Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-004850
Article Type:	Research
Date Submitted by the Author:	14-Jan-2014
Complete List of Authors:	Mosconi, Lisa; New York University School of Medicine, Murray, John; New York University, Davies, Michelle; New York University, Williams, Schantel; New York University, Pirraglia, Elizabeth; New York University, Spector, Nicole; New York University, Tsui, Wai; New York University, Li, Yi; New York University, Butler, Tracy; New York University, Osorio, Ricardo; New York University, Glodzik, Lidia; New York University, Vallabhajosula, Shankar McHugh, Pauline; New York University, Marmar, Charles; New York University, de Leon, Mony; New York University,
Primary Subject Heading:	Radiology and imaging
Secondary Subject Heading:	Neurology, Nutrition and metabolism
Keywords:	Dementia < NEUROLOGY, NUCLEAR MEDICINE, NUTRITION & DIETETICS, RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional study

Lisa Mosconi[1*], PhD, John Murray[1], BA, Michelle Davies[1], BA, Schantel Williams[1], RN, Elizabeth Pirraglia[1], MA, Nicole Spector[1], BA, Wai H. Tsui[1], MS, Yi Li[1], MD, Tracy Butler[1], MD, Ricardo S. Osorio[1], MD, Lidia Glodzik[1], MD, PhD, Shankar Vallabhajosula[2], PhD, Pauline McHugh[1], MD, Charles Marmar[1,3], MD, Mony J. de Leon[1], EdD

[1]New York University School of Medicine, NY, NY 10016; [2]Weill Cornell Medical College, NY, NY 10028; [3]Steven and Alexandra Cohen Veterans Center for PTSD and TBI, NY, NY 10016

* Dr. Lisa Mosconi, Department of Psychiatry, NYU School of Medicine, 145 East 32nd St, 2nd Floor, New York NY, 10016. Tel: (212) 263-3255, Fax: (212) 263-3270 Email: lisa.mosconi@nyumc.org

Word count

Title: 82 characters (including spaces)

Manuscript Words: 3,189

Abstract Words: 300

Tables: 3, Figures: 2, Supplemental files: 3 [eFigure 1; eFigure 2; eFigure 3]

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abstract

Objectives. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem. This study examines the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal individuals (NL) with and without AD risk factors.

Design. As part of an ongoing study on the early detection of Alzheimer's disease using brain imaging, participants had thorough clinical and laboratory exams, received a battery of neurocognitive tests, Positron Emission Tomography (PET) imaging with ^{11}C-Pittsburgh Compound B (PiB; a measure of amyloid-β (Aβ) load) and ^{18}F-fluorodeoxyglucose (FDG, a proxy of neuronal activity), and filled out semi-quantitative food frequency questionnaires.

Setting. Research center affiliated with the Alzheimer's disease Coordinating Center at New York University School of Medicine.

Participants. Forty-nine NL individuals (age 25-72 y, 69% female) with dietary information, ^{11}C-PiB and ^{18}F-FDG PET scans were examined.

Results. Across all subjects, higher intake of vitamin B12, vitamin D and ω-3 polyunsaturated fatty acid (PUFA) associated with lower Aβ load in AD-regions on PiB-PET, while higher intake of β-carotene and folate associated with higher glucose metabolism on FDG-PET. Nutrients were associated with reduced metabolism for women, *APOE*4 carriers and subjects with positive AD family history, but not for their risk-free counterparts. These effects were not found on PiB retention. The identified 'brain-protective' nutrient combination was associated with higher intake of vegetables, fruit, whole grains, fish, and legumes and lower intake of high-fat dairies, processed meat and sweets.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Conclusions. Our data indicates a relationship between nutrients and AD-biomarkers in NL persons, suggesting that dietary interventions may play a role in the prevention of AD. Longitudinal studies are warranted to investigate a causal link between food intake, brain aging and risk of AD.

Article focus

- To investigate the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal (NL) adults with and without AD risk factors.
- To define which nutrients are protective against AD pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals
- To test whether the effects would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype, a major susceptibility gene for late-onset AD.

Key messages

- Higher intake of vitamin B12, vitamin D, ω-3 polyunsaturated fatty acid (PUFA), β-carotene and folate was associated with lower amyloid-beta pathology and higher metabolic activity in AD-vulnerable brain regions.
- These associations were stronger for women, *APOE*4 carriers and subjects with positive AD family history, than for their risk-free counterparts.
- Nutrient-biomarkers associations were stronger using nutrients from foods only, which is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging and cognitive decline are less so. This study shows a relationship between nutrients and AD-biomarkers in cognitively intact persons at risk for AD, suggesting that dietary interventions may play a role in the prevention of AD
- This was a cross-sectional study whose purpose was to explore a possible link between dietary nutrients and brain biomarkers of AD in yet asymptomatic individuals; further longitudinal studies are necessary to show causal links.
- More studies with larger sample sizes and nutrient plasma measures are needed to further elucidate the inter-relationship between dietary choices, brain physiology and risk of future AD.

Funding/support: This study was supported by NIH/NIA grants AG035137, AG032554, AG022374, AG13616, Alzheimer's Association IIRG-09-132030, and an Anonymous foundation.

Financial disclosures: Drs. Mosconi, Tsui and de Leon have a patent on a technology that was licensed to Abiant Imaging Inc. by NYU and, as such, have a financial interest in this license agreement and hold stock and stock options on the company. Drs. Mosconi, Li and de Leon have received compensation for consulting services from Abiant Imaging. Dr. de Leon and Glodzik have received honoraria from the French Alzheimer Foundation. Drs. Murray, Spector, Davies,

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Pirraglia, Williams, Butler, Osorio, McHugh, Vallabhajosula and Marmar report no biomedical financial interests or potential conflicts of interest.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Introduction

"We are what we eat": the nutritional content of what we eat determines the composition of our cell membranes, bone marrow, blood, hormones, and is therefore the foundation upon which our body and brain are built. While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging are less so. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem. [1-7] However, the biological mechanisms underlying the relationship between dietary nutrients, brain aging and AD are largely unexplored. Understanding how diet promotes healthy brain aging in cognitively normal (NL) individuals, especially those at increased risk for AD, is critical prior to implementing dietary recommendations for prevention and treatment of disease.

While it is intuitive to assume a link between nutrient intake and overall brain functioning exists, to our knowledge there are no studies that examined the relationship between diet and brain amyloid-β (Aβ), a major hallmark of AD pathology, or glucose metabolism, a proxy for neuronal activity, in NL individuals.

The present brain imaging study examines the relationship between nutrient intake, brain Aβ load assessed using ^{11}C-Pittsburgh Compound-B (PiB) Positron Emission Tomography (PET), and glucose metabolism assessed using ^{18}F-fluorodeoxyglucose (FDG) PET in a cohort of young to late-middle aged NL individuals with or without risk factors for AD. Several studies demonstrated increased PiB-PET retention in AD and Mild Cognitive Impairment (MCI), often a prodromal AD phase, compared to controls[8], as well as in non-demented elderly at risk for AD [9-11]. Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with clinical symptoms [12-14]. Our goals were to define which nutrients are protective against AD

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals, and to test whether the effect would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype.

Methods

Participants

Among a larger pool of participants who underwent PET exams, this study included 49 prospectively recruited, clinically and cognitively normal (NL) individuals participating in longitudinal PET studies at NYU School of Medicine, who completed clinical, PiB- and FDG-PET exams and dietary questionnaires within 6 months of each other. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All subjects provided written informed consent to participate in this IRB approved study.

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 [14]. A family history (FH)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires [14]. *APOE* genotypes were determined using PCR.

Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Willett's semi-quantitative food frequency questionnaire (SFFQ) [15]. The SFFQ has been validated for determination of nutrient intake in the elderly and young adults [16, 17]. The 61-food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (g/day) of each food group was calculated by summing the intakes of member food items. The daily intake of nutrients was then computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion [15].

Similarly to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function[18, 19], including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), ω-3 polyunsaturated fatty acid (PUFA), ω-6 PUFA, β-carotene, vitamin B12, vitamin C, vitamin D, vitamin E, and folate. As moderate alcohol drinking may be protective against dementia [20], alcohol intake (g/day) was also calculated.

Brain imaging

All subjects received PiB- and FDG-PET scans [10, 14]. For PET, subjects were positioned in the scanner 60 min after injection of 15 mCi of ^{11}C-PiB , and scanned for 30 min in 3D-mode on an LS Discovery or BioGraph PET/CT scanner [21]. The FDG scan was performed 30 min after

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

completion of the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of ^{18}F-FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. All images were corrected for photon attenuation, scatter, and radioactive decay and smoothed for uniform resolution [22].

For each subject, summed PET images corresponding to 40-60 min of FDG data and 60-90 min of PiB data were coregistered to corresponding MRI using the Normalized Mutual Information routine implemented in Statistical Parametric Mapping (SPM8) [23]. MRI were spatially normalized to Montreal Neurological Institute space by high-dimensional warping (DARTEL) [23]. MRI-coregistered FDG and PiB scans were spatially normalized using subject-specific transformation matrixes from MRI and sampled using automated regions-of-interest (ROI) [24, 25]. We focused on pre-selected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), posterior cingulate cortex (PCC) and prefrontal cortex (PFC). Standardized uptake value ratios (SUVR) were generated for each ROI by normalizing PiB uptake by cerebellar grey matter uptake [26] and FDG by pons activity [27]. A composite cortical PiB retention mask (AVG_{PiB}) was created by combining parieto-temporal, frontal and PCC regions, and an FDG mask (AVG_{FDG}) by combining parieto-temporal and PCC regions [28].

Statistical Analysis

Clinical and demographical measures were examined with descriptive statistics. Stepwise linear regressions were used to evaluate the associations between FDG and PiB ROI measures (dependent variables), nutrients (independent variables), and covariates. We first focused on

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

nutrients from foods only. As nutrient levels are also affected by subjects' intake of supplements, analyses were repeated for the combined nutrient intake from foods and supplements, and for nutrients from supplements only. All regression models were tested for violations of the model assumptions. Variables that were not normally distributed were log-transformed which normalized the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age- and caloric intake-adjusted residuals [29]. We then examined gender [30], education, ethnicity, body mass index (BMI), alcohol consumption, FH [31] and *APOE* status [32] as covariates. Education and BMI were modeled as continuous variables. Gender (male vs. female), FH (FH+ vs. FH-), and *APOE* status (*APOE*4+ vs. *APOE*4-) were examined as dichotomous variables. Ethnic group was based on self-report using the format of the 1990 census. Ethnicity was used as a dummy variable (White/non-Hispanic vs. other ethnic groups). Alcohol intake was used as a dichotomous variable (mild-moderate (0-30 g/day) vs. no (0 g/day) or more than moderate ($\geq$30 g/day) consumption).

If one or more covariates showed significant effects on the association between nutrients and biomarkers, those covariates were separately examined for interaction effects in adjusted models by first entering the main effects, then the 2-way interactions between each nutrient and the covariate(s) in the next step. Only significant interaction terms were retained in the models.

The food sources of nutrients associated with PiB retention and FDG uptake were examined by testing for correlations between foods and nutrients [3, 4, 18, 19].

Results were considered significant at $p<0.05$ (2-sided tests). SPSS v.21 (SPPS Inc., 2013) was used for all analyses.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

Results

Subjects' characteristics are found in **Table 1**. None of the participants were diabetics, smokers, or met criteria for obesity.

FDG-PET

β-carotene and folate were positively associated with brain metabolism in several ROI ($p \leq 0.05$; **Figure 1, Table 2**). No other nutrients were associated with FDG uptake. Adjustment for education, ethnicity, BMI, and alcohol consumption did not attenuate these relationships while gender, FH and *APOE* status showed significant interaction effects with nutrients.

Gender×β-carotene effects were observed for PCC and AVG_{FDG} ($p \leq 0.03$), and gender×folate effects in all ROI ($p \leq 0.05$). Marginally significant gender×SFA effects were observed in PCC and AVG_{FDG} ($p \leq 0.08$). In these regions, women showed steeper regression slopes of metabolism on nutrients vs. men (e**Figure 1**). On post-hoc examination of gender groups, women showed significant associations between these nutrients and metabolism ($p<0.05$) while men showed no associations (**Table 3**).

FH×β-carotene interaction effects were observed for all ROI ($p \leq 0.05$), which were driven by FH+ subjects showing steeper regression slopes vs. FH- (**Table 3**). On post-hoc examination of FH groups, FH+ subjects showed significant associations between β-carotene and metabolism ($p<0.05$) while FH- subjects showed no associations (**Table 3**). Examination of gender×FH×β-carotene showed that women with a positive FH+ had the steepest regression slopes of PCC, LTL and AVG_{FDG} metabolism on β-carotene vs. other groups (**eFigure 2**). In these ROI, FH+

women showed significant associations between β-carotene and metabolism in all regions ($p<0.05$) whereas the other subjects did not (**Table 3**).

Interaction effects of *APOE*×β-carotene were marginally significant ($p\leq0.08$), with *APOE*4+ subjects showing steeper regression slopes vs. *APOE*4-. However, there were significant gender×*APOE*×β-carotene effects, as women *APOE*4+ had the steepest regression slopes of PCC and AVG_{FDG} metabolism on β-carotene vs. the other groups (**eFigure 2**). In these ROI, *APOE*4+ women showed significant associations between β-carotene and metabolism ($p<0.05$), whereas the other subjects did not (**Table 3**). The same effect was observed for gender×*APOE*×SFA (**Table 3**).

Results remained unchanged using nutrients from food and supplements, although the relationships were attenuated. There were no associations between nutrients from supplements only and metabolism.

Correlations between nutrients associated with FDG and food groups showed that β-carotene was mainly from dark leaf, green leafy and cruciferous vegetables, and fresh fruit with correlation coefficients of 0.82, 0.77, 0.69 and 0.53, respectively (p's$\leq$0.001). Folate was from grains, fish, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32, 0.30, and 0.29; p's$\leq$0.04), and SFA from high fat dairies, salad dressing, fried potatoes, sweets and processed meat (0.65, 0.52, 0.5, 0.45, 0.41, p's$\leq$0.003).

PiB-PET

PiB retention was negatively associated with vitamin B12 and vitamin D ($p<0.05$; **Table 2**, **Figure 2**). There were marginally significant negative associations between PiB retention and ω-

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

3 PUFA ($p \leq 0.10$), which prompted exploratory analyses of 3 major ω-3 PUFAs subtypes (i.e., eicosapentaenoic acid 20:4, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:2). PiB retention was negatively associated with EPA (β range -0.37 in IPL to -0.31 in PCC, SE 0.49-0.43, $p<0.04$; **Figure 2**). No other nutrients were associated with PiB retention. Including education, ethnicity, BMI, alcohol consumption, gender, FH and *APOE* status as covariates in the model did not attenuate these relationships. There were no interaction effects (**eFigure 3**). Analysis of nutrients from food and supplements attenuated the relationships between PiB retention and vitamin D, while those with vitamin B12 remained significant ($p \leq 0.05$). Only 3 subjects were taking ω-3 PUFA supplements. There were no associations between nutrients from supplements only and PiB retention.

Correlations between nutrients associated with PiB and food groups showed that vitamin B12 was mainly from meat, eggs and butter with correlation coefficients of 0.35, 0.31 and 0.36, respectively ($p<0.04$). Vitamin D was mostly from low-fat dairies and fish (0.64 and 0.55, $p<0.001$), and EPA ω-3 PUFA from fish and other vegetables (0.36 and 0.31, $p<0.01$).

Discussion

This brain imaging study shows that higher intake of vitamin B12, vitamin D, and EPA ω3-PUFA from food sources was associated with lower Aβ load, and higher β-carotene and folate intake was associated with higher brain metabolism in NL individuals. Higher consumption of SFA was also associated with lower brain metabolism, albeit weakly. This data indicates that a healthy diet rich in natural folic acid, β-carotene, ω3-PUFA, vitamin B12 and D might be particularly useful to support healthy brain aging. The identified 'AD-protective' dietary pattern (DP) was associated with higher intake of vegetables, fruit, whole grains, fish, and legumes and

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

lower intake of high-fat dairies, processed meat and sweets. A significant impact of gender, FH and *APOE* status was noted, in women, FH+ and APOE4+ individuals showed the strongest associations between metabolism and nutrients, especially β-carotene, compared to their risk-free counterparts. These effects were not evident on PiB retention.

Current evidence suggests that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly[33-39], possibly due to their ability to reduce homocysteine levels. β-carotene (a major precursor to vitamin A) might have beneficial effects via its strong antioxidant or Aβ anti-oligomerization effects [40-45]. Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculo-protective and synaptic plasticity-enhancing effects, modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products [46-48]. High intake of SFA is known to have negative effects on cognitive functions [49, 50], while PUFA decrease risk of decline [18, 19, 51, 52].

Present data is consistent with epidemiological findings of DPs that are protective against AD. Despite differences in the analytic approaches, high adherence to DPs characterized by higher intakes of fruits, vegetables, fish, nuts and legumes, and lower intake of meat, high-fat dairies and sweets, is consistently associated with reduced risk for AD[3, 4, 53 18]. The DP identified using brain PET in this study supports these epidemiological findings by providing biological evidence that a healthy diet is associated with lower risk of brain amyloidosis and with higher metabolic activity in NL individuals at risk for AD. Additionally, a community-based study of peripheral plasma Aβ in NL elderly showed that ω-3PUFA was associated with lower Aβ levels [19]. Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3PUFA EPA, further reinforcing the connection between dietary factors and AD pathology.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time [18], the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman et al[54] showed that higher levels of plasma anti-oxidants, vitamin B, D and ω3-PUFA were associated with more favorable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 y). Analysis of plasma nutrients would be very valuable in younger NL at risk for AD. In our data set, none of the nutrients were associated with neuropsychological measures, possibly because our subjects were relatively young and all high-school graduates, which resulted in a "ceiling-effect". Longitudinal studies with larger samples are warranted to assess whether the relationship between "protective" nutrients, AD-biomarkers and cognitive performance varies with age and disease.

In our data-set, gender, FH and *APOE* status all affected the relationship between nutrients, especially β-carotene, and brain metabolism. To our knowledge, there are no previous studies that examined the interaction between nutrients and AD-risk factors on brain metabolism in normal aging. Our results were in the expected direction, as at-risk individuals showed stronger effects of diet on metabolism. Several studies have shown that female gender, a 1st-degree FH, and the *APOE* ε4 genotype are all associated with reduced brain metabolism in NL individuals [13, 14, 55]. Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs [56, 57], particularly of vitamin D/calcium and folic acid, as women are susceptible to hormonal changes associated with menstruation, child-bearing and osteoporosis. While limited by the small sample, gender×FH×*APOE* interactions were observed as FH+/*APOE*4+ women showed the strongest associations between metabolism and β-carotene ($p<0.04$). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarkers associations were stronger using nutrients from foods only,

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

which reinforces the general recommendations of a lifelong diversified diet that includes an abundance of nutrient-rich foods, and is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods [58], especially for women [59]. While both FH and *APOE* status have been associated with increased Aβ load in NL [10, 11], the effects of diet on PiB retention were not exacerbated in presence of these risk factors. Overall, our data suggests that dietary interventions aimed at preserving brain activity might be particularly useful if instituted in young adulthood in NL at risk for AD, before irreversible neuronal loss. Second, these results indicate a genetic component to dietary needs, as genetic risk in conjunction with unhealthy eating habits may boost genetic predisposition [60], and support the view that AD is a multi-factorial disease resulting from genetic, life-style and environmental interactions [61]. Third, nutrient effects on Aβ load were independent of AD-risk factors, but this may differ in older populations with more substantial Aβ deposition. Nutrients may have an impact on Aß oligomers prior to plaque formation [62], which are not detectable with PiB-PET. A low SFA/glycemic index diet was shown to improve Aβ composition in cerebrospinal fluid (CSF) in MCI [63], suggesting that diet is a powerful environmental factor that modulates AD-risk through its effects on brain Aβ and associated neuronal injury.

