from 0 to 9, with higher values indicating higher adherence [28]. Table e-4 and a Figure e-3 display each food category stratified by MeDi score (low, medium, high) and sex.

Cerebrospinal fluid sampling and assessment

A subsample of 226 participants consented to undergo lumbar puncture. All procedures were guided by DZNE standard operating procedures (see supplementary methods). We focused our analyses on phosphorylated tau 181 (pTau181), amyloid-beta 1-42 (Aβ42), on their ratio Aβ42/pTau181 and on the ratio amyloid-beta 42/40 (Aβ42/40) to take into account individual differences in overall Aβ peptide concentrations [29].

Voxel-based morphometry analysis

We applied voxel-based morphometry [30] to study the relationship between gray matter volume and MeDi. All analyses were performed using the Computational Anatomy Toolbox (CAT12) and Statistical Parametric Mapping (SPM12, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) running on Matlab® 2014b (The MathWorks Inc., Natick, MA). All T1-MRI images were normalized to the Montreal Neurological Institute – MNI – standard space and segmented into gray matter, white matter and cerebrospinal fluid compartments. Modulation of preprocessed MRI images included both linear and non-linear deformations (i.e. Jacobian determinants) to account for contractions and expansions during image normalization. Image smoothing was applied with a 8 mm full-width-at-half-maximum Gaussian kernel.

Total intracranial volume and total gray matter volume were extracted from CAT12 output.

The association between MeDi score and gray matter volume was investigated via application of the general linear model (one-sample t-test in SPM12) entering age, sex, total intracranial volume and MRI scanner type as nuisance covariates. Heterogeneity in MRI devices was expressed using one-hot encoding for categorical data to avoid order effects. Additionally, we re-run the analysis correcting also for kcal, BMI, physical activity levels and ApoE-ε4 status. The model was first applied at the whole-brain level, without any *a priori* hypothesis and then restricted to hypothesis-driven regions of interest (ROI) in the mediotemporal lobe, which shows early changes in AD [31]. Anatomical ROI were selected from the Automated Anatomical Labeling – AAL – atlas using the Wake Forest University Pickatlas tool for SPM (bilateral hippocampi and parahippocampal gyri). Of note, the entorhinal cortex is included in the parahippocampal gyrus ROI as defined in the AAL atlas (Figure e-4). Correction for multiple comparisons was performed with the non-parametric threshold free cluster enhancement – TFCE – approach implemented in SPM (http://www.neuro.uni-jena.de/tfce/). We used the TFCE technique with 5000 permutations, weighting parameters for cluster extent E=0.6 and height H=2 and a significance level of $p < 0.05$ (Family-Wise Error – FWE – corrected).

Statistical analysis on CSF variables and cognitive factors

We assessed the associations between MeDi and cognition or CSF variables with linear regression models adjusted for age, sex and education. The analysis was repeated including supplementary covariates to control for potential confounding effects from BMI, caloric intake and physical activity, as well as for ApoE-ε4.

Outliers identified on CSF variables were removed from the analysis, leading to the exclusion of 12 subjects who had values at 1.5 multiplied by the interquartile range below or above the 25th or the 75th percentile, respectively. Figure e-2 displays the distributions of CSF variables. We repeated the analysis without outlier exclusion (applying log transformation to pTau181) and with robust linear regression, which is less sensitive to outliers. Finally, all linear models were corrected for the time distance between baseline visit (when biomarkers and cognitive assessment took place) and FFQ questionnaire (mean±SD: 41.5±43.17 weeks: median: 51.7 weeks).

Mediation analysis

We created hypothesis-driven models and tested them with mediation and moderated mediation analysis. All models were created with *processR* and estimated with *lavaan* package (version 0.6-5, http://lavaan.ugent.be/) in R 3.6.3.

The aim of *Model 1* was to investigate the interplay between MeDi, brain volume and memory function. Specifically, we hypothesized that the brain changes observed in the bilateral hippocampi and parahippocampal regions mediate the association between MeDi and memory identified in the regression analyses (Figure 2). The model included all the 512 subjects in the study. Gray matter values were extracted from the significant cluster from the ROI-based analysis using MarsBaR toolbox for SPM. In order to assess the specificity of the mediation effect for mediotemporal regions, we replicated a similar mediation model using total gray matter volume as mediator. A parameter to model the indirect effects of MeDi on memory via brain measures was included.