In conclusion, we identified dietary factors that are associated with healthy brain aging, which provides support for further exploration of dietary behavior for the prevention of AD. We caution that the NL population selected in our study represents a group with a high a priori risk of preclinical AD-changes, and results were found in small numbers of carefully screened subjects under controlled clinical conditions. Replication of these preliminary research findings in community-based populations with more diversified socio-economic and medical status is warranted and clinical application is not yet justified.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-483.
2. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545-1550.
3. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912-921.
4. Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216-225.
5. Rinaldi P, Polidori MC, Metastasio A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915-919.
6. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849-1853.
7. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230-3237.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
9. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469-1475.
10. Mosconi L, Rinne JO, Tsui WH, et al. Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci U S A 2010;107:5949-5954.
11. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009;106:6820-6825.
12. Langbaum JB, Fleisher AS, Chen K, et al. Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 2013;9:371-381.
13. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004;101:284-289.
14. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A 2007;104:19067-19072.
15. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
16. Morris MC, Tangney CC, Bienias JL, Evans DA, Wilson RS. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. Am J Epidemiol 2003;158:1213-1217.
17. Smith W, Mitchell P, Reay EM, Webb K, Harvey PW. Validity and reproducibility of a self-administered food frequency questionnaire in older people. Aust N Z J Public Health 1998;22:456-463.
18. Gu Y, Nieves JW, Stern Y, Luchsinger JA, Scarmeas N. Food combination and Alzheimer disease risk: a protective diet. Arch Neurol 2010;67:699-706.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

19. Gu Y, Schupf N, Cosentino SA, Luchsinger JA, Scarmeas N. Nutrient intake and plasma beta-amyloid. Neurology 2012;78:1832-1840.
20. Luchsinger JA, Tang MX, Siddiqui M, Shea S, Mayeux R. Alcohol intake and risk of dementia. J Am Geriatr Soc 2004;52:540-546.
21. Mosconi L, Rinne JO, Tsui W, et al. Amyloid and metabolic PET imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2012;in press.
22. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154-159.
23. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821.
24. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology 2005;64:1860-1867.
25. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-2181.
26. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
27. Minoshima S, Frey KA, Foster NL, Kuhl DE. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. J Comput Assist Tomogr 1995;19:541-547.
28. Mosconi L, Rinne JO, Tsui WH, et al. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2013;34:22-34.
29. Willett WC, Stampfer MJ. Implications of total energy intake for epidemiological analyses. In: Willett WC, ed. Nutritional epidemiology. New York: Oxford University Press, 1998: 273-301.
30. Vina J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. J Alzheimers Dis 2010;20 Suppl 2:S527-533.
31. Farrer LA, O'Sullivan DM, Cupples LA, Growdon JH, Myers RH. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol 1989;25:485-493.
32. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001;32:181-184.
33. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. J Nutr Health Aging 2004;8:226-232.
34. Corrada MM, Kawas CH, Hallfrisch J, Muller D, Brookmeyer R. Reduced risk of Alzheimer's disease with high folate intake: the Baltimore Longitudinal Study of Aging. Alzheimers Dement 2005;1:11-18.
35. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am J Clin Nutr 1997;65:20-29.
36. Luchsinger JA, Tang MX, Miller J, Green R, Mayeux R. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. Arch Neurol 2007;64:86-92.
37. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641-645.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

38. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 2007;369:208-216.
39. Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. J Alzheimers Dis 2006;9:429-433.
40. Kesse-Guyot E, Andreeva VA, Ducros V, et al. Carotenoid-rich dietary patterns during midlife and subsequent cognitive function. Br J Nutr 2013:1-9.
41. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids, alpha-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res 2013;2013:951786.
42. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5:56-65.
43. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. Eur J Nutr 2013;52:1553-1567.
44. de Oliveira BF, Veloso CA, Nogueira-Machado JA, et al. Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci 2012.
45. Takasaki J, Ono K, Yoshiike Y, et al. Vitamin A has anti-oligomerization effects on amyloid-beta in vitro. J Alzheimers Dis 2011;27:271-280.
46. Balion C, Griffith LE, Strifler L, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 2012;79:1397-1405.
47. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18-26.
48. Lu'o'ng KV, Nguyen LT. The role of vitamin D in Alzheimer's disease: possible genetic and cell signaling mechanisms. Am J Alzheimers Dis Other Demen 2013;28:126-136.
49. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776-782.
50. Morris MC, Evans DA, Bienias JL, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194-200.
51. Bowman GL, Shannon J, Ho E, et al. Reliability and validity of food frequency questionnaire and nutrient biomarkers in elders with and without mild cognitive impairment. Alzheimer Dis Assoc Disord 2011;25:49-57.
52. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940-946.
53. Scarmeas N, Stern Y, Mayeux R, Luchsinger JA. Mediterranean diet, Alzheimer disease, and vascular mediation. Arch Neurol 2006;63:1709-1717.
54. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241-249.
55. Murphy DG, DeCarli C, McIntosh AR, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 1996;53:585-594.
56. Vellas BJ, Hunt WC, Romero LJ, Koehler KM, Baumgartner RN, Garry PJ. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. Nutrition 1997;13:515-519.
57. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-1630.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

58. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-520S.
59. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-2342.
60. de Roos B. Personalised nutrition: ready for practice? Proc Nutr Soc 2013;72:48-52.
61. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68:270-281.
62. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
63. Bayer-Carter JL, Green PS, Montine TJ, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Arch Neurol 2011;68:743-752.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Legends

Figure 1. Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats.

Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless].

Corresponding *p* values are found in Table 2

Figure 2. Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA).

Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless].

Corresponding *p* values are found in Table 2

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 1. Demographic and clinical characteristics

N	49
Age, y, mean (SD)	54(11), range 25-72
Female gender, %	69%
Education, y, mean (SD)	16(2)
Family history of LOAD, % positive	69%
APOE ε4 status [a], % positive	39%
Ethnicity	
White	82%
Black	8%
Hispanic	6%
Other	4%
Body Mass Index [unitless], mean (SD)	26(4)
Hip to waist ratio [unitless], mean (SD)	1.2(0.2)
Blood pressure (mm/Hg), mean (SD)	
Systolic	118(15)
Diastolic	71(9)
Glucose (mg/dl)	78(12)
Cholesterol (mg/dl)	200(38)
HDL (mg/dl)	62(19)
LDL (mg/dl)	119(33)
Triglycerids (mg/dl)	91(40)
Homocystein (micromol/l)	10(2)
Neuropsychological tests, mean (SD)	
Mini Mental State Exam	29(1)
Digit symbol substitution	62(10)
Paired associates delayed recall	7(2)
Paragraph delayed recall	10(3)
Designs	8(2)
Object naming	55(12)
WAIS-vocabulary	65(14)

[a] *APOE* genotyping was obtained from 41/49 participants

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 2. Associations between nutrients and AD-biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG metabolism							
β-carotene	β	0.31[a]	0.25[c]	0.33[a]	0.31[a]	0.32[a]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35[b]	0.33[a]	0.31[a]	0.31[a]	0.36[b]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20[c]	-0.16	-0.15	-0.24[c]	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B12	β	-0.32[a]	-0.37[b]	-0.29[a]	-0.33[a]	-0.37[a]	-0.27[c]
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26[c]	-0.21	-0.28[a]	-0.13	-0.28[a]	-0.27[c]
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω3-PUFA	β	-0.18[c]	-0.28[a]	-0.07	-0.24[c]	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω3-PUFA EPA	β	-0.33[a]	-0.37[b]	-0.32[a]	-0.20	-0.31[a]	-0.28[c]
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrients from food sources

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p = 0.06\text{-}0.10$.

Abbreviations: average cortical ROI (AVG), eicosapentaenoic acid 20:4 (EPA), inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), polyunsaturated fatty acids (PUFA), posterior cingulate cortex (PCC), prefrontal cortex (PFC).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3. Effects of gender and family history on the relationship between nutrients and brain glucose metabolism

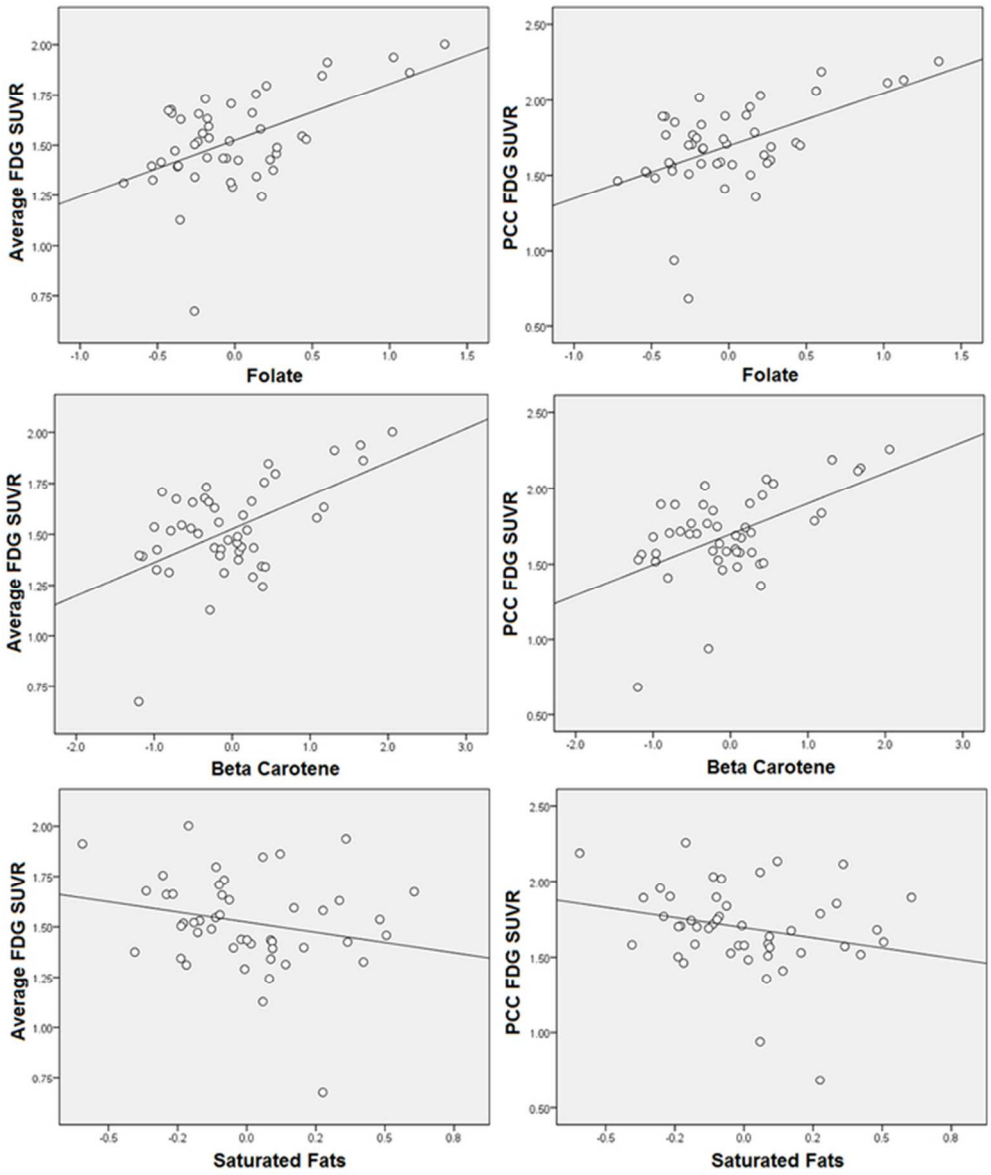
		AVG_{FDG}	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs. men=16)							
β-carotene							
Women	β	0.33 [a]	0.29	0.32 [a]	0.33 [a]	0.34 [a]	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.01	-0.01	0.08	0.08	0.06	-0.02
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate							
Women	β	0.31 [a]	0.34 [a]	0.24	0.28	0.38	0.24
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.29	0.25	0.18	0.22	0.31	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
Women	β	-0.35 [a]	-0.30	-0.32 [a]	-0.38 [a]	-0.37 [a]	-0.34 [a]
	SE	0.008	0.009	0.007	0.01	0.01	0.009
Men	β	0.15	0.16	0.17	0.12	0.09	0.12
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Family history (FH+, n=33 vs. FH-=16)							
β-carotene							
FH+	β	0.42 [b]	0.35 [a]	0.42 [b]	0.43 [b]	0.45 [b]	0.42 [b]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
FH-	β	-0.10	-0.10	-0.17	0.12	0.06	-0.2
Gender × Family history (FH+ women, n=23 vs. other subjects=26)							
β-carotene							
FH+ women	β	0.48 [a]	0.43 [a]	0.46 [a]	0.49 [b]	0.50 [b]	0.47 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.04	-0.006	0.08	0.09	0.04	0.05
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Gender × APOE genotype (APOE4+ women, n=13 vs. other subjects=36)							
β-carotene							
*APOE*4+ women	β	0.52 [a]	0.46 [a]	0.48 [a]	0.54 [a]	0.53 [a]	0.48 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.10	0.10	0.10	-0.08	0.03	-0.08
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
*APOE*4+ women	β	-0.66 [b]	-0.60 [a]	-0.59 [b]	-0.57 [b]	-0.68 [b]	-0.57 [a]
	SE	0.02	0.03	0.02	0.03	0.03	0.03
Other	β	0.11	0.11	0.16	0.18	0.08	0.19

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrient values

[a] $p<0.05$, [b] $p\leq0.01$. See Legend to Table 2.

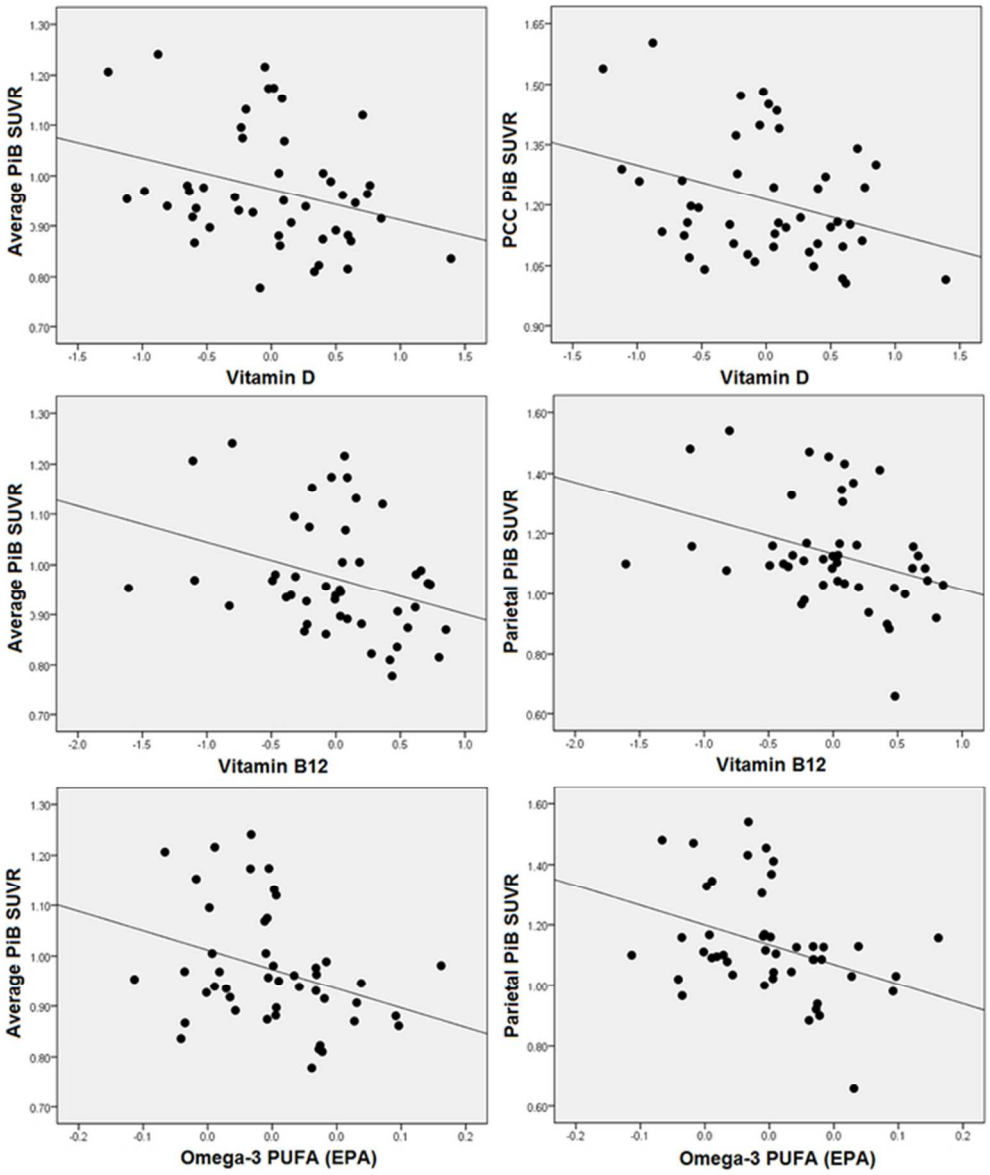
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats
59x70mm (300 x 300 DPI)

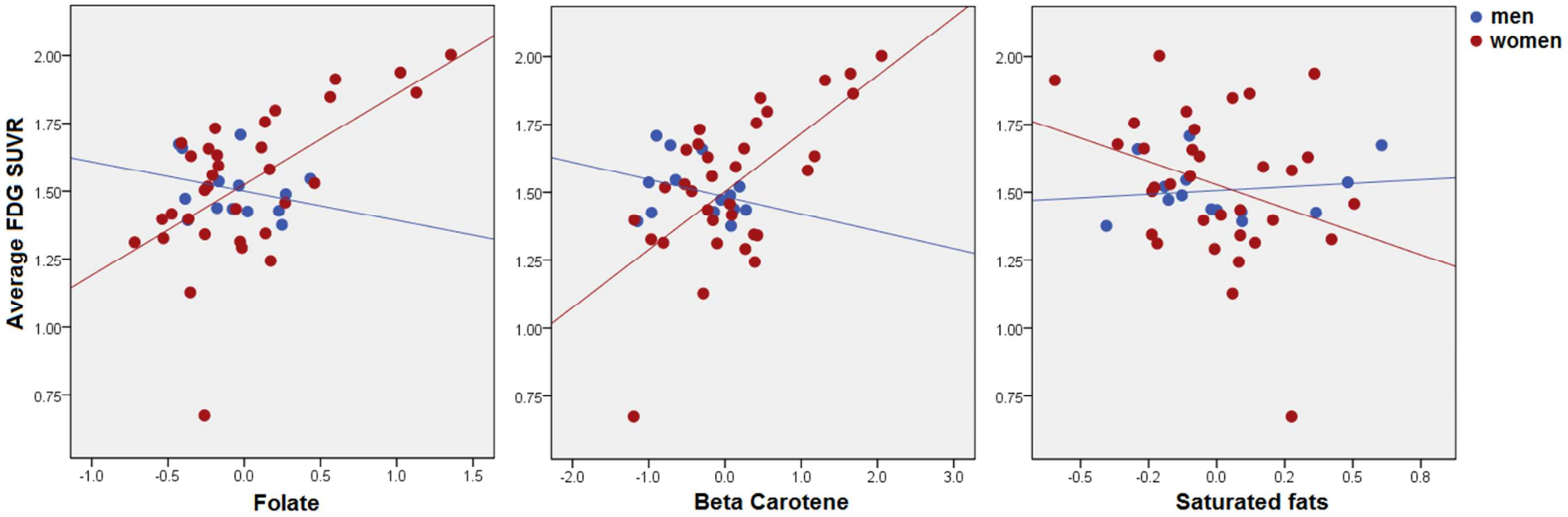
BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA)
59x70mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16)

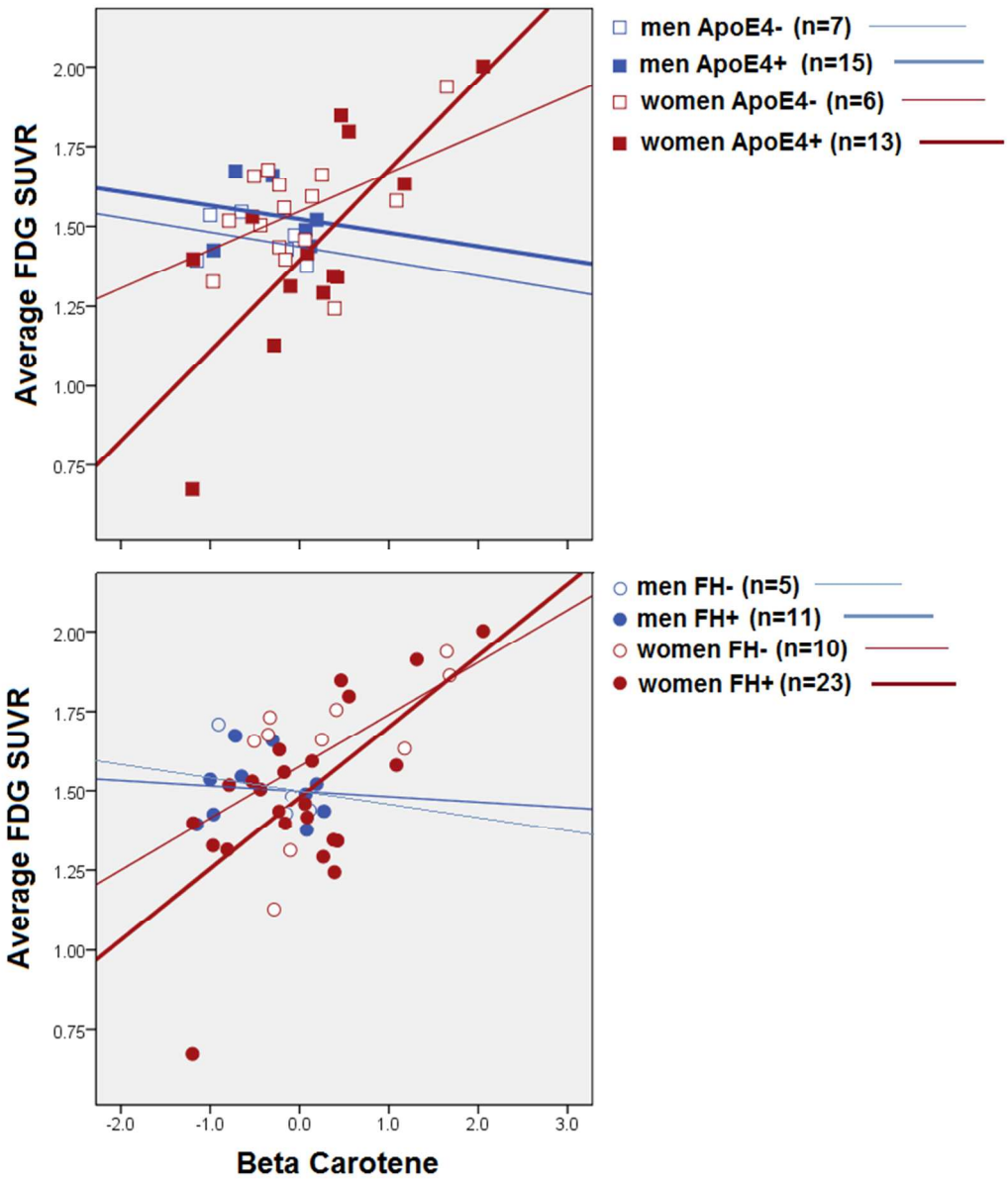


Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)