We then designed additional models to disentangle the moderation effect of MeDi on the associations between Aβ42/40 ratio and pTau181 and brain volume in

mediotemporal regions. In particular, we adopted the theoretical framework of the amyloid cascade hypothesis according to which amyloidosis is the earliest upstream pathological event that leads to tau phosphorylation and finally to brain atrophy [32]. The following models were therefore performed on the sub-sample with CSF information. The rationale for these models is that MeDi adherence might sustain brain maintenance, thus reducing the development of disease-related brain changes and pathology [33]. In particular, we expected that MeDi moderates the paths connecting neuropathology and brain atrophy as defined by the amyloid cascade model. First, we tested a mediation model reflecting the amyloid cascade hypothesis itself, i.e. Aβ42/40 → pTau181 → brain volume (*Model2.0*). Then, we tested two additional models where MeDi score was added as moderator either of the path connecting Aβ42/40 to pTau181 (*Model2.1*, first stage mediation) or on the path connecting pTau181 to brain volume (*Model 2.2*, second stage mediation). This analysis allows to test if the associations between Aβ42/40 and pTau181 and between pTau181 and brain volume vary at different levels of MeDi. A schematic visualization of the models is presented in Figure 2.

In all models we included age, sex and education level as background confounds and brain measures were additionally corrected for total intracranial volume. Additionally, we tested the influence of ApoE-ε4 as covariate. The significance of the associations was based on confidence intervals generated with bias corrected bootstrap with 10000 replicates. In the moderated mediation models, all predictors were mean centered. For *Model 2.1* and *2.2* direct and indirect effects were evaluated at different levels of the moderator (i.e. MeDi) using the mean ± 1 standard deviation approach. In addition, we report the index of moderated mediation, which reflects if the indirect effects vary at different levels of the moderator.

Exploratory analysis of MeDi diet components

To explore the individual contribution of each of the nine MeDi score components, we run additional linear regression models. Dependent variables were the memory factor score, brain volume in hippocampal and para-hippocampal regions, pTau181 or Aβ42/40 ratio. In each model, we entered all dichotomous MeDi components at once, correcting for age, sex, education, caloric intake, BMI and physical activity.

Data availability

Anonymized data generated and analyzed in the current study will be made available upon reasonable request from qualified investigators.

Results

Brain volume

Whole-brain results. The MeDi score showed a significant positive association with brain gray matter volume in the right parahippocampal gyrus and right hippocampus ($p<0.05$ FWE corrected). The opposite contrast did not show any negative. Results are shown in Figure 1, left panel and in Table 2. Figure e-1 shows the results corrected using the less conservative $p<0.05$ FDR approach (Drylad-link).

ROI-based results. Restricting the analysis to *a priori* ROI revealed a bilateral association between higher MeDi and increased gray matter volume in hippocampi and parahippocampal gyri ($p<0.05$ FWE corrected). Of note, we observed also in this analysis a right>left asymmetry (Figure 1, right panel and Table 2). The reverse

contrast did not reveal any inverse association. Of note, a 1-point increase in MeDi corresponds to an increase in brain volume in the significant cluster associated with -0.84 years of age. The result of whole-brain and ROI-based analyses were stable correcting for kcal, BMI, physical activity and ApoE-ε4 status. The unthresholded T-maps of whole-brain models are available at Neurovault (https://neurovault.org/collections/KMIELIOW/).

Cognition

The models adjusted for age, sex and education showed an association between MeDi and both memory ($F(4,507)=57.87$, $p<0.001$, $R^2=0.31$) and language ($F(4,507)=59.22$, $p<0.001$, $R^2=0.32$) but not for the other domains (Table 3). In the models additionally corrected for BMI, caloric intake and physical activity, only the association between an increased adherence to MeDi and an improved memory performance remained ($F(7,482)=30.57$, $p< 0.001$, $R^2=0.31$). Here, a 1-point increase of MeDi corresponded to an increase of memory performance associated with almost -1 year of age. Correcting for ApoE-ε4 and time distance between baseline visit and FFQ did not change the results (Table 3 and Table e-7).

CSF biomarkers

The linear regression models showed significant associations of MeDi with pTau181 ($F(4,209)=6.02$, $p<0.001$, $R^2=0.103$), Aβ42/40 ($F(4,209)=6.15$, $p<0.001$, $R^2=0.105$) and Aβ42/pTau181 ($F(4,209)=6.29$, $p<0.001$, $R^2=0.107$).