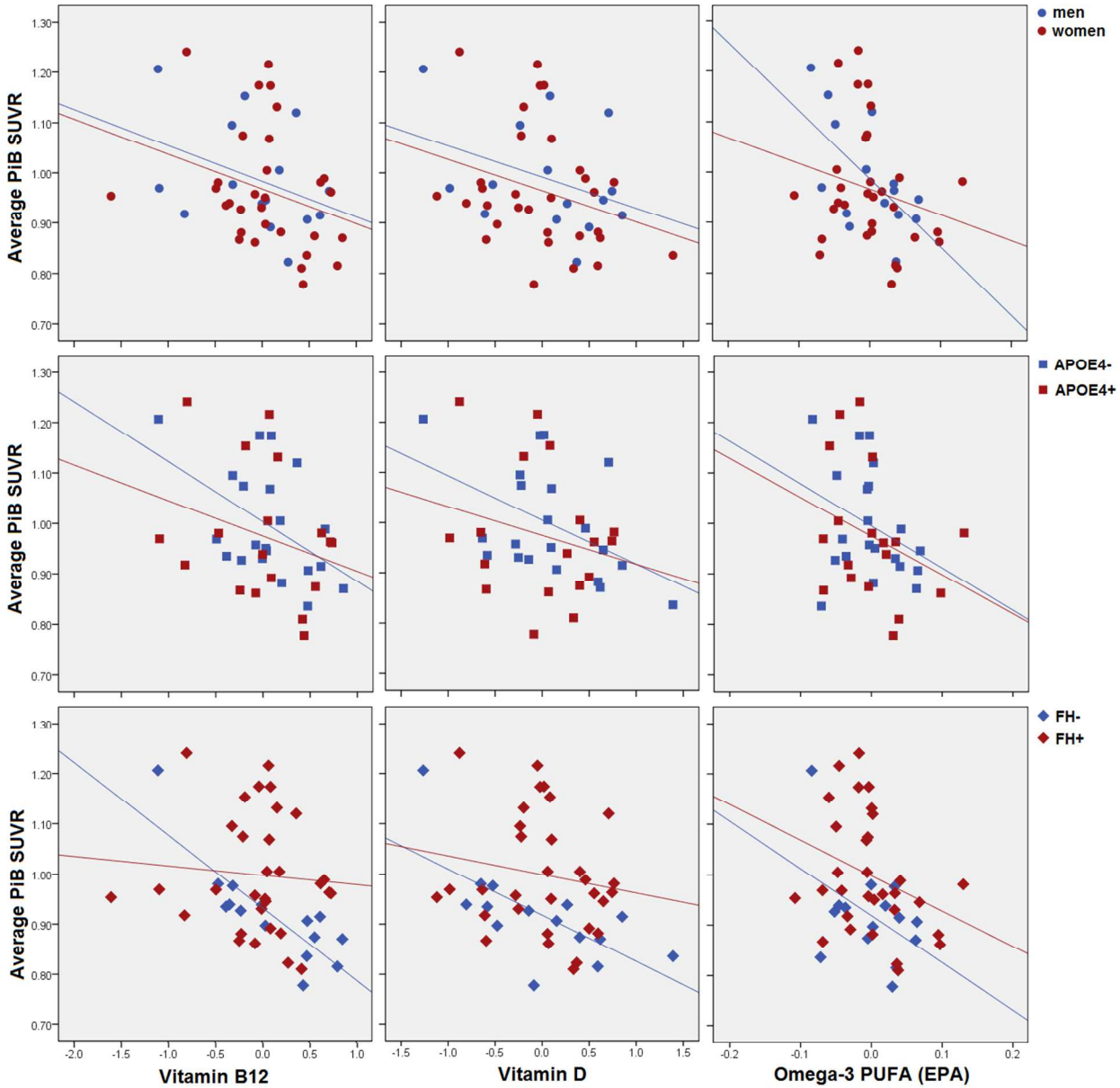
β-carotene values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2.

Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status



Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract DONE p.1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found DONE p.2-3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported DONE p.6
Objectives	3	State specific objectives, including any prespecified hypotheses DONE p.7
Methods		
Study design	4	Present key elements of study design early in the paper DONE
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection DONE p.7-9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants DONE p.7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable DONE p.10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group DONE p.8,9 and 10-11
Bias	9	Describe any efforts to address potential sources of bias DONE p.8,9 and 10-11
Study size	10	Explain how the study size was arrived at DONE p.7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why DONE p.10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding DONE p.10-11
		(*b*) Describe any methods used to examine subgroups and interactions DONE p.10-11
		(*c*) Explain how missing data were addressed n.a.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Continued on next page

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed N.A.
		(b) Give reasons for non-participation at each stage N.A.
		(c) Consider use of a flow diagram N.A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders DONE p.7-8
		(b) Indicate number of participants with missing data for each variable of interest N.A.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) N.A.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures DONE p.11-13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included DONE p.11-13
		(*b*) Report category boundaries when continuous variables were categorized DONE p.11-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives DONE p. 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias DONE p.15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence DONE
Generalisability	21	Discuss the generalisability (external validity) of the study results DONE
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based DONE p.4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-004850.R1
Article Type:	Research
Date Submitted by the Author:	10-Apr-2014
Complete List of Authors:	Mosconi, Lisa; New York University School of Medicine, Psychiatry Murray, John; New York University, Psychiatry Davies, Michelle; New York University, Psychiatry Williams, Schantel; New York University, Psychiatry Pirraglia, Elizabeth; New York University, Psychiatry Spector, Nicole; New York University, Psychiatry Tsui, Wai; New York University, Psychiatry Li, Yi; New York University, Psychiatry Butler, Tracy; New York University, Psychiatry Osorio, Ricardo; New York University, Psychiatry Glodzik, Lidia; New York University, Psychiatry Vallabhajosula, Shankar; Weill Cornell Medical Center, Radiology McHugh, Pauline; New York University, Psychiatry Marmar, Charles; New York University School of Medicine, Psychiatry de Leon, Mony; New York University, Psychiatry
Primary Subject Heading:	Radiology and imaging
Secondary Subject Heading:	Neurology, Nutrition and metabolism
Keywords:	Dementia < NEUROLOGY, NUCLEAR MEDICINE, NUTRITION & DIETETICS, RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Lisa Mosconi[1*], PhD, John Murray[1], BA, Michelle Davies[1], BA, Schantel Williams[1], RN, Elizabeth Pirraglia[1], MA, Nicole Spector[1], BA, Wai H. Tsui[1], MS, Yi Li[1], MD, Tracy Butler[1], MD, Ricardo S. Osorio[1], MD, Lidia Glodzik[1], MD, PhD, Shankar Vallabhajosula[2], PhD, Pauline McHugh[1], MD, Charles Marmar[1,3], MD, Mony J. de Leon[1], EdD

[1]Department of Psychiatry, New York University School of Medicine, New York, NY 10016; [2]Department of Radiology, Citigroup Biomedical Imaging Center (CBIC), Weill Cornell Medical College, New York, NY 10021; [3]Steven and Alexandra Cohen Veterans Center for PTSD and TBI, New York, NY 10016

* Dr. Lisa Mosconi, Department of Psychiatry, NYU School of Medicine, 145 East 32nd St, 2nd Floor, New York NY, 10016. Tel: (212) 263-3255, Fax: (212) 263-3270 Email: lisa.mosconi@nyumc.org

Word count

Title: 137 characters (including spaces)

Manuscript Words: 4034

Abstract Words: 300

Tables: 3, Figures: 2, Supplemental files: 3 [eFigure 1; eFigure 2; eFigure 3]

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abstract

Objectives. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD). This study examines the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal individuals (NL) with and without AD risk factors.

Design. As part of an ongoing brain imaging study, participants received clinical and laboratory exams, a neurocognitive test battery, Positron Emission Tomography (PET) with ^{11}C-Pittsburgh Compound B (PiB; a measure of amyloid-β (Aβ) load) and ^{18}F-fluorodeoxyglucose (FDG; a proxy of neuronal activity), and completed semi-quantitative food frequency questionnaires.

Setting. Research center affiliated with the Alzheimer's disease Core Center at New York University School of Medicine.

Participants. Forty-nine NL individuals (age 25-72 y, 69% female) with dietary information, ^{11}C-PiB and ^{18}F-FDG PET scans were examined.

Results. Controlling for age and total caloric intake, higher intake of vitamin B12, vitamin D and ω-3 polyunsaturated fatty acid (PUFA) associated with lower Aβ load in AD-regions on PiB-PET, while higher intake of β-carotene and folate associated with higher glucose metabolism on FDG-PET. β-carotene and folate were associated with reduced glucose metabolism for women, *APOE*4 carriers and subjects with positive AD family history, but not for their risk-free counterparts. The associations of vitamin B12, vitamin D and ω-3PUFA with PiB retention were independent of gender, APOE and family history. The identified nutrient combination was associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, meat and sweets.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Conclusions. Our data provide a potential pathophysiological mechanism for epidemiological findings showing that dietary interventions may play a role in the prevention of AD. Longitudinal studies are needed to determine whether there is a direct link between nutrient intake, brain biomarkers and risk of AD.

Article focus

- To investigate the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal (NL) adults with and without AD risk factors.
- To define which nutrients are protective against AD pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals
- To test whether the effects would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype, a major susceptibility gene for late-onset AD.

Key messages

- Higher intake of vitamin B12, vitamin D, ω-3 polyunsaturated fatty acid (PUFA), β-carotene and folate was associated with lower amyloid-beta pathology and higher metabolic activity in AD-vulnerable brain regions.
- These associations were stronger for women, *APOE*4 carriers and subjects with positive AD family history, than for their risk-free counterparts.
- Nutrient-biomarkers associations were stronger using nutrients from foods only, which is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging and cognitive decline are less so. This study shows a relationship between nutrients and AD-biomarkers in cognitively intact persons at risk for AD, suggesting that dietary interventions may play a role in the prevention of AD
- This was a cross-sectional study whose purpose was to explore a possible link between dietary nutrients and brain biomarkers of AD in yet asymptomatic individuals; further longitudinal studies are necessary to show causal links.
- More studies with larger sample sizes and plasma nutrient measures are needed to further elucidate the inter-relationship between dietary choices, brain physiology and risk of future AD.
- Our preliminary findings of interaction effects between nutrients and AD risk factors such as gender, APOE genotype and family history need to be replicated with larger samples.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Introduction

"We are what we eat": the nutritional content of what we eat determines the composition of our cell membranes, bone marrow, blood, hormones, and is therefore the foundation upon which our body and brain are built. While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging are less so. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem[1-7]. However, the biological mechanisms underlying the relationship between dietary nutrients, brain aging and AD are largely unexplored. Understanding how diet and nutrition promote healthy brain aging in cognitively normal (NL) individuals, especially those at increased risk for AD, is critical prior to implementing dietary recommendations for prevention and treatment of disease.

While it is intuitive to assume a link between nutrient intake and overall brain functioning exists, to our knowledge there are no studies that examined the relationship between nutrient intake and brain amyloid-β (Aβ), a major hallmark of AD pathology, or glucose metabolism, a proxy for neuronal activity, in NL individuals.

The present brain imaging study examines the relationship between nutrient intake, brain Aβ load assessed using ^{11}C-Pittsburgh Compound-B (PiB) Positron Emission Tomography (PET), and glucose metabolism assessed using ^{18}F-fluorodeoxyglucose (FDG) PET in a cohort of young to late-middle aged NL individuals with or without risk factors for AD. Several studies demonstrated increased PiB-PET retention in AD and Mild Cognitive Impairment (MCI), often a prodromal AD phase, compared to controls[8], as well as in non-demented elderly at risk for AD[9-11]. Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

clinical symptoms[12-14]. Our goals were to define which nutrients are associated with lower AD-burden (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals, and to test whether the associations are stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype.

Methods

Participants

Among a larger pool of participants who underwent PET exams, this study included 49 prospectively recruited, clinically and cognitively normal (NL) individuals participating in longitudinal PET studies at NYU School of Medicine, who completed clinical, PiB- and FDG-PET exams and dietary questionnaires within 6 months of each other. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All subjects provided written informed consent to participate in this IRB approved study.

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[14]. A family history of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires[14]. *APOE* genotypes were determined using standard

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

quantitative polymerase chain reaction (qPCR)[15]. Individuals with at least one copy of the ε4 allele were grouped as *APOE* ε4 carriers (APOE4+) and compared to ε4 non-carriers (APOE4-).

Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Willett's semi-quantitative food frequency questionnaire (SFFQ)[16]. The SFFQ has been validated for determination of nutrient intake in the elderly and young adults[16-20]. The 61-food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (g/day) of each food group was calculated by summing the intakes of member food items. The daily intake of nutrients was then computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion[16].

Similarly to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function[21, 22], including saturated fatty acids, monounsaturated fatty acids, ω-3 polyunsaturated fatty acid (PUFA), ω-6 PUFA, β-carotene, vitamin B12, vitamin C, vitamin D, vitamin E, and folate. As moderate alcohol drinking may be protective against dementia[23], alcohol intake (g/day) was also calculated.

The nutrient intakes from foods and from supplements were separately estimated. The nutrient intake from foods was the main focus of this analysis. A total of 29/49 (59%) participants reported taking no supplements for >1 year prior to PET, and the remaining 20/49 (41%) subjects reported taking a multivitamin at least 3 times a week, for >1 year prior to PET. Of these 20 subjects, 8 (16%) reported taking additional fish oil supplements, 5 (10%) reported taking vitamin D (300-1000 IU) and/or vitamin E (>600 IU), and 2 (4%) reported taking vitamin B12

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

(500 mcg) regularly. Twelve subjects reported taking vitamin C (1000 mg) and vitamin D (300-1000 IU) on a seasonal basis only, and mostly occasionally. None of the subjects were taking saturated fats or monounsaturated fatty acids supplementation. As some subjects reported taking supplements consistently, we additionally looked at nutrient intake from supplements, and the combined nutrient intake from food and supplements.

Brain imaging

All subjects received PiB- and FDG-PET scans using standardized procedures[10, 14, 24]. Briefly, subjects were positioned in the scanner 60 min after injection of 15 mCi of ^{11}C-PiB , and scanned for 30 min in 3D-mode on an LS Discovery or BioGraph PET/CT scanner. The FDG scan was performed 30 min after completion of the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of ^{18}F-FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. All images were corrected for photon attenuation, scatter, and radioactive decay and smoothed for uniform resolution[25].

For each subject, summed PET images corresponding to 40-60 min of FDG data and 60-90 min of PiB data were coregistered to corresponding MRI using the Normalized Mutual Information routine implemented in Statistical Parametric Mapping (SPM8)[26]. MRI were spatially normalized to Montreal Neurological Institute space by high-dimensional warping (DARTEL)[26]. MRI-coregistered FDG and PiB scans were spatially normalized using subject-specific transformation matrixes from MRI and sampled using automated regions-of-interest (ROI)[27, 28]. We focused on pre-selected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), posterior cingulate cortex (PCC) and prefrontal cortex (PFC). Standardized uptake value ratios (SUVR) were generated for each ROI

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

by normalizing PiB uptake by cerebellar grey matter uptake[29] and FDG by pons activity[30]. A composite cortical PiB retention mask (AVG_{PiB}) was created by combining parieto-temporal, frontal and PCC regions, and an FDG mask (AVG_{FDG}) by combining parieto-temporal and PCC regions[24].

Statistical Analysis

Clinical and demographical measures were examined with descriptive statistics. Stepwise forward and backward linear regressions were used to evaluate the associations between FDG and PiB ROI measures (independent variables), nutrients (dependent variables), and covariates. We first focused on nutrients from foods only. As nutrient levels are also affected by subjects' intake of supplements, analyses were repeated for the combined nutrient intake from foods and supplements, and for nutrients from supplements only. All regression models were tested for violations of the model assumptions. Variables that were not normally distributed were log-transformed which normalized the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age- and caloric intake-adjusted residuals[31]. We then examined gender[32], education, ethnicity, body mass index (BMI), alcohol consumption, family history[33] and *APOE* status[34] as covariates. Education and BMI were modeled as continuous variables. Gender (male vs. female), FH (positive vs. negative), and *APOE* status (*APOE*4+ vs. *APOE*4-) were examined as dichotomous variables. Ethnic group was based on self-report using the format of the 1990 census. Ethnicity was used as a dichotomous variable (White/non-Hispanic vs. other ethnic groups). Alcohol intake was used as a dichotomous variable (mild-moderate (0-30 g/day) vs. no (0 g/day) or more than moderate ($\geq$30 g/day) consumption).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

If one or more covariates showed significant effects on the association between nutrients and biomarkers, those covariates were separately examined for interaction effects in adjusted models by first entering the main effects, then the 2-way interactions between each nutrient and the covariate(s) in the next step. Only significant interaction terms were retained in the models.

The food sources of nutrients associated with PiB retention and FDG uptake were examined by testing for correlations between food sources (g/day) and nutrient intake[3, 4, 21, 22].

Results were considered significant at $p<0.05$ (2-sided tests). SPSS v.21 (SPPS Inc., 2013) was used for all analyses.

Results

Subjects' characteristics are found in **Table 1**. None of the participants were diabetics, smokers, or met criteria for obesity.

FDG-PET

Among all nutrients examined, β-carotene and folate from food sources were positively associated with brain glucose metabolism in several ROIs ($p\leq0.05$; **Figure 1, Table 2**). Results remained substantially unchanged using the combined nutrient intake from food and supplements. There were no associations between nutrients from supplements only and glucose metabolism in any ROI. As supplements did not appear to have significant effects, and less than half of our subjects were taking multivitamins at the time of the exam, results from β-carotene and folate from food sources are presented in what follows.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Adjustment for education, ethnicity, BMI, and alcohol consumption did not attenuate the relationships between β-carotene, folate and glucose metabolism, while gender, family history and *APOE* status showed significant interaction effects with β-carotene and folate.

Gender × β-carotene interaction effects on brain glucose metabolism were observed in PCC and AVG_{FDG} ($p \leq 0.03$, **Table 3**). In these regions, women showed significant positive associations between β-carotene and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**), resulting in steeper regression slopes of glucose metabolism on β-carotene in women compared to men (e**Figure 1**). Interaction effects of gender × folate on glucose metabolism were observed in all ROIs ($p \leq 0.05$), with women showing significant positive associations between folate and glucose metabolism ($p<0.05$) and men showing no associations (**Table 3,** e**Figure 1**). Additionally, marginally significant gender × saturated fats interaction effects were observed on PCC and AVG_{FDG} glucose metabolism ($p \leq 0.08$), as women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**).

Family history × β-carotene interaction effects on glucose metabolism were observed for all ROIs ($p \leq 0.05$). In these regions, subjects with positive family history showed significant associations between β-carotene and glucose metabolism ($p<0.05$), while those with negative family history showed no associations (**Table 3**). Interaction effects of gender × family history × β-carotene on glucose metabolism were observed ($p<0.05$), as women with a positive family history showed significant associations between β-carotene and glucose metabolism in PCC, LTL and AVG_{FDG} ($p<0.05$) whereas the other groups (i.e., men with positive family history, men and women with negative family history) did not show significant associations (**Table 3**), resulting in significant interaction effects ($p<0.05$, **eFigure 2**).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Interaction effects of *APOE* × β-carotene were marginally significant ($p \leq 0.08$), with *APOE*4+ subjects showing steeper regression slopes vs. *APOE*4-. Nonetheless, there were significant gender × *APOE* × β-carotene interaction effects, as women *APOE*4+ had the steepest regression slopes of PCC and AVG_{FDG} glucose metabolism on β-carotene as compared to the other groups (i.e., APOE4+ men, APOE4- men and women) ($p<0.05$; **Table 3, eFigure 2**). Additionally, there were significant gender × *APOE* × saturated fat interactions in glucose metabolism, as *APOE*4+ women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$), whereas the other groups (i.e., APOE4+ men, APOE4- men and women) did not (**Table 3**).