The associations of MeDi with pTau181 ($F(7,197)=4.118$, $p<0.001$, $R^2=0.128$), Aβ42/40 ($F(7,197)=3.509$, $p=0.0014$, $R^2=0.111$) and Aβ42/pTau181

($F(7,197)=3.933$, $p<0.001$, $R^2=0.123$) were stable additionally controlling for BMI, caloric intake and physical activity (Table 3). Higher adherence to MeDi showed associations with pTau181 and both Aβ42/Aβ40 and Aβ42/pTau181 ratios. Specifically, in the adjusted models, a unity increase in MeDi score was associated with a decrease of 1.96 pg/mL of pTau181 and with an increase of 0.0027 and of 0.71 in Aβ42/Aβ40 and Aβ42/pTau181 ratios, respectively. For comparison, a 1-point increase in MeDi corresponded to a decrease of the neuropathological burden on Aβ42/Aβ40 and pTau181 associated with over -3 years of age (-3.5 and -3.33 years, respectively). Correcting for ApoE-ε4 reduced the associations between MeDi and CSF biomarkers for amyloid (but showing a consistent pattern of results, Table 3), while the time distance between baseline visit and FFQ did not influence the results (Table e-7). We observed very similar results in the analysis without outlier exclusion and using both linear and robust linear regressions (Table-e5).

Mediation models

Model 1 revealed a significant indirect effect of MeDi on memory via brain volume in hippocampal and para-hippocampal regions (est=0.017, ci= 0.007 to 0.03). Notably, the direct effect of MeDi on memory was no longer significant (est=0.025, ci= -0.005 to 0.056), thus suggesting complete mediation. The indirect pathway representing the effect of MeDi on memory via hippocampal and para-hippocampal volume accounted for 40% of the total effect. The replication of *Model 1* using total gray matter volume showed a significant direct effect, while the indirect effect was weak and accounted only for 4.6% of the total effect (Table e-6).

Model 2.0 showed a complete mediation of Aβ42/40 on brain volume through pTau181, in that only the indirect effect (est=0.109, ci=0.009 to 0.0239) was

significant and explained 34% of the total effect. In *Model 2.1* we observed a significant index of moderated mediation (est=-0.02, ci= -0.065 to -0.001) and significant indirect effects at all levels of the moderator. The indirect effect was larger for lower values of MeDi and decreased for higher MeDi score. The proportion of the total effect mediated by the Aβ42/40 → pTau181 → brain volume path at different levels of MeDi was 39% at -1 standard deviation, 32% at the mean level and 23% at +1 standard deviation. *Model 2.2* showed a significant index of moderated mediation (est= -0.047, ci= -0.101 to -0.004) and a significant indirect effect only at the lowest level of the moderator, i.e. at -1 standard deviation. Complete details are displayed in Table 4. All mediation and moderated-mediation models showed consistent results when correcting for ApoE-ε4 (Table 4).

Individual contributions of MeDi diet components

Table e-9 displays the results of the exploratory analysis on individual MeDi components. With MEM as dependent variable we observed a significant positive association only for cereals (p=0.013). Congruently, only cereals showed a marginally significant positive association with mediotemporal volume (p=0.056). For both pTau181 and Aβ42/40 ratio a significant association was found with the ratio of monounsaturated/saturated fat (p=0.021 and p=0.038, respectively). Specifically, an increased ratio of monounsaturated/saturated fat was associated with increased levels of Aβ42/40 and decreased burden of pTau181.

Discussion

Overall, our results suggest that the favorable association between MeDi adherence and memory performance, found here as in many previous studies, could be mediated

by preservation of brain volume in mediotemporal regions. Moreover, we showed that MeDi adherence is inversely associated with both pathological biomarkers for amyloidosis and tauopathy, which underlie AD. Finally, our data shows that a healthier diet moderates the associations between Aβ42/40, pTau181 and brain atrophy, suggesting that MeDi contributes to brain maintenance [33].

First, we observed a significant association between MeDi and hippocampal and para-hippocampal regions in both whole-brain and in ROI-based analyses. This is in line with studies that reported positive associations between MeDi and brain morphology in cognitively normal mid- and old-aged subjects and in non-demented elderly individuals [11–15]. However, one study reported no significant association between MeDi and brain volume [34] and one other reported an association only with meat consumption, but not with MeDi as a whole [35].Compared to these studies, we analyzed a larger sample enriched for AD risk, thus possibly making our analysis more sensitive to capture brain structural variations related to MeDi. Moreover, in both negative studies there was a larger temporal distance between dietary and MRI data assessments (5 and 9 years, respectively) which might have influenced the results. Several hypotheses could be advanced concerning the link between diet and brain structural integrity. Considering our moderated mediation results, we hypothesize that the adherence to MeDi protects brain structures from the adverse effects of upstream pathological events, i.e. accumulation of amyloid plaques and tau phosphorylation. This hypothesis would clarify why the association between MeDi and brain structure is specific for the mediotemporal regions, as AD-related atrophy starts in these regions and co-localizes with tau accumulation.