Correlations between nutrients associated with FDG uptake and food groups showed that β-carotene was mainly from dark leaf, green leafy and cruciferous vegetables and fresh fruit, with correlation coefficients of 0.82, 0.77, 0.69 and 0.53, respectively (p's$\leq$0.001). Folate was from grains, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32 and 0.30; p's$\leq$0.04), and saturated fats were from high fat dairies, salad dressing, fried potatoes, sweets and processed meat (0.65, 0.52, 0.5, 0.45, 0.41, p's$\leq$0.003).

PiB-PET

Among all nutrients examined, vitamin B12 and vitamin D levels from food sources were negatively associated with PiB retention in all ROIs ($p<0.05$; **Table 2**, **Figure 2**). There were marginally significant negative associations between PiB retention and ω-3 PUFA ($p \leq 0.10$), which prompted exploratory analyses of 3 major ω-3 PUFAs subtypes (i.e., eicosapentaenoic acid 20:5, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:3, ALA). PiB retention

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

was negatively associated with EPA (β range -0.37 in IPL to -0.31 in PCC, SE 0.49-0.43, $p<0.04$; **Figure 2**), but not with DHA or ALA ($p>0.20$, n.s.).

Analysis of nutrients from food and supplements attenuated the relationships between PiB retention and vitamin D, while those with vitamin B12 and ω-3PUFA EPA remained significant ($p\leq0.05$). There were no associations between nutrients from supplements only and PiB retention in any ROI. Since supplements did not appear to have significant effects other than to attenuate the relationship between nutrients and PiB retention, and only 41% of our subjects were taking supplements at the time of the exam, results using nutrients from food sources only are presented in what follows.

Including education, ethnicity, BMI, alcohol consumption, gender, family history and *APOE* status as covariates in the model did not attenuate the relationships between vitamin B12, vitamin D, ω-3PUFA EPA and PiB retention. There were no interaction effects between possible AD-risk factors and nutrient intake on PiB retention in any ROI (**eFigure 3**).

Correlations between nutrients associated with PiB and food groups showed that vitamin B12 was mainly from meat, eggs and butter with correlation coefficients of 0.35, 0.31 and 0.36, respectively ($p<0.04$). Vitamin D was mostly from low-fat dairies and fish (0.64 and 0.55, $p<0.001$), and ω-3PUFA EPA from fish and other vegetables (0.36 and 0.31, $p<0.01$).

Discussion

This brain imaging study shows that higher intake of vitamin B12, vitamin D, and ω3-PUFA EPA from food sources was associated with lower Aβ load, and higher β-carotene and folate

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

intake was associated with higher brain glucose metabolism in NL individuals. Higher consumption of saturated fats was associated with lower brain glucose metabolism, albeit weakly. This data indicates that a healthy diet rich in natural folate, β-carotene, ω3-PUFA, vitamin B12 and vitamin D might be particularly useful to support healthy brain aging. The identified 'AD-protective' nutrients were associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and with lower intake of high-fat dairies, processed meat and sweets. A significant impact of risk factors such as gender, *APOE* status, and family history of late-onset AD was noted on the associations between nutrients and glucose metabolism in AD-regions. Specifically, women, individuals with positive family history and APOE ε4 carriers showed the strongest associations between β-carotene, folate and saturated fats on glucose metabolism as compared to their risk-free counterparts. These effects were not evident on PiB retention, as the associations between Aβ load and intake of vitamin B12, vitamin D and ω3-PUFA EPA were independent of these risk factors for late-onset AD.

Current evidence suggests that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly[35-41], possibly due to their ability to reduce homocysteine levels. β-carotene (a major precursor to vitamin A) might have beneficial effects via its antioxidant or Aβ anti-oligomerization effects[42-47]. Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculo-protective and synaptic plasticity-enhancing effects, modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products[48-50]. On the other hand, higher intake of saturated fats is known to have negative effects on cognitive functions[51, 52], while intake of polyunsaturated fatty acids, especially ω3-PUFA, is known to decrease risk of decline[21, 22, 53, 54].

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

A distinction must be made between diet and nutrition, where nutrition refers to the components of the foods which one may absorb while diet refers to patterns of foods eaten. Knowledge of AD-protective nutrients is important to identify the food sources of these nutrients in order to develop and implement dietary recommendations. Several epidemiological studies have identified dietary patterns (i.e., food combinations) that are protective against AD. Despite differences in the analytic approaches, high adherence to dietary patterns characterized by higher intakes of fruits, vegetables, fish, nuts and legumes, and lower intake of meat, high-fat dairies and sweets, is consistently associated with reduced risk for AD[3, 4, 21, 55-59]. The food sources associated with the nutrients identified as being AD-protective using brain PET in this study are consistent with prior epidemiological findings. Additionally, present PET data provide biological evidence that higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, processed meat and sweets are associated with lower risk of brain amyloidosis and with higher metabolic activity in NL individuals at risk for AD. A community-based study of NL elderly showed that ω-3PUFA was associated with lower peripheral plasma Aβ levels [22]. Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3PUFA EPA, further reinforcing the connection between ω-3PUFA and AD pathology. Other studies with larger samples and a wider age range are needed to assess whether other ω-3PUFA markers are also associated with brain Aβ load in the elderly.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time[21], the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman et al[60] showed that higher levels of plasma anti-oxidants, vitamin B, D and ω3-PUFA were associated with more favorable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 y). Analysis of plasma

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

nutrients would be very valuable in younger NL at risk for AD. In our data set, none of the nutrients were associated with neuropsychological measures, possibly because our subjects were relatively young and all high-school graduates, which resulted in a "ceiling-effect". Longitudinal studies with larger samples are warranted to assess whether the relationship between "protective" nutrients, AD-biomarkers and cognitive performance varies with age and disease.

In our data-set, gender, family history and *APOE* status affected the relationships between brain glucose metabolism, β-carotene and to a lesser extent folate levels. To our knowledge, there are no previous studies that examined the interaction between nutrients and AD-risk factors on glucose metabolism in normal aging. Our results were in the expected direction, as at-risk individuals showed stronger effects of nutrient levels on glucose metabolism. Several studies have shown that female gender, a 1st-degree family history of late-onset AD, and the *APOE* ε4 genotype are all associated with reduced glucose metabolism in NL individuals[13, 14, 61]. Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs[62, 63], particularly of vitamin D/calcium and folic acid, as women are susceptible to hormonal changes associated with menstruation, child-bearing and osteoporosis. While limited by the small sample, APOE4+ women with a positive family history showed the strongest associations between glucose metabolism and β-carotene ($p<0.04$). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarkers associations were stronger using nutrients from foods only, which reinforces the general recommendations of a lifelong diversified diet that includes an abundance of nutrient-rich foods, and is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods[64], especially for women[65]. While both family history and *APOE* status have been associated with increased Aβ load in NL[10, 11], the effects of vitamin B12,

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

vitamin D12 and ω3-PUFA EPA on PiB retention were not exacerbated in presence of these risk factors or female gender. Overall, our data suggests that nutritional interventions aimed at preserving brain activity might be particularly useful if instituted in young adulthood in NL at risk for AD, before irreversible neuronal loss. Second, these results indicate a genetic component to dietary needs, as genetic risk in conjunction with unhealthy eating habits may boost genetic predisposition[66], and support the view that AD is a multi-factorial disease resulting from genetic, life-style and environmental interactions[67]. Third, nutrient effects on Aβ load were independent of AD-risk factors, but this may differ in older populations with more substantial Aβ deposition. Nutrients may have an impact on Aß oligomers, which are not detectable with PiB-PET, prior to plaque formation[68]. A low saturated fat/glycemic index diet was shown to improve Aβ composition in cerebrospinal fluid (CSF) in MCI [69], suggesting that diet is a powerful environmental factor that modulates AD-risk through its effects on brain Aβ and associated neuronal injury.

This study has several limitations. The NL population selected in our study consists of a group with a high a priori risk of preclinical AD-changes, and results were found in small numbers of carefully screened subjects under controlled clinical conditions. While a sample of 49 subjects with FDG and PiB-PET scans is quite sizeable from a neuroimaging perspective, larger samples are warranted to replicate these preliminary studies. The small sample led us to examine predictors and confounds in separate analyses rather than simultaneously so as to avoid over-fitting. Simultaneous statistical control of potential confounds would be informative because of conjoint confounding that stepwise approaches do not capture. Nonetheless, only gender, family history and APOE genotype had a significant impact on the associations between nutrient levels and glucose metabolism, yielding significant interaction effects even in this small sample. The

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

sample size also may have decreased the likelihood of a wide distribution of nutrient intake to fully test for potential associations. For instance, we did not report associations between biomarkers and vitamin E, possibly because vitamin E intake in our healthy population did not include the level below or above which an association may be observed. However, while there is evidence for an association between higher intakes of vitamin E or α-tocopherol equivalents and reduced incidence of AD[70], a clinical trial showed no clinical benefits of vitamin E supplementation (2000 IU/day) in MCI[71]. Likewise, we observed positive associations between folate and brain glucose metabolism in a NL, albeit at risk population, although folate levels were generally moderate. The literature on dementia indicates that low to moderate folate levels (obtained through foods) may be beneficial, while high intake levels may be harmful particularly in individuals with low vitamin B12 status[41], as high dosages of folic acid may mask vitamin B12 deficiencies[72].

Second, given the small sample, we did not test for non-linear associations between nutrients and biomarkers. An interesting approach would be to analyze indicator variables of quantiles (i.e., tertiles, quartiles) of the nutrients to test for quadratic, rather than linear associations. Third, dietary intake assessments may lead to incorrect quantification of bioavailable nutrient levels. For instance, vitamin B12 intake may be challenging to characterize by dietary intake assessment as its serum level can be affected by gastrointestinal malabsorption syndromes, such as those seen with pernicious anemia, Crohn's disease, or gastric bypass surgery; by excessive alcohol intake; or by common medications, including antacids and antidiabetic agents, among others. Although none of our subjects showed vitamin B12 deficiencies in plasma on standard blood tests, we cannot exclude that intake may have been underestimated in some participants. Moreover, a significant component to vitamin D serum levels is sunlight exposure as well as

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

dietary intake; residents of large cities have been shown to be particularly at risk for vitamin D deficiency due to a chronic lack of sunlight exposure. Malabsorption syndromes may also affect vitamin D bioavailability regardless of sunlight exposure or dietary intake, as well as conditions such as obesity or renal insufficiency, which may interfere with vitamin D extraction or conversion to its active form. A few participants reported taking vitamin D on a seasonal basis. Overall, our preliminary results using SFFQs warrant replication by direct quantification of nutrient levels from plasma to better address these potential confounds, as well as confirmation on medical screening that medical conditions or medications that may interfere with vitamin bioavailability are not present. Finally, replication of these preliminary research findings in community-based populations with more diversified socio-economic and medical status is warranted and clinical application is not yet justified.

In conclusion, we identified specific nutrients associated with healthy brain aging, which provides support for further exploration of nutritional status in the prevention of AD.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Funding/support: This study was supported by NIH/NIA grants AG035137, AG13616, Alzheimer's Association IIRG-09-132030, and an Anonymous foundation.

Contributorship Statement: Study concept and design: Mosconi, McHugh, Marmar. Acquisition of data: Mosconi, Murray, Davies, Williams, Spector, Osorio, McHugh, Vallabhajosula. Analysis and interpretation of data: Mosconi, Pirraglia, Butler, Osorio, Glodzik, McHugh, Marmar. Drafting of the manuscript: Mosconi and McHugh. Critical revision of the manuscript for important intellectual content: Mosconi, Butler, Osorio, Glodzik, Vallabhajosula, McHugh, Marmar and de Leon. Statistical analysis: Mosconi and Pirraglia. Obtained funding: Mosconi and de Leon. Administrative, technical, and material support: Mosconi and de Leon. Study supervision: Mosconi.

Financial disclosures: Drs. Mosconi, Tsui and de Leon have a patent on a technology that was licensed to Abiant Imaging Inc. by NYU and, as such, have a financial interest in this license agreement and hold stock and stock options on the company. Drs. Mosconi, Li and de Leon have received compensation for consulting services from Abiant Imaging. Dr. de Leon and Glodzik have received honoraria from the French Alzheimer Foundation. Drs. Murray, Spector, Davies, Pirraglia, Williams, Butler, Osorio, McHugh, Vallabhajosula and Marmar report no biomedical financial interests or potential conflicts of interest.

Data Sharing Statement: All clinical data, images, nutrition data and image analysis methods are freely available for academic collaboration and sharing. There are no intellectual property restrictions for academic uses.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

Legends

Figure 1. Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats.

Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless].

Corresponding *p* values are found in Table 2

Figure 2. Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA).

Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless].

Corresponding *p* values are found in Table 2.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16).

Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

β-carotene values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status

Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-483.
2. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545-1550.
3. Scarmeas N, Stern Y, Tang MX, et al. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912-921.
4. Scarmeas N, Stern Y, Mayeux R, et al. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216-225.
5. Rinaldi P, Polidori MC, Metastasio A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915-919.
6. Morris MC, Evans DA, Tangney CC, et al. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849-1853.
7. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230-3237.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
9. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469-1475.
10. Mosconi L, Rinne JO, Tsui WH, et al. Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci U S A 2010;107:5949-5954.
11. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009;106:6820-6825.
12. Langbaum JB, Fleisher AS, Chen K, et al. Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 2013;9:371-381.
13. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004;101:284-289.
14. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A 2007;104:19067-19072.
15. Hixson JE, Powers PK. Restriction isotyping of human apolipoprotein A-IV: rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. J Lipid Res 1991;32:1529-1535.
16. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
17. Hendrickson SJ, Willett WC, Rosner BA, Eliassen AH. Food predictors of plasma carotenoids. Nutrients 2013;5:4051-4066.
18. Morris MC, Tangney CC, Bienias JL, et al. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. Am J Epidemiol 2003;158:1213-1217.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

19. Newby PK, Hu FB, Rimm EB, et al. Reproducibility and validity of the Diet Quality Index Revised as assessed by use of a food-frequency questionnaire. Am J Clin Nutr 2003;78:941-949.
20. Willett W, Stampfer M, Chu NF, et al. Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. Am J Epidemiol 2001;154:1107-1112.
21. Gu Y, Nieves JW, Stern Y, et al. Food combination and Alzheimer disease risk: a protective diet. Arch Neurol 2010;67:699-706.
22. Gu Y, Schupf N, Cosentino SA, et al. Nutrient intake and plasma beta-amyloid. Neurology 2012;78:1832-1840.
23. Luchsinger JA, Tang MX, Siddiqui M, Shea S, Mayeux R. Alcohol intake and risk of dementia. J Am Geriatr Soc 2004;52:540-546.
24. Mosconi L, Rinne JO, Tsui WH, et al. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2013;34:22-34.
25. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154-159.
26. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821.
27. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology 2005;64:1860-1867.
28. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-2181.
29. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
30. Minoshima S, Frey KA, Foster NL, et al. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. J Comput Assist Tomogr 1995;19:541-547.
31. Willett WC, Stampfer MJ. Implications of total energy intake for epidemiological analyses. In: Willett WC, ed. Nutritional epidemiology. New York: Oxford University Press, 1998: 273-301.
32. Vina J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. J Alzheimers Dis 2010;20 Suppl 2:S527-533.
33. Farrer LA, O'Sullivan DM, Cupples LA, et al. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol 1989;25:485-493.
34. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001;32:181-184.
35. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. J Nutr Health Aging 2004;8:226-232.
36. Corrada MM, Kawas CH, Hallfrisch J, et al. Reduced risk of Alzheimer's disease with high folate intake: the Baltimore Longitudinal Study of Aging. Alzheimers Dement 2005;1:11-18.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

37. La Rue A, Koehler KM, Wayne SJ, et al. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am J Clin Nutr 1997;65:20-29.
38. Luchsinger JA, Tang MX, Miller J, et al. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. Arch Neurol 2007;64:86-92.
39. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641-645.
40. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 2007;369:208-216.
41. Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. J Alzheimers Dis 2006;9:429-433.
42. Kesse-Guyot E, Andreeva VA, Ducros V, et al. Carotenoid-rich dietary patterns during midlife and subsequent cognitive function. Br J Nutr 2013:1-9.
43. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids, alpha-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res 2013;2013:951786.
44. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5:56-65.
45. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. Eur J Nutr 2013;52:1553-1567.
46. de Oliveira BF, Veloso CA, Nogueira-Machado JA, et al. Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci 2012.
47. Takasaki J, Ono K, Yoshiike Y, et al. Vitamin A has anti-oligomerization effects on amyloid-beta in vitro. J Alzheimers Dis 2011;27:271-280.
48. Balion C, Griffith LE, Strifler L, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 2012;79:1397-1405.
49. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18-26.
50. Lu'o'ng KV, Nguyen LT. The role of vitamin D in Alzheimer's disease: possible genetic and cell signaling mechanisms. Am J Alzheimers Dis Other Demen 2013;28:126-136.
51. Kalmijn S, Launer LJ, Ott A, et al. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776-782.
52. Morris MC, Evans DA, Bienias JL, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194-200.
53. Bowman GL, Shannon J, Ho E, et al. Reliability and validity of food frequency questionnaire and nutrient biomarkers in elders with and without mild cognitive impairment. Alzheimer Dis Assoc Disord 2011;25:49-57.
54. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940-946.
55. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007;69:1921-1930.
56. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302:638-648.
57. Gardener S, Gu Y, Rainey-Smith SR, et al. Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. Transl Psychiatry 2012;2:e164.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

58. Gu Y, Scarmeas N. Dietary patterns in Alzheimer's disease and cognitive aging. Curr Alzheimer Res 2011;8:510-519.
59. Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599-2608.
60. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241-249.
61. Murphy DG, DeCarli C, McIntosh AR, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 1996;53:585-594.
62. Vellas BJ, Hunt WC, Romero LJ, et al. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. Nutrition 1997;13:515-519.
63. Trumbo P, Schlicker S, Yates AA, et al. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-1630.
64. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-520S.
65. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-2342.
66. de Roos B. Personalised nutrition: ready for practice? Proc Nutr Soc 2013;72:48-52.
67. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68:270-281.
68. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
69. Bayer-Carter JL, Green PS, Montine TJ, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Arch Neurol 2011;68:743-752.
70. Morris MC, Evans DA, Tangney CC, et al. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. Am J Clin Nutr 2005;81:508-514.
71. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005;352:2379-2388.
72. Smith AD. Folic acid fortification: the good, the bad, and the puzzle of vitamin B12. Am J Clin Nutr 2007; 85:3-5.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 1. Demographic and clinical characteristics

N	49
Age, y, mean (SD)	54(11), range 25-72
Female gender, %	69%
Education, y, mean (SD)	16(2)
Family history of LOAD, % positive	69%
APOE ε4 status [a], % positive	39%
Ethnicity	
White	82%
Black	8%
Hispanic	6%
Other	4%
Body Mass Index [unitless], mean (SD)	26(4)
Hip to waist ratio [unitless], mean (SD)	1.2(0.2)
Blood pressure (mm/Hg), mean (SD)	
Systolic	118(15)
Diastolic	71(9)
Glucose (mg/dl)	78(12)
Cholesterol (mg/dl)	200(38)
HDL (mg/dl)	62(19)
LDL (mg/dl)	119(33)
Triglycerids (mg/dl)	91(40)
Homocystein (micromol/l)	10(2)
Neuropsychological tests, mean (SD)	
Mini Mental State Exam	29(1)
Digit symbol substitution	62(10)
Paired associates delayed recall	7(2)
Paragraph delayed recall	10(3)
Designs	8(2)
Object naming	55(12)
WAIS-vocabulary	65(14)
Nutrients levels, mean (SD), range	
β-carotene (mcg)	
Foods	6556(8593), 825-35451
Foods and supplements	7438(8447), 1331-35957
ω-3 polyunsaturated fatty acid (g)	
Foods	1.9(1.4), 0.5-8.2
Foods and supplements	2.3(1.8), 0.8-10.9
ω-6 polyunsaturated fatty acid (g)	
Foods	19.7(17.3), 5.2-75.5
Foods and supplements	20.1(17.2), 5.3-75.5
Folate (mcg)	
Foods	486.6(345.1), 181.4-1674.2
Foods and supplements	648.1(335.9), 187.6-1759.9

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Monounsaturated fatty acids (g)	
Foods	38.7(31.5), 10.8-164.5
Foods and supplements	n.a.
Saturated fatty acids (g)	
Foods	23.5(13.0), 8.6-61.3
Foods and supplements	n.a.
Vitamin B12 (mcg)	
Foods	4.5(2.7), 1.3-12.1
Foods and supplements	15.4(9.9), 2.6-58.3
Vitamin C (mg)	
Foods	129(100), 35-521
Foods and supplements	305(267), 69-1565
Vitamin D (mcg)	
Foods	4.7(2.9), 1.1-16.3
Foods and supplements	729(445), 1.8-1806
Vitamin E (mg)	
Foods	13.2(11.5), 3.6-57.4
Foods and supplements	697(37), 632-797

[a] *APOE* genotyping was obtained from 41/49 participants

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 2. Associations between nutrients and AD-biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG glucose metabolism							
β-carotene	β	0.31[a]	0.25[c]	0.33[a]	0.31[a]	0.32[a]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35[b]	0.33[a]	0.31[a]	0.31[a]	0.36[b]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20[c]	-0.16	-0.15	-0.24[c]	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B12	β	-0.32[a]	-0.37[b]	-0.29[a]	-0.33[a]	-0.37[a]	-0.27[c]
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26[c]	-0.21	-0.28[a]	-0.13	-0.28[a]	-0.27[c]
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω3-PUFA	β	-0.18[c]	-0.28[a]	-0.07	-0.24[c]	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω3-PUFA EPA	β	-0.33[a]	-0.37[b]	-0.32[a]	-0.20	-0.31[a]	-0.28[c]
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrients from food sources

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p = 0.06\text{-}0.10$.

Abbreviations: average cortical ROI (AVG), eicosapentaenoic acid 20:4 (EPA), inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), polyunsaturated fatty acids (PUFA), posterior cingulate cortex (PCC), prefrontal cortex (PFC).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3. Effects of gender and family history on the relationship between nutrients and brain glucose metabolism

		AVG_{FDG}	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs. men=16)							
β-carotene							
Women	β	0.33 [a]	0.29	0.32 [a]	0.33 [a]	0.34 [a]	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.01	-0.01	0.08	0.08	0.06	-0.02
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate							
Women	β	0.31 [a]	0.34 [a]	0.24	0.28	0.38	0.24
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.29	0.25	0.18	0.22	0.31	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
Women	β	-0.35 [a]	-0.30	-0.32 [a]	-0.38 [a]	-0.37 [a]	-0.34 [a]
	SE	0.008	0.009	0.007	0.01	0.01	0.009
Men	β	0.15	0.16	0.17	0.12	0.09	0.12
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Family history (FH+, n=33 vs. FH-=16)							
β-carotene							
FH+	β	0.42 [b]	0.35 [a]	0.42 [b]	0.43 [b]	0.45 [b]	0.42 [b]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
FH-	β	-0.10	-0.10	-0.17	0.12	0.06	-0.2
Gender × Family history (FH+ women, n=23 vs. other subjects=26)							
β-carotene							
FH+ women	β	0.48 [a]	0.43 [a]	0.46 [a]	0.49 [b]	0.50 [b]	0.47 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.04	-0.006	0.08	0.09	0.04	0.05
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Gender × APOE genotype (APOE4+ women, n=13 vs. other subjects=36)							
β-carotene							
*APOE*4+ women	β	0.52 [a]	0.46 [a]	0.48 [a]	0.54 [a]	0.53 [a]	0.48 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.10	0.10	0.10	-0.08	0.03	-0.08
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
*APOE*4+ women	β	-0.66 [b]	-0.60 [a]	-0.59 [b]	-0.57 [b]	-0.68 [b]	-0.57 [a]
	SE	0.02	0.03	0.02	0.03	0.03	0.03
Other	β	0.11	0.11	0.16	0.18	0.08	0.19

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrient values

[a] $p<0.05$, [b] $p\leq0.01$. See Legend to Table 2.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Lisa Mosconi[1*], PhD, John Murray[1], BA, Michelle Davies[1], BA, Schantel Williams[1], RN, Elizabeth Pirraglia[1], MA, Nicole Spector[1], BA, Wai H. Tsui[1], MS, Yi Li[1], MD, Tracy Butler[1], MD, Ricardo S. Osorio[1], MD, Lidia Glodzik[1], MD, PhD, Shankar Vallabhajosula[2], PhD, Pauline McHugh[1], MD, Charles Marmar[1,3], MD, Mony J. de Leon[1], EdD

[1]Department of Psychiatry, New York University School of Medicine, New York, NY 10016; [2]Department of Radiology, Citigroup Biomedical Imaging Center (CBIC), Weill Cornell Medical College, New York, NY 10021; [3]Steven and Alexandra Cohen Veterans Center for PTSD and TBI, New York, NY 10016

* Dr. Lisa Mosconi, Department of Psychiatry, NYU School of Medicine, 145 East 32nd St, 2nd Floor, New York NY, 10016. Tel: (212) 263-3255, Fax: (212) 263-3270 Email: lisa.mosconi@nyumc.org

Word count

Title: 137 characters (including spaces)

Manuscript Words: 4034

Abstract Words: 300

Tables: 3, Figures: 2, Supplemental files: 3 [eFigure 1; eFigure 2; eFigure 3]

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abstract

Objectives. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD). This study examines the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal individuals (NL) with and without AD risk factors.

Design. As part of an ongoing brain imaging study, participants received clinical and laboratory exams, a neurocognitive test battery, Positron Emission Tomography (PET) with ^{11}C-Pittsburgh Compound B (PiB; a measure of amyloid-β (Aβ) load) and ^{18}F-fluorodeoxyglucose (FDG; a proxy of neuronal activity), and completed semi-quantitative food frequency questionnaires.

Setting. Research center affiliated with the Alzheimer's disease Core Center at New York University School of Medicine.

Participants. Forty-nine NL individuals (age 25-72 y, 69% female) with dietary information, ^{11}C-PiB and ^{18}F-FDG PET scans were examined.

Results. Controlling for age and total caloric intake, higher intake of vitamin B12, vitamin D and ω-3 polyunsaturated fatty acid (PUFA) associated with lower Aβ load in AD-regions on PiB-PET, while higher intake of β-carotene and folate associated with higher glucose metabolism on FDG-PET. β-carotene and folate were associated with reduced glucose metabolism for women, *APOE*4 carriers and subjects with positive AD family history, but not for their risk-free counterparts. The associations of vitamin B12, vitamin D and ω-3PUFA with PiB retention were independent of gender, APOE and family history. The identified nutrient combination was associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, meat and sweets.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Conclusions. Our data provide a potential pathophysiological mechanism for epidemiological findings showing that dietary interventions may play a role in the prevention of AD. Longitudinal studies are needed to determine whether there is a direct link between nutrient intake, brain biomarkers and risk of AD.

Article focus

- To investigate the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal (NL) adults with and without AD risk factors.
- To define which nutrients are protective against AD pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals
- To test whether the effects would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype, a major susceptibility gene for late-onset AD.

Key messages

- Higher intake of vitamin B12, vitamin D, ω-3 polyunsaturated fatty acid (PUFA), β-carotene and folate was associated with lower amyloid-beta pathology and higher metabolic activity in AD-vulnerable brain regions.
- These associations were stronger for women, *APOE*4 carriers and subjects with positive AD family history, than for their risk-free counterparts.
- Nutrient-biomarkers associations were stronger using nutrients from foods only, which is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging and cognitive decline are less so. This study shows a relationship between nutrients and AD-biomarkers in cognitively intact persons at risk for AD, suggesting that dietary interventions may play a role in the prevention of AD
- This was a cross-sectional study whose purpose was to explore a possible link between dietary nutrients and brain biomarkers of AD in yet asymptomatic individuals; further longitudinal studies are necessary to show causal links.
- More studies with larger sample sizes and plasma nutrient measures are needed to further elucidate the inter-relationship between dietary choices, brain physiology and risk of future AD.
- Our preliminary findings of interaction effects between nutrients and AD risk factors such as gender, APOE genotype and family history need to be replicated with larger samples.

Funding/support: This study was supported by NIH/NIA grants AG035137, AG13616, Alzheimer's Association IIRG-09-132030, and an Anonymous foundation.

Financial disclosures: Drs. Mosconi, Tsui and de Leon have a patent on a technology that was licensed to Abiant Imaging Inc. by NYU and, as such, have a financial interest in this license agreement and hold stock and stock options on the company. Drs. Mosconi, Li and de Leon have received compensation for consulting services from Abiant Imaging. Dr. de Leon and Glodzik

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

have received honoraria from the French Alzheimer Foundation. Drs. Murray, Spector, Davies, Pirraglia, Williams, Butler, Osorio, McHugh, Vallabhajosula and Marmar report no biomedical financial interests or potential conflicts of interest.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Introduction

"We are what we eat": the nutritional content of what we eat determines the composition of our cell membranes, bone marrow, blood, hormones, and is therefore the foundation upon which our body and brain are built. While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging are less so. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem[1-7]. However, the biological mechanisms underlying the relationship between dietary nutrients, brain aging and AD are largely unexplored. Understanding how diet and nutrition promote healthy brain aging in cognitively normal (NL) individuals, especially those at increased risk for AD, is critical prior to implementing dietary recommendations for prevention and treatment of disease.

While it is intuitive to assume a link between nutrient intake and overall brain functioning exists, to our knowledge there are no studies that examined the relationship between nutrient intake and brain amyloid-β (Aβ), a major hallmark of AD pathology, or glucose metabolism, a proxy for neuronal activity, in NL individuals.

The present brain imaging study examines the relationship between nutrient intake, brain Aβ load assessed using ^{11}C-Pittsburgh Compound-B (PiB) Positron Emission Tomography (PET), and glucose metabolism assessed using ^{18}F-fluorodeoxyglucose (FDG) PET in a cohort of young to late-middle aged NL individuals with or without risk factors for AD. Several studies demonstrated increased PiB-PET retention in AD and Mild Cognitive Impairment (MCI), often a prodromal AD phase, compared to controls[8], as well as in non-demented elderly at risk for AD[9-11]. Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

clinical symptoms[12-14]. Our goals were to define which nutrients are associated with lower AD-burden (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals, and to test whether the associations are stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype.

Methods

Participants

Among a larger pool of participants who underwent PET exams, this study included 49 prospectively recruited, clinically and cognitively normal (NL) individuals participating in longitudinal PET studies at NYU School of Medicine, who completed clinical, PiB- and FDG-PET exams and dietary questionnaires within 6 months of each other. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All subjects provided written informed consent to participate in this IRB approved study.

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[14]. A family history of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires[14]. *APOE* genotypes were determined using standard

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

quantitative polymerase chain reaction (qPCR)[15]. Individuals with at least one copy of the ε4 allele were grouped as *APOE* ε4 carriers (APOE4+) and compared to ε4 non-carriers (APOE4-).

Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Willett's semi-quantitative food frequency questionnaire (SFFQ)[16]. The SFFQ has been validated for determination of nutrient intake in the elderly and young adults[16-20]. The 61-food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (g/day) of each food group was calculated by summing the intakes of member food items. The daily intake of nutrients was then computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion[16].

Similarly to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function[21, 22], including saturated fatty acids, monounsaturated fatty acids, ω-3 polyunsaturated fatty acid (PUFA), ω-6 PUFA, β-carotene, vitamin B12, vitamin C, vitamin D, vitamin E, and folate. As moderate alcohol drinking may be protective against dementia[23], alcohol intake (g/day) was also calculated.

The nutrient intakes from foods and from supplements were separately estimated. The nutrient intake from foods was the main focus of this analysis. A total of 29/49 (59%) participants reported taking no supplements for >1 year prior to PET, and the remaining 20/49 (41%) subjects reported taking a multivitamin at least 3 times a week, for >1 year prior to PET. Of these 20 subjects, 8 (16%) reported taking additional fish oil supplements, 5 (10%) reported taking vitamin D (300-1000 IU) and/or vitamin E (>600 IU), and 2 (4%) reported taking vitamin B12

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

(500 mcg) regularly. Twelve subjects reported taking vitamin C (1000 mg) and vitamin D (300-1000 IU) on a seasonal basis only, and mostly occasionally. None of the subjects were taking saturated fats or monounsaturated fatty acids supplementation. As some subjects reported taking supplements consistently, we additionally looked at nutrient intake from supplements, and the combined nutrient intake from food and supplements.

Brain imaging

All subjects received PiB- and FDG-PET scans using standardized procedures[10, 14, 24]. Briefly, subjects were positioned in the scanner 60 min after injection of 15 mCi of ^{11}C-PiB , and scanned for 30 min in 3D-mode on an LS Discovery or BioGraph PET/CT scanner. The FDG scan was performed 30 min after completion of the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of ^{18}F-FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. All images were corrected for photon attenuation, scatter, and radioactive decay and smoothed for uniform resolution[25].

For each subject, summed PET images corresponding to 40-60 min of FDG data and 60-90 min of PiB data were coregistered to corresponding MRI using the Normalized Mutual Information routine implemented in Statistical Parametric Mapping (SPM8)[26]. MRI were spatially normalized to Montreal Neurological Institute space by high-dimensional warping (DARTEL)[26]. MRI-coregistered FDG and PiB scans were spatially normalized using subject-specific transformation matrixes from MRI and sampled using automated regions-of-interest (ROI)[27, 28]. We focused on pre-selected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), posterior cingulate cortex (PCC) and prefrontal cortex (PFC). Standardized uptake value ratios (SUVR) were generated for each ROI

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

by normalizing PiB uptake by cerebellar grey matter uptake[29] and FDG by pons activity[30]. A composite cortical PiB retention mask (AVG_{PiB}) was created by combining parieto-temporal, frontal and PCC regions, and an FDG mask (AVG_{FDG}) by combining parieto-temporal and PCC regions[24].

Statistical Analysis

Clinical and demographical measures were examined with descriptive statistics. Stepwise forward and backward linear regressions were used to evaluate the associations between FDG and PiB ROI measures (independent variables), nutrients (dependent variables), and covariates. We first focused on nutrients from foods only. As nutrient levels are also affected by subjects' intake of supplements, analyses were repeated for the combined nutrient intake from foods and supplements, and for nutrients from supplements only. All regression models were tested for violations of the model assumptions. Variables that were not normally distributed were log-transformed which normalized the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age- and caloric intake-adjusted residuals[31]. We then examined gender[32], education, ethnicity, body mass index (BMI), alcohol consumption, family history[33] and *APOE* status[34] as covariates. Education and BMI were modeled as continuous variables. Gender (male vs. female), FH (positive vs. negative), and *APOE* status (*APOE*4+ vs. *APOE*4-) were examined as dichotomous variables. Ethnic group was based on self-report using the format of the 1990 census. Ethnicity was used as a dichotomous variable (White/non-Hispanic vs. other ethnic groups). Alcohol intake was used as a dichotomous variable (mild-moderate (0-30 g/day) vs. no (0 g/day) or more than moderate ($\geq$30 g/day) consumption).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

If one or more covariates showed significant effects on the association between nutrients and biomarkers, those covariates were separately examined for interaction effects in adjusted models by first entering the main effects, then the 2-way interactions between each nutrient and the covariate(s) in the next step. Only significant interaction terms were retained in the models.

The food sources of nutrients associated with PiB retention and FDG uptake were examined by testing for correlations between food sources (g/day) and nutrient intake[3, 4, 21, 22].

Results were considered significant at $p<0.05$ (2-sided tests). SPSS v.21 (SPPS Inc., 2013) was used for all analyses.

Results

Subjects' characteristics are found in **Table 1**. None of the participants were diabetics, smokers, or met criteria for obesity.

FDG-PET

Among all nutrients examined, β-carotene and folate from food sources were positively associated with brain glucose metabolism in several ROIs ($p\leq0.05$; **Figure 1, Table 2**). Results remained substantially unchanged using the combined nutrient intake from food and supplements. There were no associations between nutrients from supplements only and glucose metabolism in any ROI. As supplements did not appear to have significant effects, and less than half of our subjects were taking multivitamins at the time of the exam, results from β-carotene and folate from food sources are presented in what follows.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Adjustment for education, ethnicity, BMI, and alcohol consumption did not attenuate the relationships between β-carotene, folate and glucose metabolism, while gender, family history and *APOE* status showed significant interaction effects with β-carotene and folate.

Gender × β-carotene interaction effects on brain glucose metabolism were observed in PCC and AVG_{FDG} ($p \leq 0.03$, **Table 3**). In these regions, women showed significant positive associations between β-carotene and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**), resulting in steeper regression slopes of glucose metabolism on β-carotene in women compared to men (e**Figure 1**). Interaction effects of gender × folate on glucose metabolism were observed in all ROIs ($p \leq 0.05$), with women showing significant positive associations between folate and glucose metabolism ($p<0.05$) and men showing no associations (**Table 3,** e**Figure 1**). Additionally, marginally significant gender × saturated fats interaction effects were observed on PCC and AVG_{FDG} glucose metabolism ($p \leq 0.08$), as women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**).

Family history × β-carotene interaction effects on glucose metabolism were observed for all ROIs ($p \leq 0.05$). In these regions, subjects with positive family history showed significant associations between β-carotene and glucose metabolism ($p<0.05$), while those with negative family history showed no associations (**Table 3**). Interaction effects of gender × family history × β-carotene on glucose metabolism were observed ($p<0.05$), as women with a positive family history showed significant associations between β-carotene and glucose metabolism in PCC, LTL and AVG_{FDG} ($p<0.05$) whereas the other groups (i.e., men with positive family history, men and women with negative family history) did not show significant associations (**Table 3**), resulting in significant interaction effects ($p<0.05$, **eFigure 2**).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Interaction effects of *APOE* × β-carotene were marginally significant (p≤0.08), with *APOE*4+ subjects showing steeper regression slopes vs. *APOE*4-. Nonetheless, there were significant gender × *APOE* × β-carotene interaction effects, as women *APOE*4+ had the steepest regression slopes of PCC and AVG_{FDG} glucose metabolism on β-carotene as compared to the other groups (i.e., APOE4+ men, APOE4- men and women) (p<0.05; **Table 3, eFigure 2**). Additionally, there were significant gender × *APOE* × saturated fat interactions in glucose metabolism, as *APOE*4+ women showed significant negative associations between saturated fats and glucose metabolism (p<0.05), whereas the other groups (i.e., APOE4+ men, APOE4- men and women) did not (**Table 3**).

Correlations between nutrients associated with FDG uptake and food groups showed that β-carotene was mainly from dark leaf, green leafy and cruciferous vegetables and fresh fruit, with correlation coefficients of 0.82, 0.77, 0.69 and 0.53, respectively (p's≤0.001). Folate was from grains, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32 and 0.30; p's≤0.04), and saturated fats were from high fat dairies, salad dressing, fried potatoes, sweets and processed meat (0.65, 0.52, 0.5, 0.45, 0.41, p's≤0.003).

PiB-PET

Among all nutrients examined, vitamin B12 and vitamin D levels from food sources were negatively associated with PiB retention in all ROIs (p<0.05; **Table 2**, **Figure 2**). There were marginally significant negative associations between PiB retention and ω-3 PUFA (p≤0.10), which prompted exploratory analyses of 3 major ω-3 PUFAs subtypes (i.e., eicosapentaenoic acid 20:5, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:3, ALA). PiB retention

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

was negatively associated with EPA (β range -0.37 in IPL to -0.31 in PCC, SE 0.49-0.43, p<0.04; **Figure 2**), but not with DHA or ALA (p>0.20, n.s.).

Analysis of nutrients from food and supplements attenuated the relationships between PiB retention and vitamin D, while those with vitamin B12 and ω-3PUFA EPA remained significant (p≤0.05). There were no associations between nutrients from supplements only and PiB retention in any ROI. Since supplements did not appear to have significant effects other than to attenuate the relationship between nutrients and PiB retention, and only 41% of our subjects were taking supplements at the time of the exam, results using nutrients from food sources only are presented in what follows.

Including education, ethnicity, BMI, alcohol consumption, gender, family history and *APOE* status as covariates in the model did not attenuate the relationships between vitamin B12, vitamin D, ω-3PUFA EPA and PiB retention. There were no interaction effects between possible AD-risk factors and nutrient intake on PiB retention in any ROI (**eFigure 3**).

Correlations between nutrients associated with PiB and food groups showed that vitamin B12 was mainly from meat, eggs and butter with correlation coefficients of 0.35, 0.31 and 0.36, respectively (p<0.04). Vitamin D was mostly from low-fat dairies and fish (0.64 and 0.55, p<0.001), and ω-3PUFA EPA from fish and other vegetables (0.36 and 0.31, p<0.01).