The second main finding is the favorable association between MeDi and memory performance. In particular, we show a significant positive association between diet

and a composite memory factor score which, capitalizing on an in-depth memory assessment, was used to quantify the level of memory performance in our sample [26]. This finding replicates previous work performed on a smaller interim release of DELCODE [36] and is in agreement with the view of MeDi as a protective lifestyle factor against cognitive decline and dementia [1–3]. Despite a protective effect of MeDi has been reported for general cognition and for different cognitive domains, memory seems to be the one that benefits more from a healthy diet [15,37,38], in line with the regional specific association with brain volume. The analysis of the individual MeDi score components showed a significant association between memory and the item 'cereals'. This supports previous studies showing a protective effect of cereals, and in particular whole grains, on cognition [37,39]. We propose that the specificity of our findings for the memory domain should be interpreted in light of the mediation analysis, showing that the mediotemporal volume mediates the association between MeDi and memory. Of note, the mediation effect was specific for the mediotemporal regions, in that the mediating effect of total gray matter volume was very weak.

Finally, the analysis of the sub-sample with CSF information allowed us to investigate the associations between MeDi and AD-related biomarkers as well as to model their interplay with brain volume. First, we reported that MeDi is associated with lower levels of amyloid as expressed by the Aβ42/40 ratio and with reduced pTau181. In agreement with our observations, previous studies in middle- and old-age cognitively normal subjects reported that diet is associated with reduced amyloid levels and amyloid accumulation as studied with PiB-PET assessments [17,20]. Of note, we observed a significant association between MeDi and Aβ42/40 ratio, but not with Aβ42. Previous studies suggested that Aβ42/40 ratio is a more sensitive biomarker for AD as compared to Aβ42 [29]. Moreover, a recent study on a cell culture model of AD

showed the relevance Aβ42/40 ratio, but not total amyloid, as driver of tau pathology [40]. The mediation *Model 2* is in line with the amyloid cascade hypothesis, showing a link between Aβ42/40, pTau181 and brain atrophy [32]. Then, in *Model 2.1* and *2.2* we showed that MeDi exerts a significant moderation effect both on the association between Aβ42/40 ratio and pTau181 and, to a lesser extent, on the one between pTau181 levels and brain atrophy, specifically mitigating their associations. However, these models should be interpreted with caution as they rely on cross-sectional data and cannot therefore prove causal pathways. A possible (and speculative) mechanistic interpretation of these observations is that MeDi acts on the triggers that connect these pathological events, for example inflammation [41] and oxidative stress [42]. MeDi is indeed based on higher consumption of fruits and vegetables, whole grains, fish and olive oil that are known for their anti-inflammatory and antioxidant actions [43]. Future studies could include markers for inflammation or oxidative stress to test more fine-grained hypotheses concerning the underlying biological processes.

Notably, the exploratory analysis of the individual MeDi components showed a beneficial association between the ratio of monounsaturated/saturated fat and both pTau181 and Aβ42/40 ratio. Monounsaturated fats are found in many food sources such as plant oils, nuts, seeds, and animal products and a combination of them likely accounted for the total level in our study. In Mediterranean regions higher scores of monounsaturated/saturated fat ratio most likely reflect higher consumption of extra-virgin olive, which has been associated with reduced AD-pathology in mice [44] and with better cognitive performance in human subjects of the PREDIMED trial [8].

A strength of the present study is the availability of multiple data types, which enabled the integration of dietary information, cognitive data, brain morphometry and CSF biomarkers. This allowed us to model not only the associations between MeDi

and the single variables of interest, but also their interplay. Another strength is that the sample is enriched for AD risk. While this constrains generalization to the old population at large, it allows studying the interaction of diet with substantial variation of amyloid, tau, and brain neurodegeneration in a group that could be a target for nutritional intervention trials. We additionally repeated the regression models excluding individuals with MCI, the highest-risk clinical group. This showed a stable association of MeDi with mediotempoal brain volume, but not with other outcomes, pTau181, Aβ42/40 ratio and memory (Table e-10). This might indicate that the beneficial association between MeDi and AD-related biomarkers and cognition are more pronounced in the prodromal AD stages. However, these negative findings might also be attributable to reduced power in the sub-sample analysis and to lower variability in the outcomes.

A limitation of the present cross-sectional study is that it does not allow causal inference. However, MeDi diet scores are stable over years in older adults, even in the years before a diagnosis of incident dementia [1,45] and Maude et al. showed that the longitudinal trajectories of MeDi over 15 years are comparable between women who showed cognitive decline and those who did not in the Nurses' Health Study [46]. Therefore, we posit that MeDi adherence reflects the past aggregate exposure to the MeDi ingredients, so that the statistical associations with MeDi described above could result from accumulated long-term causal effects of diet. The extension to longitudinal data, including data from DELCODE follow-ups, should be the next step to address this limitation and validate the proposed models. Moreover, it has to be noted that the analysis of the single components presented here is exploratory and should be validated by more focused studies. Future studies in humans and animal models could focus on specific hypothesis-driven dietary components and leverage on

modern techniques to directly measure their effects on the metabolome and microbiome [47]. On the same line, recent efforts to map the chemical complexity of diets provide a promising avenue for a deeper understanding of the effects of diet on health and disease [48]. It has to be mentioned that previous studies reported an association between different dietary patterns (i.e. Western diet and the Alternative Healthy Eating Index 2010) and risk of dementia and cognitive decline [49] or AD-related markers, such as hippocampal volume [5]. This might question if the results reported in our study are specific for MeDi or rather reflect a more general advantage of a healthy diet. This is linked to another limitation of our and similar studies where MeDi adherence is defined on sample medians, thus representing the relative adherence to dietary guidelines and not the high consumption of beneficial foods in absolute terms as in Mediterranean regions. Moreover, it is possible that MeDi has systemic effects on health (e.g. modulating inflammation or cardiovascular health [50]) that might in turn influence AD-specific mechanisms. Our results were stable when controlling for factors associated with cardiovascular risk (BMI, physical activity and smoking, see Table e-8), but a deeper investigation of this topic is needed. The study of many other biomarkers such as diffusion tensor imaging, resting-state functional connectivity and markers for neuroinflammation, especially in longitudinal study design, could help generating a more comprehensive and mechanistic understanding of the effects of MeDi on cognition in old age and early AD.

In conclusion, our study supports the view of MeDi as a protective lifestyle factor against AD-related neurodegeneration and memory impairment. Longitudinal studies with AD biomarker outcomes could further examine this conjecture and pave the way for dietary interventions to delay AD.

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Appendix 1 – Authors contribution to the manuscript

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Table 1. Demographic and basic clinical characteristics (n=512)

Variables	Mean	Std	Min	Max
Age (years)	69.49	5.86	59	86
Education (years)	14.57	2.91	8	20
MMSE, range 0-30	29.10	1.30	18	30
CDR sum of boxes, range 0-18	0.43	0.86	0	7.5
BMI (kg/m^2) †	25.76	3.83	16.00	47.00
Daily energy intake (kcal/day)	2298.95	743.26	765.10	4954.60
Physical activity score (PASE)†	31.10	11.95	4.67	78.75
Mediterranean diet, range 0-9	4.53	1.64	0	8
MEM score	0.31	0.7	-2.2	3.83
	Frequencies (%)			
Sex female/male	270/242 (52.7%/47.3%)			
ApoE-ε4 carriers/non-carriers†	143/358 (28.54%/71.46%)			
Cognitive status (n)				
Cognitively normal	431 (84.2%)			
MCI	81 (15.8%)			

Abbreviations: BMI body mass index; CDR clinical dementia rating; MCI mild cognitive impairment; MEM memory summary factor score; MMSE mini-mental state examination; PASE: physical activity scale for the elderly
†incomplete data: 508 cases for BMI, 504 for CDR, 494 for PASE, 501 for APOE-ε4 status

Table 2. MNI coordinates and statistics from neuroimaging analysis

Whole-brain results					
K_E	*p(FWE)*	*p(FDR)*	*TFCE*	*p(unc)*	*x z y*
1339	0.032	0.043	2747.53	0.001	22 -39 -14
	0.035	0.043	2676.16	0.001	22 -32 -21
	0.036	0.043	2670.39	0.002	22 -21 -24
ROI-based results					
K_E	*p(FWE)*	*p(FDR)*	*TFCE*	*p(unc)*	*x z y*
2343	0.004	0.007	841.96	<0.001	22 -38 -12
	0.006	0.007	774.82	<0.001	38 -30 -14
	0.006	0.007	772.33	<0.001	22 -21 -24
1366	0.011	0.007	644.53	0.001	-20 -21 -26
	0.026	0.008	489.51	0.002	-18 -9 -12
	0.027	0.008	483.28	0.003	-30 -9 -16