Discussion

This brain imaging study shows that higher intake of vitamin B12, vitamin D, and ω3-PUFA EPA from food sources was associated with lower Aβ load, and higher β-carotene and folate

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

intake was associated with higher brain glucose metabolism in NL individuals. Higher consumption of saturated fats was associated with lower brain glucose metabolism, albeit weakly. This data indicates that a healthy diet rich in natural folate, β-carotene, ω3-PUFA, vitamin B12 and vitamin D might be particularly useful to support healthy brain aging. The identified 'AD-protective' nutrients were associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and with lower intake of high-fat dairies, processed meat and sweets. A significant impact of risk factors such as gender, *APOE* status, and family history of late-onset AD was noted on the associations between nutrients and glucose metabolism in AD-regions. Specifically, women, individuals with positive family history and APOE ε4 carriers showed the strongest associations between β-carotene, folate and saturated fats on glucose metabolism as compared to their risk-free counterparts. These effects were not evident on PiB retention, as the associations between Aβ load and intake of vitamin B12, vitamin D and ω3-PUFA EPA were independent of these risk factors for late-onset AD.

Current evidence suggests that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly[35-41], possibly due to their ability to reduce homocysteine levels. β-carotene (a major precursor to vitamin A) might have beneficial effects via its antioxidant or Aβ anti-oligomerization effects[42-47]. Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculo-protective and synaptic plasticity-enhancing effects, modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products[48-50]. On the other hand, higher intake of saturated fats is known to have negative effects on cognitive functions[51, 52], while intake of polyunsaturated fatty acids, especially ω3-PUFA, is known to decrease risk of decline[21, 22, 53, 54].

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

A distinction must be made between diet and nutrition, where nutrition refers to the components of the foods which one may absorb while diet refers to patterns of foods eaten. Knowledge of AD-protective nutrients is important to identify the food sources of these nutrients in order to develop and implement dietary recommendations. Several epidemiological studies have identified dietary patterns (i.e., food combinations) that are protective against AD. Despite differences in the analytic approaches, high adherence to dietary patterns characterized by higher intakes of fruits, vegetables, fish, nuts and legumes, and lower intake of meat, high-fat dairies and sweets, is consistently associated with reduced risk for AD[3, 4, 21, 55-59]. The food sources associated with the nutrients identified as being AD-protective using brain PET in this study are consistent with prior epidemiological findings. Additionally, present PET data provide biological evidence that higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, processed meat and sweets are associated with lower risk of brain amyloidosis and with higher metabolic activity in NL individuals at risk for AD. A community-based study of NL elderly showed that ω-3PUFA was associated with lower peripheral plasma Aβ levels [22]. Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3PUFA EPA, further reinforcing the connection between ω-3PUFA and AD pathology. Other studies with larger samples and a wider age range are needed to assess whether other ω-3PUFA markers are also associated with brain Aβ load in the elderly.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time[21], the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman et al[60] showed that higher levels of plasma anti-oxidants, vitamin B, D and ω3-PUFA were associated with more favorable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 y). Analysis of plasma

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

nutrients would be very valuable in younger NL at risk for AD. In our data set, none of the nutrients were associated with neuropsychological measures, possibly because our subjects were relatively young and all high-school graduates, which resulted in a "ceiling-effect". Longitudinal studies with larger samples are warranted to assess whether the relationship between "protective" nutrients, AD-biomarkers and cognitive performance varies with age and disease.

In our data-set, gender, family history and *APOE* status affected the relationships between brain glucose metabolism, β-carotene and to a lesser extent folate levels. To our knowledge, there are no previous studies that examined the interaction between nutrients and AD-risk factors on glucose metabolism in normal aging. Our results were in the expected direction, as at-risk individuals showed stronger effects of nutrient levels on glucose metabolism. Several studies have shown that female gender, a 1st-degree family history of late-onset AD, and the *APOE* ε4 genotype are all associated with reduced glucose metabolism in NL individuals[13, 14, 61]. Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs[62, 63], particularly of vitamin D/calcium and folic acid, as women are susceptible to hormonal changes associated with menstruation, child-bearing and osteoporosis. While limited by the small sample, APOE4+ women with a positive family history showed the strongest associations between glucose metabolism and β-carotene ($p<0.04$). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarkers associations were stronger using nutrients from foods only, which reinforces the general recommendations of a lifelong diversified diet that includes an abundance of nutrient-rich foods, and is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods[64], especially for women[65]. While both family history and *APOE* status have been associated with increased Aβ load in NL[10, 11], the effects of vitamin B12,

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

vitamin D12 and ω3-PUFA EPA on PiB retention were not exacerbated in presence of these risk factors or female gender. Overall, our data suggests that nutritional interventions aimed at preserving brain activity might be particularly useful if instituted in young adulthood in NL at risk for AD, before irreversible neuronal loss. Second, these results indicate a genetic component to dietary needs, as genetic risk in conjunction with unhealthy eating habits may boost genetic predisposition[66], and support the view that AD is a multi-factorial disease resulting from genetic, life-style and environmental interactions[67]. Third, nutrient effects on Aβ load were independent of AD-risk factors, but this may differ in older populations with more substantial Aβ deposition. Nutrients may have an impact on Aß oligomers, which are not detectable with PiB-PET, prior to plaque formation[68]. A low saturated fat/glycemic index diet was shown to improve Aβ composition in cerebrospinal fluid (CSF) in MCI [69], suggesting that diet is a powerful environmental factor that modulates AD-risk through its effects on brain Aβ and associated neuronal injury.

This study has several limitations. The NL population selected in our study consists of a group with a high a priori risk of preclinical AD-changes, and results were found in small numbers of carefully screened subjects under controlled clinical conditions. While a sample of 49 subjects with FDG and PiB-PET scans is quite sizeable from a neuroimaging perspective, larger samples are warranted to replicate these preliminary studies. The small sample led us to examine predictors and confounds in separate analyses rather than simultaneously so as to avoid over-fitting. Simultaneous statistical control of potential confounds would be informative because of conjoint confounding that stepwise approaches do not capture. Nonetheless, only gender, family history and APOE genotype had a significant impact on the associations between nutrient levels and glucose metabolism, yielding significant interaction effects even in this small sample. The

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

sample size also may have decreased the likelihood of a wide distribution of nutrient intake to fully test for potential associations. For instance, we did not report associations between biomarkers and vitamin E, possibly because vitamin E intake in our healthy population did not include the level below or above which an association may be observed. However, while there is evidence for an association between higher intakes of vitamin E or α-tocopherol equivalents and reduced incidence of AD[70], a clinical trial showed no clinical benefits of vitamin E supplementation (2000 IU/day) in MCI[71]. Likewise, we observed positive associations between folate and brain glucose metabolism in a NL, albeit at risk population, although folate levels were generally moderate. The literature on dementia indicates that low to moderate folate levels (obtained through foods) may be beneficial, while high intake levels may be harmful particularly in individuals with low vitamin B12 status[41], as high dosages of folic acid may mask vitamin B12 deficiencies[72].

Second, given the small sample, we did not test for non-linear associations between nutrients and biomarkers. An interesting approach would be to analyze indicator variables of quantiles (i.e., tertiles, quartiles) of the nutrients to test for quadratic, rather than linear associations. Third, dietary intake assessments may lead to incorrect quantification of bioavailable nutrient levels. For instance, vitamin B12 intake may be challenging to characterize by dietary intake assessment as its serum level can be affected by gastrointestinal malabsorption syndromes, such as those seen with pernicious anemia, Crohn's disease, or gastric bypass surgery; by excessive alcohol intake; or by common medications, including antacids and antidiabetic agents, among others. Although none of our subjects showed vitamin B12 deficiencies in plasma on standard blood tests, we cannot exclude that intake may have been underestimated in some participants. Moreover, a significant component to vitamin D serum levels is sunlight exposure as well as

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

dietary intake; residents of large cities have been shown to be particularly at risk for vitamin D deficiency due to a chronic lack of sunlight exposure. Malabsorption syndromes may also affect vitamin D bioavailability regardless of sunlight exposure or dietary intake, as well as conditions such as obesity or renal insufficiency, which may interfere with vitamin D extraction or conversion to its active form. A few participants reported taking vitamin D on a seasonal basis. Overall, our preliminary results using SFFQs warrant replication by direct quantification of nutrient levels from plasma to better address these potential confounds, as well as confirmation on medical screening that medical conditions or medications that may interfere with vitamin bioavailability are not present. Finally, replication of these preliminary research findings in community-based populations with more diversified socio-economic and medical status is warranted and clinical application is not yet justified.

In conclusion, we identified specific nutrients associated with healthy brain aging, which provides support for further exploration of nutritional status in the prevention of AD.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Legends

Figure 1. Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats.

Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless].

Corresponding *p* values are found in Table 2

Figure 2. Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA).

Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless].

Corresponding *p* values are found in Table 2.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16).

Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

β-carotene values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status

Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-483.
2. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545-1550.
3. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912-921.
4. Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216-225.
5. Rinaldi P, Polidori MC, Metastasio A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915-919.
6. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849-1853.
7. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230-3237.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
9. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469-1475.
10. Mosconi L, Rinne JO, Tsui WH, et al. Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci U S A 2010;107:5949-5954.
11. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009;106:6820-6825.
12. Langbaum JB, Fleisher AS, Chen K, et al. Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 2013;9:371-381.
13. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004;101:284-289.
14. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A 2007;104:19067-19072.
15. Hixson JE, Powers PK. Restriction isotyping of human apolipoprotein A-IV: rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. J Lipid Res 1991;32:1529-1535.
16. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
17. Hendrickson SJ, Willett WC, Rosner BA, Eliassen AH. Food predictors of plasma carotenoids. Nutrients 2013;5:4051-4066.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

18. Morris MC, Tangney CC, Bienias JL, Evans DA, Wilson RS. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. Am J Epidemiol 2003;158:1213-1217.
19. Newby PK, Hu FB, Rimm EB, et al. Reproducibility and validity of the Diet Quality Index Revised as assessed by use of a food-frequency questionnaire. Am J Clin Nutr 2003;78:941-949.
20. Willett W, Stampfer M, Chu NF, Spiegelman D, Holmes M, Rimm E. Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. Am J Epidemiol 2001;154:1107-1112.
21. Gu Y, Nieves JW, Stern Y, Luchsinger JA, Scarmeas N. Food combination and Alzheimer disease risk: a protective diet. Arch Neurol 2010;67:699-706.
22. Gu Y, Schupf N, Cosentino SA, Luchsinger JA, Scarmeas N. Nutrient intake and plasma beta-amyloid. Neurology 2012;78:1832-1840.
23. Luchsinger JA, Tang MX, Siddiqui M, Shea S, Mayeux R. Alcohol intake and risk of dementia. J Am Geriatr Soc 2004;52:540-546.
24. Mosconi L, Rinne JO, Tsui WH, et al. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2013;34:22-34.
25. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154-159.
26. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821.
27. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology 2005;64:1860-1867.
28. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-2181.
29. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
30. Minoshima S, Frey KA, Foster NL, Kuhl DE. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. J Comput Assist Tomogr 1995;19:541-547.
31. Willett WC, Stampfer MJ. Implications of total energy intake for epidemiological analyses. In: Willett WC, ed. Nutritional epidemiology. New York: Oxford University Press, 1998: 273-301.
32. Vina J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. J Alzheimers Dis 2010;20 Suppl 2:S527-533.
33. Farrer LA, O'Sullivan DM, Cupples LA, Growdon JH, Myers RH. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol 1989;25:485-493.
34. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001;32:181-184.
35. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. J Nutr Health Aging 2004;8:226-232.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

36. Corrada MM, Kawas CH, Hallfrisch J, Muller D, Brookmeyer R. Reduced risk of Alzheimer's disease with high folate intake: the Baltimore Longitudinal Study of Aging. Alzheimers Dement 2005;1:11-18.
37. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am J Clin Nutr 1997;65:20-29.
38. Luchsinger JA, Tang MX, Miller J, Green R, Mayeux R. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. Arch Neurol 2007;64:86-92.
39. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641-645.
40. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 2007;369:208-216.
41. Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. J Alzheimers Dis 2006;9:429-433.
42. Kesse-Guyot E, Andreeva VA, Ducros V, et al. Carotenoid-rich dietary patterns during midlife and subsequent cognitive function. Br J Nutr 2013:1-9.
43. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids, alpha-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res 2013;2013:951786.
44. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5:56-65.
45. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. Eur J Nutr 2013;52:1553-1567.
46. de Oliveira BF, Veloso CA, Nogueira-Machado JA, et al. Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci 2012.
47. Takasaki J, Ono K, Yoshiike Y, et al. Vitamin A has anti-oligomerization effects on amyloid-beta in vitro. J Alzheimers Dis 2011;27:271-280.
48. Balion C, Griffith LE, Strifler L, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 2012;79:1397-1405.
49. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18-26.
50. Lu'o'ng KV, Nguyen LT. The role of vitamin D in Alzheimer's disease: possible genetic and cell signaling mechanisms. Am J Alzheimers Dis Other Demen 2013;28:126-136.
51. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776-782.
52. Morris MC, Evans DA, Bienias JL, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194-200.
53. Bowman GL, Shannon J, Ho E, et al. Reliability and validity of food frequency questionnaire and nutrient biomarkers in elders with and without mild cognitive impairment. Alzheimer Dis Assoc Disord 2011;25:49-57.
54. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940-946.
55. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007;69:1921-1930.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

56. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302:638-648.
57. Gardener S, Gu Y, Rainey-Smith SR, et al. Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. Transl Psychiatry 2012;2:e164.
58. Gu Y, Scarmeas N. Dietary patterns in Alzheimer's disease and cognitive aging. Curr Alzheimer Res 2011;8:510-519.
59. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599-2608.
60. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241-249.
61. Murphy DG, DeCarli C, McIntosh AR, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 1996;53:585-594.
62. Vellas BJ, Hunt WC, Romero LJ, Koehler KM, Baumgartner RN, Garry PJ. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. Nutrition 1997;13:515-519.
63. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-1630.
64. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-520S.
65. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-2342.
66. de Roos B. Personalised nutrition: ready for practice? Proc Nutr Soc 2013;72:48-52.
67. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68:270-281.
68. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
69. Bayer-Carter JL, Green PS, Montine TJ, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Arch Neurol 2011;68:743-752.
70. Morris MC, Evans DA, Tangney CC, et al. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. Am J Clin Nutr 2005;81:508-514.
71. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005;352:2379-2388.
72. Smith AD. Folic acid fortification: the good, the bad, and the puzzle of vitamin B12. Am J Clin Nutr 2007; 85:3-5.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 1. Demographic and clinical characteristics

N	49
Age, y, mean (SD)	54(11), range 25-72
Female gender, %	69%
Education, y, mean (SD)	16(2)
Family history of LOAD, % positive	69%
APOE ε4 status [a], % positive	39%
Ethnicity	
White	82%
Black	8%
Hispanic	6%
Other	4%
Body Mass Index [unitless], mean (SD)	26(4)
Hip to waist ratio [unitless], mean (SD)	1.2(0.2)
Blood pressure (mm/Hg), mean (SD)	
Systolic	118(15)
Diastolic	71(9)
Glucose (mg/dl)	78(12)
Cholesterol (mg/dl)	200(38)
HDL (mg/dl)	62(19)
LDL (mg/dl)	119(33)
Triglycerids (mg/dl)	91(40)
Homocystein (micromol/l)	10(2)
Neuropsychological tests, mean (SD)	
Mini Mental State Exam	29(1)
Digit symbol substitution	62(10)
Paired associates delayed recall	7(2)
Paragraph delayed recall	10(3)
Designs	8(2)
Object naming	55(12)
WAIS-vocabulary	65(14)
Nutrients levels, mean (SD), range	
β-carotene (mcg)	
Foods	6556(8593), 825-35451
Foods and supplements	7438(8447), 1331-35957
ω-3 polyunsaturated fatty acid (g)	
Foods	1.9(1.4), 0.5-8.2
Foods and supplements	2.3(1.8), 0.8-10.9
ω-6 polyunsaturated fatty acid (g)	
Foods	19.7(17.3), 5.2-75.5
Foods and supplements	20.1(17.2), 5.3-75.5
Folate (mcg)	
Foods	486.6(345.1), 181.4-1674.2

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Foods and supplements	648.1(335.9), 187.6-1759.9
Monounsaturated fatty acids (g)	
Foods	38.7(31.5), 10.8-164.5
Foods and supplements	n.a.
Saturated fatty acids (g)	
Foods	23.5(13.0), 8.6-61.3
Foods and supplements	n.a.
Vitamin B12 (mcg)	
Foods	4.5(2.7), 1.3-12.1
Foods and supplements	15.4(9.9), 2.6-58.3
Vitamin C (mg)	
Foods	129(100), 35-521
Foods and supplements	305(267), 69-1565
Vitamin D (mcg)	
Foods	4.7(2.9), 1.1-16.3
Foods and supplements	729(445), 1.8-1806
Vitamin E (mg)	
Foods	13.2(11.5), 3.6-57.4
Foods and supplements	697(37), 632-797

[a] *APOE* genotyping was obtained from 41/49 participants

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 2. Associations between nutrients and AD-biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG glucose metabolism							
β-carotene	β	0.31[a]	0.25[c]	0.33[a]	0.31[a]	0.32[a]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35[b]	0.33[a]	0.31[a]	0.31[a]	0.36[b]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20[c]	-0.16	-0.15	-0.24[c]	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B12	β	-0.32[a]	-0.37[b]	-0.29[a]	-0.33[a]	-0.37[a]	-0.27[c]
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26[c]	-0.21	-0.28[a]	-0.13	-0.28[a]	-0.27[c]
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω3-PUFA	β	-0.18[c]	-0.28[a]	-0.07	-0.24[c]	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω3-PUFA EPA	β	-0.33[a]	-0.37[b]	-0.32[a]	-0.20	-0.31[a]	-0.28[c]
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrients from food sources

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p = 0.06\text{-}0.10$.

Abbreviations: average cortical ROI (AVG), eicosapentaenoic acid 20:4 (EPA), inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), polyunsaturated fatty acids (PUFA), posterior cingulate cortex (PCC), prefrontal cortex (PFC).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3. Effects of gender and family history on the relationship between nutrients and brain glucose metabolism

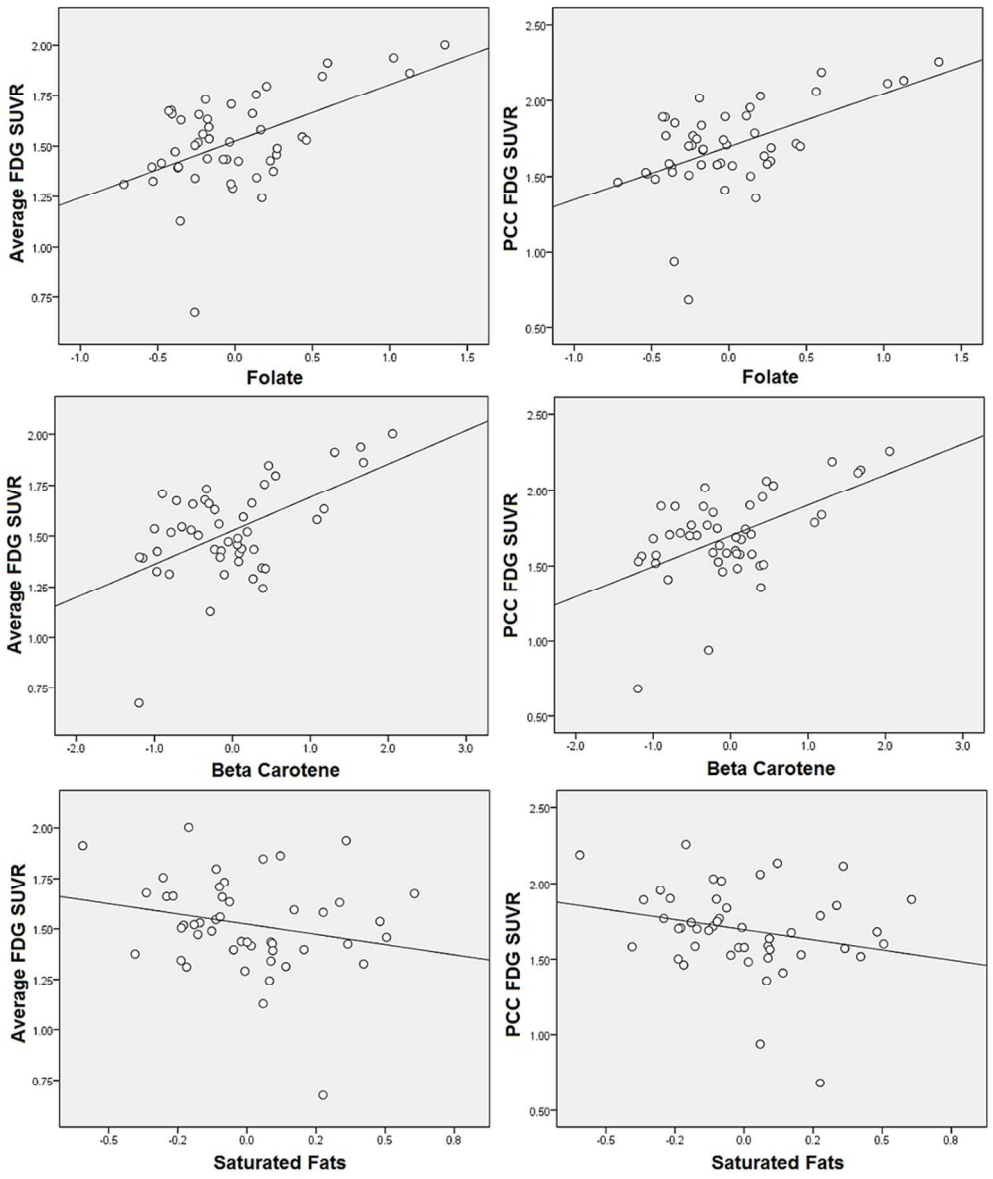
		AVG_{FDG}	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs. men=16)							
β-carotene							
Women	β	0.33 [a]	0.29	0.32 [a]	0.33 [a]	0.34 [a]	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.01	-0.01	0.08	0.08	0.06	-0.02
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate							
Women	β	0.31 [a]	0.34 [a]	0.24	0.28	0.38	0.24
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.29	0.25	0.18	0.22	0.31	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
Women	β	-0.35 [a]	-0.30	-0.32 [a]	-0.38 [a]	-0.37 [a]	-0.34 [a]
	SE	0.008	0.009	0.007	0.01	0.01	0.009
Men	β	0.15	0.16	0.17	0.12	0.09	0.12
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Family history (FH+, n=33 vs. FH-=16)							
β-carotene							
FH+	β	0.42 [b]	0.35 [a]	0.42 [b]	0.43 [b]	0.45 [b]	0.42 [b]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
FH-	β	-0.10	-0.10	-0.17	0.12	0.06	-0.2
Gender × Family history (FH+ women, n=23 vs. other subjects=26)							
β-carotene							
FH+ women	β	0.48 [a]	0.43 [a]	0.46 [a]	0.49 [b]	0.50 [b]	0.47 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.04	-0.006	0.08	0.09	0.04	0.05
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Gender × APOE genotype (APOE4+ women, n=13 vs. other subjects=36)							
β-carotene							
*APOE*4+ women	β	0.52 [a]	0.46 [a]	0.48 [a]	0.54 [a]	0.53 [a]	0.48 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.10	0.10	0.10	-0.08	0.03	-0.08
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
*APOE*4+ women	β	-0.66 [b]	-0.60 [a]	-0.59 [b]	-0.57 [b]	-0.68 [b]	-0.57 [a]
	SE	0.02	0.03	0.02	0.03	0.03	0.03
Other	β	0.11	0.11	0.16	0.18	0.08	0.19

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrient values

[a] $p<0.05$, [b] $p\leq0.01$. See Legend to Table 2.

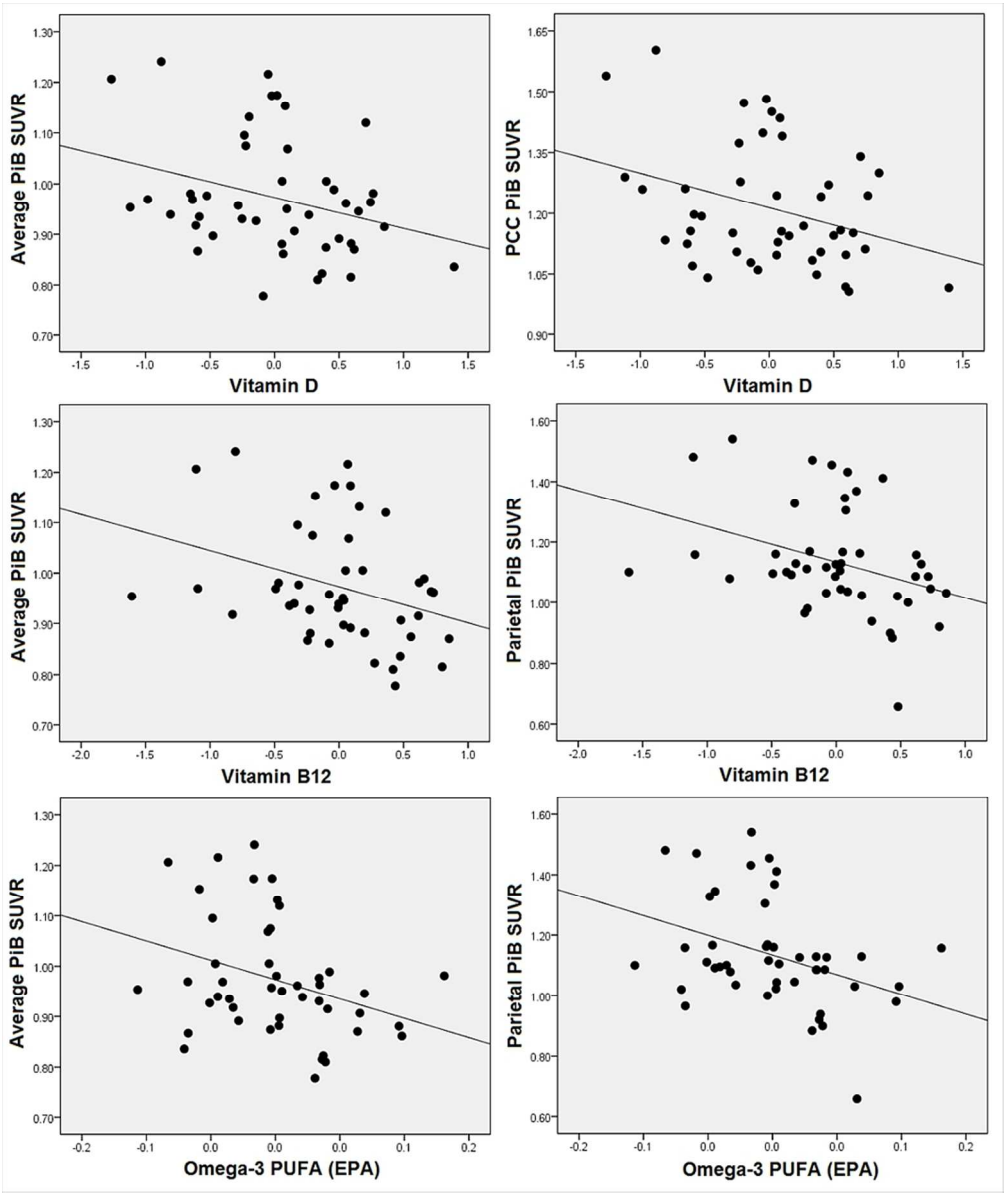
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats
90x106mm (300 x 300 DPI)

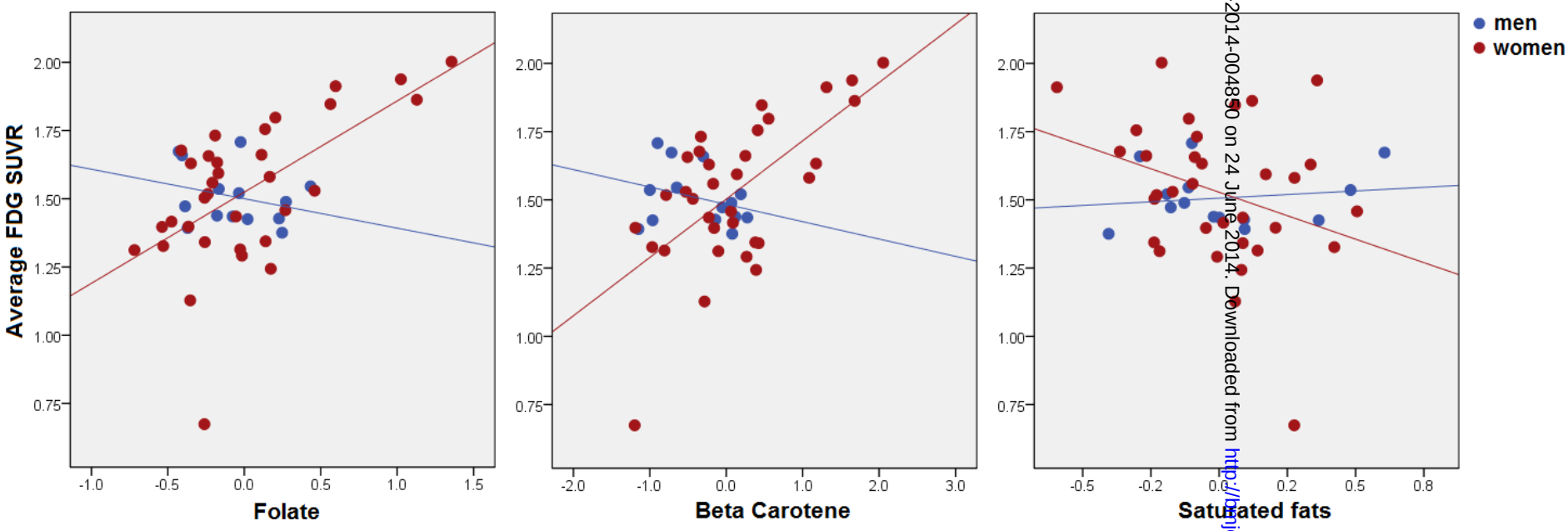
BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA)
90x106mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16)

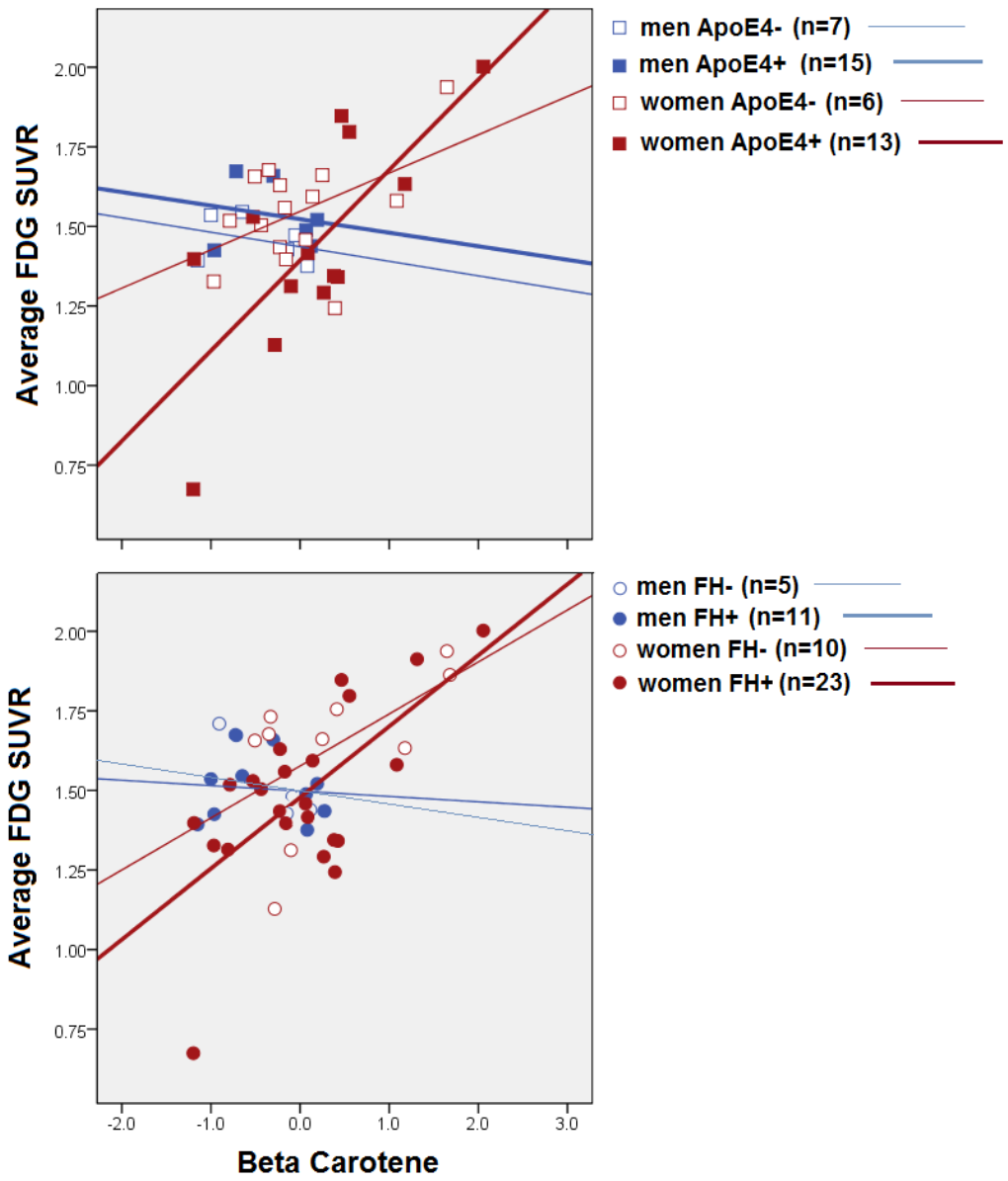


Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

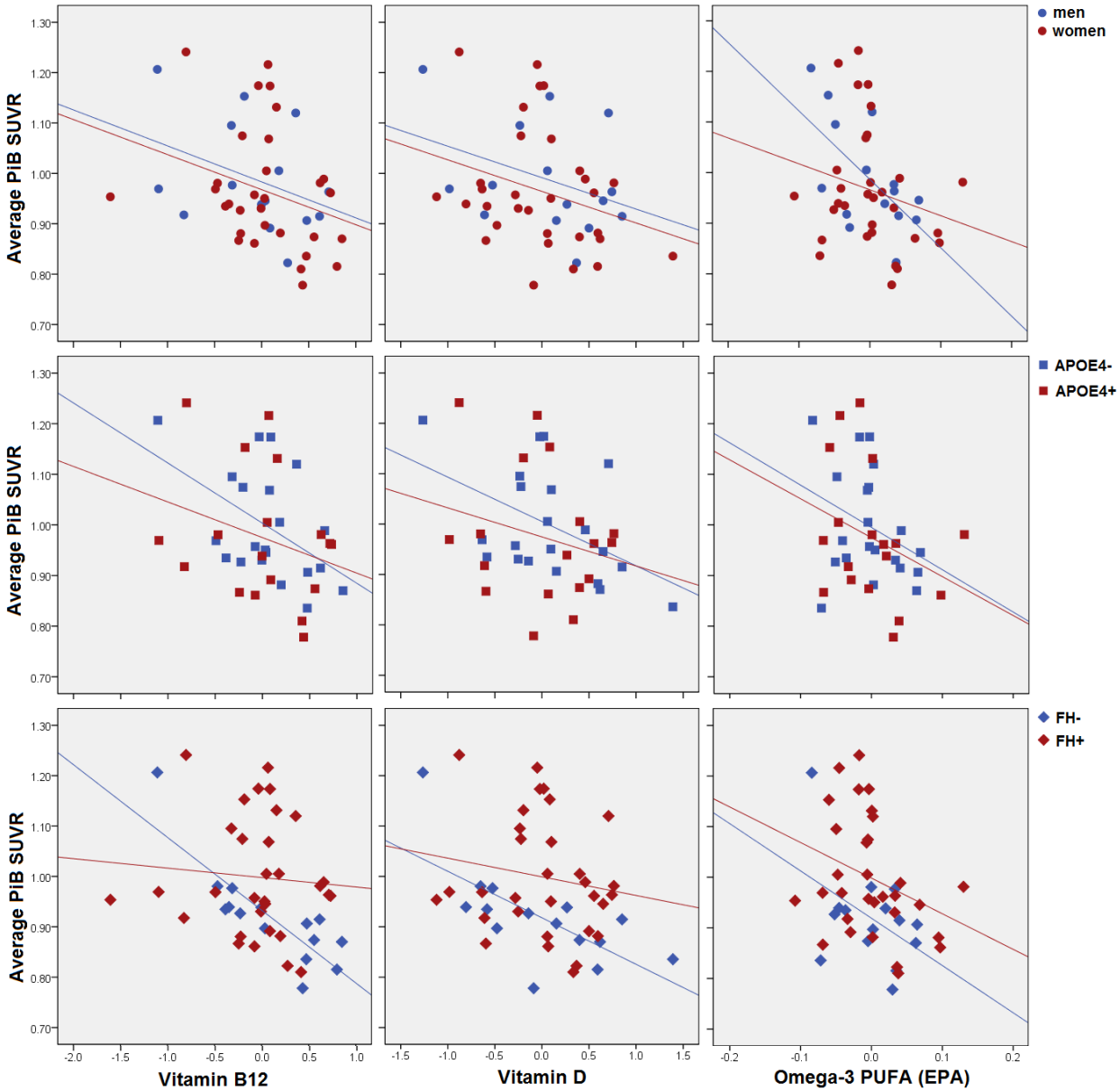


β-carotene values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status



Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract DONE p.1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found DONE p.2-3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported DONE p.6
Objectives	3	State specific objectives, including any prespecified hypotheses DONE p.7
Methods		
Study design	4	Present key elements of study design early in the paper DONE
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection DONE p.7-9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants DONE p.7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable DONE p.10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group DONE p.8,9 and 10-11
Bias	9	Describe any efforts to address potential sources of bias DONE p.8,9 and 10-11
Study size	10	Explain how the study size was arrived at DONE p.7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why DONE p.10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding DONE p.10-11
		(*b*) Describe any methods used to examine subgroups and interactions DONE p.10-11
		(*c*) Explain how missing data were addressed n.a.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Continued on next page

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed N.A.
		(b) Give reasons for non-participation at each stage N.A.
		(c) Consider use of a flow diagram N.A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders DONE p.7-8
		(b) Indicate number of participants with missing data for each variable of interest N.A.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) N.A.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures DONE p.11-13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included DONE p.11-13
		(*b*) Report category boundaries when continuous variables were categorized DONE p.11-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives DONE p. 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias DONE p.15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence DONE
Generalisability	21	Discuss the generalisability (external validity) of the study results DONE
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based DONE p.4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-004850.R2
Article Type:	Research
Date Submitted by the Author:	07-May-2014
Complete List of Authors:	Mosconi, Lisa; New York University School of Medicine, Psychiatry Murray, John; New York University, Psychiatry Davies, Michelle; New York University, Psychiatry Williams, Schantel; New York University, Psychiatry Pirraglia, Elizabeth; New York University, Psychiatry Spector, Nicole; New York University, Psychiatry Tsui, Wai; New York University, Psychiatry Li, Yi; New York University, Psychiatry Butler, Tracy; New York University, Psychiatry Osorio, Ricardo; New York University, Psychiatry Glodzik, Lidia; New York University, Psychiatry Vallabhajosula, Shankar; Weill Cornell Medical Center, Radiology McHugh, Pauline; New York University, Psychiatry Marmar, Charles; New York University School of Medicine, Psychiatry de Leon, Mony; New York University, Psychiatry
Primary Subject Heading:	Radiology and imaging
Secondary Subject Heading:	Neurology, Nutrition and metabolism
Keywords:	Dementia < NEUROLOGY, NUCLEAR MEDICINE, NUTRITION & DIETETICS, RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Lisa Mosconi[1*], PhD, John Murray[1], BA, Michelle Davies[1], BA, Schantel Williams[1], RN, Elizabeth Pirraglia[1], MA, Nicole Spector[1], BA, Wai H. Tsui[1], MS, Yi Li[1], MD, Tracy Butler[1], MD, Ricardo S. Osorio[1], MD, Lidia Glodzik[1], MD, PhD, Shankar Vallabhajosula[2], PhD, Pauline McHugh[1], MD, Charles Marmar[1,3], MD, Mony J. de Leon[1], EdD

[1]Department of Psychiatry, New York University School of Medicine, New York, NY 10016; [2]Department of Radiology, Citigroup Biomedical Imaging Center (CBIC), Weill Cornell Medical College, New York, NY 10021; [3]Steven and Alexandra Cohen Veterans Center for PTSD and TBI, New York, NY 10016

* Dr. Lisa Mosconi, Department of Psychiatry, NYU School of Medicine, 145 East 32nd St, 2nd Floor, New York NY, 10016. Tel: (212) 263-3255, Fax: (212) 263-3270 Email: lisa.mosconi@nyumc.org

Word count

Title: 137 characters (including spaces)

Manuscript Words: 3965

Abstract Words: 300

Tables: 3, Figures: 2, Supplemental files: 3 [eFigure 1; eFigure 2; eFigure 3]

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abstract

Objectives. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD). This study examines the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal individuals (NL) with and without AD risk factors.

Design. As part of an ongoing brain imaging study, participants received clinical and laboratory exams, a neurocognitive test battery, Positron Emission Tomography (PET) with ^{11}C-Pittsburgh Compound B (PiB; a measure of amyloid-β (Aβ) load) and ^{18}F-fluorodeoxyglucose (FDG; a proxy of neuronal activity), and completed semi-quantitative food frequency questionnaires.

Setting. Research center affiliated with the Alzheimer's disease Core Center at New York University School of Medicine.

Participants. Forty-nine NL individuals (age 25-72 y, 69% female) with dietary information, ^{11}C-PiB and ^{18}F-FDG PET scans were examined.