Abbreviations: ROI region of interest; FWE family-wise error rate; FDR false discovery rate; unc uncorrected; K_E equivalent cluster size; TFCE threshold free cluster enhancement value

Table 3. Associations between MeDi score, cognitive outcomes and CSF biomarkers

	Model	*Estimate*	*Standard Error*	*C.I.*	*p*
Memory	1	0.05	0.02	0.01 – 0.08	**0.005**
	2	0.03	0.02	0.00 – 0.07	**0.038**
	1 + ApoE	0.04	0.02	0.01 – 0.07	**0.007**
	2 + ApoE	0.04	0.02	0.00 – 0.07	**0.031**
Language	1	0.03	0.02	0.00 – 0.06	**0.027**
	2	0.02	0.02	-0.01 – 0.05	0.261
	1 + ApoE	0.03	0.02	-0.00 – 0.06	0.055
	2 + ApoE	0.02	0.02	-0.01 – 0.05	0.291
Executive Functions	1	0.01	0.02	-0.02 – 0.04	0.510
	2	0.00	0.02	-0.03 – 0.04	0.866
	1 + ApoE	0.01	0.02	-0.02 – 0.04	0.561
	2 + ApoE	0.00	0.02	-0.03 – 0.04	0.837
Working Memory	1	0.02	0.02	-0.01 – 0.05	0.254
	2	0.02	0.02	-0.02 – 0.05	0.317
	1 + ApoE	0.02	0.02	-0.02 – 0.05	0.327
	2 + ApoE	0.02	0.02	-0.02 – 0.05	0.337
Visuospatial abilities	1	0.02	0.02	-0.01 – 0.05	0.241
	2	0.01	0.02	-0.02 – 0.04	0.482
	1 + ApoE	0.02	0.02	-0.02 – 0.05	0.339
	2 + ApoE	0.01	0.02	-0.02 – 0.04	0.543
pTau181	1	-2.26	0.65	-3.54 – -0.99	**<0.001**
	2	-1.96	0.68	-3.29 – -0.63	**0.004**
	1 + ApoE	-1.89	0.64	-3.15 – -0.62	**0.004**
	2 + ApoE	-1.64	0.67	-2.96 – -0.33	**0.015**
Aβ42	1	24.24	12.00	0.58 – 47.90	**0.045**
	2	17.77	12.45	-6.79 – 42.33	0.155
	1 + ApoE	12.58	11.54	-10.17 – 35.33	0.277
	2 + ApoE	8.16	11.93	-15.36 – 31.68	0.494
Aβ42/Aβ40	1	0.0034	0.00098	0.00 – 0.01	**0.001**
	2	0.0027	0.001	0.00 – 0.00	**0.008**
	1 + ApoE	0.0022	0.0009	0.0004 – 0.0039	**0.014**
	2 + ApoE	0.0017	0.0009	-0.0001 – 0.0035	0.064
Aβ42/pTau181	1	0.94	0.26	0.43 – 1.45	**<0.001**
	2	0.71	0.27	0.18 – 1.24	**0.009**
	1 + ApoE	0.63	0.24	0.16 – 1.09	**0.009**
	2 + ApoE	0.46	0.25	-0.03 – 0.94	0.063

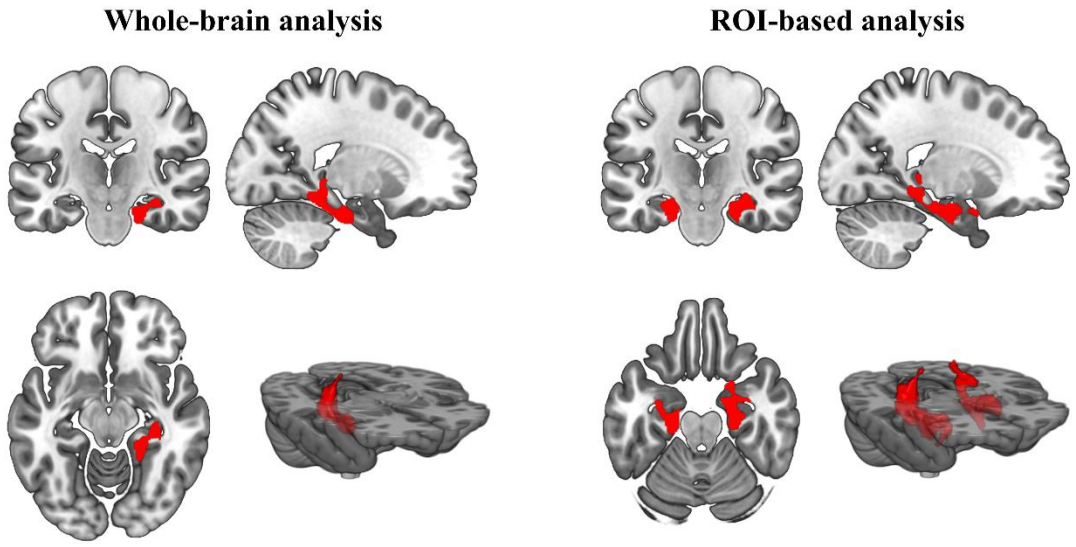
Results of linear regression models. Covariates in Model 1: age, sex, years of education and in Model 2: age, sex, years of education, BMI, total daily caloric intake, level of physical activity. Model 1 and 2 + ApoE-ε4 show the results after additionally correcting for ApoE-ε4 status (carriers or non-carriers).

Abbreviations: C.I. confidence interval

Table 4. Result of mediation and moderated-mediation models

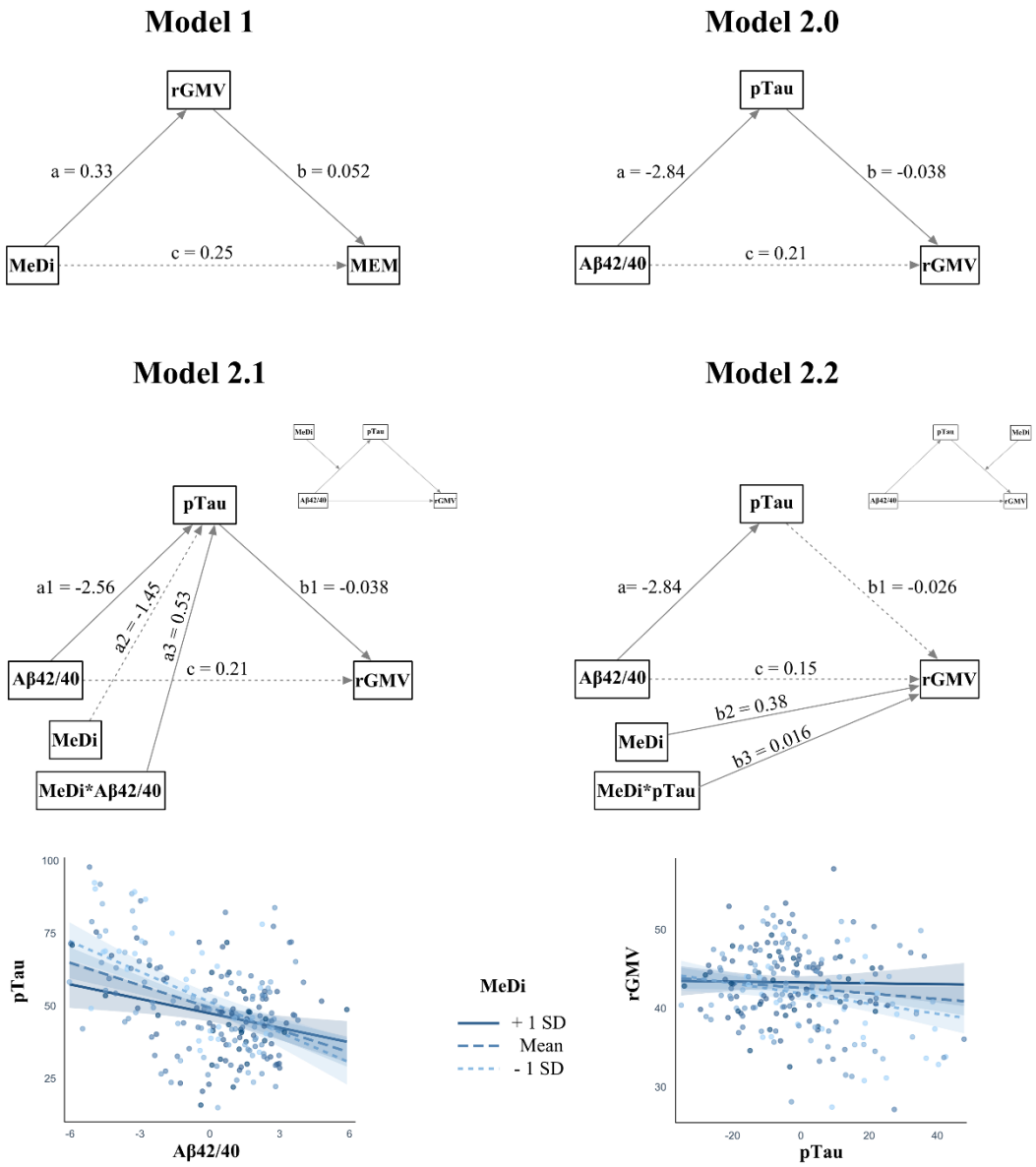
				Controlling for ApoE-ε4 status	
	Effect	*Estimate*	*95% Bootstrap CI*	*Estimate*	*95% Bootstrap CI*
Model 1	indirect	0.017	**(0.007 to 0.030)**	0.016	**(0.006 to 0.028)**
	direct	0.025	(-0.005 to 0.056)	0.024	(-0.006 to 0.054)
	total	0.042	**(0.009 to 0.075)**	0.040	**(0.008 to 0.073)**
	%	40%		40%	
Model 2	Indirect	0.109	**(0.009 to 0.239)**	0.116	**(0.025 to 0.249)**
	direct	0.210	(-0.070 to 0.471)	0.195	(-0.094 to 0.473)
	total	0.319	**(0.071 to 0.562)**	0.311	**(0.048 to 0.580)**
	%	34%		37%	
Model 2.1					
Below	indirect	0.133	**(0.011 to 0.308)**	0.142	**(0.030 to 0.314)**
	%	39%		42%	
Mean	indirect	0.098	**(0.010 to 0.220)**	0.105	**(0.024 to 0.229)**
	%	32%		35%	
Above	indirect	0.063	**(0.008 to 0.172)**	0.068	**(0.010 to 0.180)**
	%	23%		26%	
	IMM	-0.020	**(-0.065 to -0.001)**	-0.022	**(-0.065 to -0.001)**
Model 2.2					
Below	indirect	0.154	**(0.044 to 0.292)**	0.164	**(0.068 to 0.306)**
	%	51%		54%	
mean	indirect	0.075	(-0.029 to 0.205)	0.083	(-0.008 to 0.214)
	%	34%		37%	
above	indirect	-0.005	(-0.159 to 0.160)	0.002	(-0.142 to 0.160)
	%	3%		1%	
	IMM	-0.047	**(-0.101 to -0.004)**	-0.048	**(-0.101 to -0.009)**

Effects for the moderated mediation models are shown at different levels of the moderator. Mean: at mean level of MeDi; below and above: at -1 and +1 standard deviations from the mean of MeDi, respectively. Bold text highlights significant paths according to confidence intervals generated with bias corrected bootstrap with 10000 replicates.
Abbreviations: IMM index moderated mediation; % proportion of mediated effect



Legend to Figure 1. Positive association between Mediterranean diet and brain volume

Left panel Positive association between MeDi score and brain gray matter volume at the whole-brain level. ***Right panel*** Positive association between MeDi score and gray matter volume in *a priori* defined regions of interest covering the bilateral hippocampi and parahippocampal gyri. All results are corrected for age, sex, total intracranial volume and MRI scanner heterogeneity. Results are shown at $p<0.05$ FWE. Images are displayed in neurological convention: left of the brain on the left of the image. The unthresholded T-map is available at Neurovault (https://neurovault.org/collections/KMIELIOW/).



Legend to Figure 2. Graphical display of mediation and moderated mediation models.

Names of the paths and associated regression estimates are reported. Solid lines represent significant paths according to confidence intervals generated with bias corrected bootstrap with 10000 replicates. Dashed lines mark non-significant regression paths. For *Model 2.1* and *Model 2.2*, in addition to the statistical models, the conceptual models are shown in the upper right corners and simple slopes

representing the interactions effects are shown below. A complete overview of direct and indirect effects is reported in Table 4.

Abbreviations: rGMV regional gray matter volume in bilateral hippocampi and para-hippocampi, MeDi Mediterranean diet; MEM memory function; pTau phosphorylated Tau; Aβ42/40 ratio between Aβ42 and Aβ40.