Results. Controlling for age and total caloric intake, higher intake of vitamin B12, vitamin D and ω-3 polyunsaturated fatty acid (PUFA) associated with lower Aβ load in AD-regions on PiB-PET, while higher intake of β-carotene and folate associated with higher glucose metabolism on FDG-PET. β-carotene and folate were associated with reduced glucose metabolism for women, *APOE*4 carriers and subjects with positive AD family history, but not for their risk-free counterparts. The associations of vitamin B12, vitamin D and ω-3PUFA with PiB retention were independent of gender, APOE and family history. The identified nutrient combination was associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, meat and sweets.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Conclusions. Our data provide a potential pathophysiological mechanism for epidemiological findings showing that dietary interventions may play a role in the prevention of AD. Longitudinal studies are needed to determine whether there is a direct link between nutrient intake, brain biomarkers and risk of AD.

Article focus

- To investigate the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal (NL) adults with and without AD risk factors.
- To define which nutrients are protective against AD pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals
- To test whether the effects would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype, a major susceptibility gene for late-onset AD.

Key messages

- Higher intake of vitamin B12, vitamin D, ω-3 polyunsaturated fatty acid (PUFA), β-carotene and folate was associated with lower amyloid-beta pathology and higher metabolic activity in AD-vulnerable brain regions.
- These associations were stronger for women, *APOE*4 carriers and subjects with positive AD family history, than for their risk-free counterparts.
- Nutrient-biomarkers associations were stronger using nutrients from foods only, which is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging and cognitive decline are less so. This study shows a relationship between nutrients and AD-biomarkers in cognitively intact persons at risk for AD, suggesting that dietary interventions may play a role in the prevention of AD
- This was a cross-sectional study whose purpose was to explore a possible link between dietary nutrients and brain biomarkers of AD in yet asymptomatic individuals; further longitudinal studies are necessary to show causal links.
- More studies with larger sample sizes and plasma nutrient measures are needed to further elucidate the inter-relationship between dietary choices, brain physiology and risk of future AD.
- Our preliminary findings of interaction effects between nutrients and AD risk factors such as gender, APOE genotype and family history need to be replicated with larger samples.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Introduction

"We are what we eat": the nutritional content of what we eat determines the composition of our cell membranes, bone marrow, blood, hormones, and is therefore the foundation upon which our body and brain are built. While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging are less so. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem[1-7]. However, the biological mechanisms underlying the relationship between dietary nutrients, brain aging and AD are largely unexplored. Understanding how diet and nutrition promote healthy brain aging in cognitively normal (NL) individuals, especially those at increased risk for AD, is critical prior to implementing dietary recommendations for prevention and treatment of disease.

While it is intuitive to assume a link between nutrient intake and overall brain functioning exists, to our knowledge there are no studies that examined the relationship between nutrient intake and brain amyloid-β (Aβ), a major hallmark of AD pathology, or glucose metabolism, a proxy for neuronal activity, in NL individuals.

The present brain imaging study examines the relationship between nutrient intake, brain Aβ load assessed using ^{11}C-Pittsburgh Compound-B (PiB) Positron Emission Tomography (PET), and glucose metabolism assessed using ^{18}F-fluorodeoxyglucose (FDG) PET in a cohort of young to late-middle aged NL individuals with or without risk factors for AD. Several studies demonstrated increased PiB-PET retention in AD and Mild Cognitive Impairment (MCI), often a prodromal AD phase, compared to controls[8], as well as in non-demented elderly at risk for AD[9-11]. Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

clinical symptoms[12-14]. Our goals were to define which nutrients are associated with lower AD-burden (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals, and to test whether the associations are stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype.

Methods

Participants

Among a larger pool of participants who underwent PET exams, this study included 49 prospectively recruited, clinically and cognitively normal (NL) individuals participating in longitudinal PET studies at NYU School of Medicine, who completed clinical, PiB- and FDG-PET exams and dietary questionnaires within 6 months of each other. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All subjects provided written informed consent to participate in this IRB approved study.

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[14]. A family history of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires[14]. *APOE* genotypes were determined using standard

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

quantitative polymerase chain reaction (qPCR)[15]. Individuals with at least one copy of the ε4 allele were grouped as *APOE* ε4 carriers (APOE4+) and compared to ε4 non-carriers (APOE4-).

Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Willett's semi-quantitative food frequency questionnaire (SFFQ)[16-20]. The 61-food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (g/day) of each food group was calculated by summing the intakes of member food items. The daily intake of nutrients was then computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion[19].

Similarly to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function[21, 22], including saturated fatty acids, monounsaturated fatty acids, ω-3 polyunsaturated fatty acid (PUFA), ω-6 PUFA, β-carotene, vitamin B12, vitamin C, vitamin D, vitamin E, and folate. As moderate alcohol drinking may be protective against dementia[23], alcohol intake (g/day) was also calculated.

The nutrient intakes from foods and from supplements were separately estimated. The nutrient intake from foods was the main focus of this analysis. A total of 29/49 (59%) participants reported taking no supplements for >1 year prior to PET, and the remaining 20/49 (41%) subjects reported taking a multivitamin at least 3 times a week, for >1 year prior to PET. Of these 20 subjects, 8 (16%) reported taking additional fish oil supplements, 5 (10%) reported taking vitamin D (300-1000 IU) and/or vitamin E (>600 IU), and 2 (4%) reported taking vitamin B12 (500 mcg) regularly. Twelve subjects reported taking vitamin C (1000 mg) and vitamin D (300-

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1000 IU) on a seasonal basis only, and mostly occasionally. None of the subjects were taking saturated fats or monounsaturated fatty acids supplementation. As some subjects reported taking supplements consistently, we additionally looked at nutrient intake from supplements, and the combined nutrient intake from food and supplements.

Brain imaging

All subjects received PiB- and FDG-PET scans using standardized procedures[10, 14, 24]. Briefly, subjects were positioned in the scanner 60 min after injection of 15 mCi of ^{11}C-PiB , and scanned for 30 min in 3D-mode on an LS Discovery or BioGraph PET/CT scanner. The FDG scan was performed 30 min after completion of the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of ^{18}F-FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. All images were corrected for photon attenuation, scatter, and radioactive decay and smoothed for uniform resolution[25].

For each subject, summed PET images corresponding to 40-60 min of FDG data and 60-90 min of PiB data were coregistered to corresponding MRI using the Normalized Mutual Information routine implemented in Statistical Parametric Mapping (SPM8)[26]. MRI were spatially normalized to Montreal Neurological Institute space by high-dimensional warping (DARTEL)[26]. MRI-coregistered FDG and PiB scans were spatially normalized using subject-specific transformation matrixes from MRI and sampled using automated regions-of-interest (ROI)[27, 28]. We focused on pre-selected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), posterior cingulate cortex (PCC) and prefrontal cortex (PFC). Standardized uptake value ratios (SUVR) were generated for each ROI by normalizing PiB uptake by cerebellar grey matter uptake[29] and FDG by pons activity[30]. A

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

composite cortical PiB retention mask (AVG_{PiB}) was created by combining parieto-temporal, frontal and PCC regions, and an FDG mask (AVG_{FDG}) by combining parieto-temporal and PCC regions[24].

Statistical Analysis

Clinical and demographical measures were examined with descriptive statistics. Stepwise forward and backward linear regressions were used to evaluate the associations between FDG and PiB ROI measures (independent variables), nutrients (dependent variables), and covariates. We first focused on nutrients from foods only. As nutrient levels are also affected by subjects' intake of supplements, analyses were repeated for the combined nutrient intake from foods and supplements, and for nutrients from supplements only. All regression models were tested for violations of the model assumptions. Variables that were not normally distributed were log-transformed which normalized the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age- and caloric intake-adjusted residuals[31]. We then examined gender[32], education, ethnicity, body mass index (BMI), alcohol consumption, family history[33] and *APOE* status[34] as covariates. Education and BMI were modeled as continuous variables. Gender (male vs. female), FH (positive vs. negative), and *APOE* status (*APOE*4+ vs. *APOE*4-) were examined as dichotomous variables. Ethnic group was based on self-report using the format of the 1990 census. Ethnicity was used as a dichotomous variable (White/non-Hispanic vs. other ethnic groups). Alcohol intake was used as a dichotomous variable (mild-moderate (0-30 g/day) vs. no (0 g/day) or more than moderate ($\geq$30 g/day) consumption).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

If one or more covariates showed significant effects on the association between nutrients and biomarkers, those covariates were separately examined for interaction effects in adjusted models by first entering the main effects, then the 2-way interactions between each nutrient and the covariate(s) in the next step. Only significant interaction terms were retained in the models.

The food sources of nutrients associated with PiB retention and FDG uptake were examined by testing for correlations between food sources (g/day) and nutrient intake[3, 4, 21, 22].

Results were considered significant at $p<0.05$ (2-sided tests). SPSS v.21 (SPPS Inc., 2013) was used for all analyses.

Results

Subjects' characteristics are found in **Table 1**. None of the participants were diabetics, smokers, or met criteria for obesity.

FDG-PET

Among all nutrients examined, β-carotene and folate from food sources were positively associated with brain glucose metabolism in several ROIs ($p\leq0.05$; **Figure 1, Table 2**). Results remained unchanged using the combined nutrient intake from food and supplements. There were no associations between nutrients from supplements only and glucose metabolism in any ROI. As supplements did not appear to have significant effects, and less than half of our subjects were taking multivitamins at the time of the exam, results from β-carotene and folate from food sources are presented in what follows.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Adjustment for education, ethnicity, BMI, and alcohol consumption did not attenuate the relationships between β-carotene, folate and glucose metabolism, while gender, family history and *APOE* status showed significant interaction effects with β-carotene and folate.

Gender × β-carotene interaction effects on brain glucose metabolism were observed in PCC and AVG_{FDG} ($p \leq 0.03$, **Table 3**). In these regions, women showed significant positive associations between β-carotene and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**), resulting in steeper regression slopes of glucose metabolism on β-carotene in women compared to men (e**Figure 1**). Interaction effects of gender × folate on glucose metabolism were observed in all ROIs ($p \leq 0.05$), with women showing significant positive associations between folate and glucose metabolism ($p<0.05$) and men showing no associations (**Table 3,** e**Figure 1**). Additionally, marginally significant gender × saturated fats interaction effects were observed on PCC and AVG_{FDG} glucose metabolism ($p \leq 0.08$), as women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**).

Family history × β-carotene interaction effects on glucose metabolism were observed for all ROIs ($p \leq 0.05$). In these regions, subjects with positive family history showed significant associations between β-carotene and glucose metabolism ($p<0.05$), while those with negative family history showed no associations (**Table 3**). Interaction effects of gender × family history × β-carotene on glucose metabolism were observed ($p<0.05$), as women with a positive family history showed significant associations between β-carotene and glucose metabolism in PCC, LTL and AVG_{FDG} ($p<0.05$) whereas the other groups (i.e., men with positive family history, men and women with negative family history) did not show significant associations (**Table 3**), resulting in significant interaction effects ($p<0.05$, **eFigure 2**).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Interaction effects of *APOE* × β-carotene were marginally significant ($p \leq 0.08$), with *APOE*4+ subjects showing steeper regression slopes vs. *APOE*4-. Nonetheless, there were significant gender × *APOE* × β-carotene interaction effects, as women *APOE*4+ had the steepest regression slopes of PCC and AVG_{FDG} glucose metabolism on β-carotene as compared to the other groups (i.e., APOE4+ men, APOE4- men and women) ($p<0.05$; **Table 3, eFigure 2**). Additionally, there were significant gender × *APOE* × saturated fat interactions in glucose metabolism, as *APOE*4+ women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$), whereas the other groups (i.e., APOE4+ men, APOE4- men and women) did not (**Table 3**).

Correlations between nutrients associated with FDG uptake and food groups showed that β-carotene was mainly from dark leaf, green leafy and cruciferous vegetables and fresh fruit, with correlation coefficients of 0.82, 0.77, 0.69 and 0.53, respectively (p's$\leq$0.001). Folate was from grains, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32 and 0.30; p's$\leq$0.04), and saturated fats were from high fat dairies, salad dressing, fried potatoes, sweets and processed meat (0.65, 0.52, 0.5, 0.45, 0.41, p's$\leq$0.003).

PiB-PET

Among all nutrients examined, vitamin B12 and vitamin D levels from food sources were negatively associated with PiB retention in all ROIs ($p<0.05$; **Table 2**, **Figure 2**). There were marginally significant negative associations between PiB retention and ω-3 PUFA ($p \leq 0.10$), which prompted exploratory analyses of 3 major ω-3 PUFAs subtypes (i.e., eicosapentaenoic acid 20:5, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:3, ALA). PiB retention

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

was negatively associated with EPA (β range -0.37 in IPL to -0.31 in PCC, SE 0.49-0.43, $p<0.04$; **Figure 2**), but not with DHA or ALA ($p>0.20$, n.s.).

Analysis of nutrients from food and supplements attenuated the relationships between PiB retention and vitamin D, while those with vitamin B12 and ω-3PUFA EPA remained significant ($p\leq0.05$). There were no associations between nutrients from supplements only and PiB retention in any ROI. Since supplements did not appear to have significant effects other than to attenuate the relationship between nutrients and PiB retention, and only 41% of our subjects were taking supplements at the time of the exam, results using nutrients from food sources only are presented in what follows.

Including education, ethnicity, BMI, alcohol consumption, gender, family history and *APOE* status as covariates in the model did not attenuate the relationships between vitamin B12, vitamin D, ω-3PUFA EPA and PiB retention. There were no interaction effects between possible AD-risk factors and nutrient intake on PiB retention in any ROI (**eFigure 3**).

Correlations between nutrients associated with PiB and food groups showed that vitamin B12 was mainly from meat, eggs and butter with correlation coefficients of 0.35, 0.31 and 0.36, respectively ($p<0.04$). Vitamin D was mostly from low-fat dairies and fish (0.64 and 0.55, $p<0.001$), and ω-3PUFA EPA from fish and other vegetables (0.36 and 0.31, $p<0.01$).

Discussion

This brain imaging study shows that higher intake of vitamin B12, vitamin D, and ω3-PUFA EPA from food sources was associated with lower Aβ load, and higher β-carotene and folate

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

intake was associated with higher brain glucose metabolism in NL individuals. Higher consumption of saturated fats was associated with lower brain glucose metabolism, albeit weakly. This data indicates that a healthy diet rich in natural folate, β-carotene, ω3-PUFA, vitamin B12 and vitamin D might be particularly useful to support healthy brain aging. The identified 'AD-protective' nutrients were associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and with lower intake of high-fat dairies, processed meat and sweets. A significant impact of risk factors such as gender, *APOE* status, and family history of late-onset AD was noted on the associations between nutrients and glucose metabolism in AD-regions. Specifically, women, individuals with positive family history and APOE ε4 carriers showed the strongest associations between β-carotene, folate and saturated fats on glucose metabolism as compared to their risk-free counterparts. These effects were not evident on PiB retention, as the associations between Aβ load and intake of vitamin B12, vitamin D and ω3-PUFA EPA were independent of these risk factors for late-onset AD.

Some studies have shown that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly[35-41], possibly due to their ability to reduce homocysteine levels, although results are not conclusive[41]. β-carotene (a major precursor to vitamin A) might have beneficial effects via its antioxidant or Aβ anti-oligomerization effects[42-47]. Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculo-protective and synaptic plasticity-enhancing effects, modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products[48-50]. On the other hand, higher intake of saturated fats is known to have negative effects on cognitive functions[51, 52], while intake of polyunsaturated fatty acids, especially ω3-PUFA, is known to decrease risk of decline[21, 22, 53, 54].

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

A distinction must be made between diet and nutrition, where nutrition refers to the components of the foods which one may absorb while diet refers to patterns of foods eaten. Knowledge of AD-protective nutrients is important to identify the food sources of these nutrients in order to develop and implement dietary recommendations. Several epidemiological studies have identified dietary patterns (i.e., food combinations) that are protective against AD. Despite differences in the analytic approaches, high adherence to dietary patterns characterized by higher intakes of fruits, vegetables, fish, nuts and legumes, and lower intake of meat, high-fat dairies and sweets, is consistently associated with reduced risk for AD[3, 4, 21, 55-59]. The food sources associated with the nutrients identified as being AD-protective using brain PET in this study are consistent with prior epidemiological findings. Other studies are needed to test for specific associations between PET biomarkers, specific food groups and risk for AD.

A community-based study of NL elderly showed that ω-3PUFA was associated with lower peripheral plasma Aβ levels [22]. Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3PUFA EPA, further reinforcing the connection between ω-3PUFA and AD pathology. Other studies with larger samples and a wider age range are needed to assess whether other ω-3PUFA markers are also associated with brain Aβ load in the elderly.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time[21], the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman et al[60] showed that higher levels of plasma anti-oxidants, vitamin B, D and ω3-PUFA were associated with more favorable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 y). Analysis of plasma nutrients would be very valuable in younger NL at risk for AD. In our data set, none of the nutrients were associated with neuropsychological measures, possibly because our subjects were

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

relatively young and all high-school graduates, which resulted in a "ceiling-effect". Longitudinal studies with larger samples are warranted to assess whether the relationship between "protective" nutrients, AD-biomarkers and cognitive performance varies with age and disease.

In our data-set, gender, family history and *APOE* status affected the relationships between brain glucose metabolism, β-carotene and to a lesser extent folate levels. To our knowledge, there are no previous studies that examined the interaction between nutrients and AD-risk factors on glucose metabolism in normal aging. Our results were in the expected direction, as at-risk individuals showed stronger effects of nutrient levels on glucose metabolism. Several studies have shown that female gender, a 1st-degree family history of late-onset AD, and the *APOE* ε4 genotype are all associated with reduced glucose metabolism in NL individuals[13, 14, 61]. Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs[62, 63], particularly of vitamin D/calcium and folic acid, as women are susceptible to hormonal changes associated with menstruation, child-bearing and osteoporosis. While limited by the small sample, APOE4+ women with a positive family history showed the strongest associations between glucose metabolism and β-carotene ($p<0.04$). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarkers associations were stronger using nutrients from foods only, which reinforces the general recommendations of a lifelong diversified diet that includes an abundance of nutrient-rich foods, and is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods[64], especially for women[65]. While both family history and *APOE* status have been associated with increased Aβ load in NL[10, 11], the effects of vitamin B12, vitamin D12 and ω3-PUFA EPA on PiB retention were not exacerbated in presence of these risk factors or female gender. Overall, our data suggests that nutritional interventions aimed at

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

preserving brain activity might be particularly useful if instituted in young adulthood in NL at risk for AD, before irreversible neuronal loss. Second, these results indicate a genetic component to dietary needs, as genetic risk in conjunction with unhealthy eating habits may boost genetic predisposition[66], and support the view that AD is a multi-factorial disease resulting from genetic, life-style and environmental interactions[67]. Third, nutrient effects on Aβ load were independent of AD-risk factors, but this may differ in older populations with more substantial Aβ deposition. Nutrients may have an impact on Aß oligomers, which are not detectable with PiB-PET, prior to plaque formation[68]. A low saturated fat/glycemic index diet was shown to improve Aβ composition in cerebrospinal fluid (CSF) in MCI [69], suggesting that diet is a powerful environmental factor that modulates AD-risk through its effects on brain Aβ and associated neuronal injury.

This study has several limitations. The NL population selected in our study consists of a group with a high a priori risk of preclinical AD-changes, and results were found in small numbers of carefully screened subjects under controlled clinical conditions. While a sample of 49 subjects with FDG and PiB-PET scans is quite sizeable from a neuroimaging perspective, larger samples are warranted to replicate these preliminary studies. The small sample led us to examine predictors and confounds in separate analyses rather than simultaneously so as to avoid over-fitting. Simultaneous statistical control of potential confounds would be informative because of conjoint confounding that stepwise approaches do not capture. Nonetheless, only gender, family history and APOE genotype had a significant impact on the associations between nutrient levels and glucose metabolism, yielding significant interaction effects even in this small sample. The sample size also may have decreased the likelihood of a wide distribution of nutrient intake to fully test for potential associations. For instance, we did not report associations between

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

biomarkers and vitamin E, possibly because vitamin E intake in our healthy population did not include the level below or above which an association may be observed. Likewise, we observed positive associations between folate and brain glucose metabolism in a NL, albeit at risk population, although folate levels were generally moderate. The literature indicates that whereas higher food intake of folate may be beneficial, high intake levels of folic acid (the synthetic form of folate) may be harmful, particularly in individuals with low B12 status[41], as high dosages of folic acid may mask vitamin B12 deficiencies.

Second, the sample size was not large enough to detect non-linear associations that may be present for nutrients and imaging biomarker measures. An interesting approach would be to analyze indicator variables of quantiles (i.e., tertiles, quartiles) of the nutrients to test for quadratic, rather than linear associations. Third, dietary intake assessments may lead to incorrect quantification of bioavailable nutrient levels. For instance, vitamin B12 intake may be challenging to characterize by dietary intake assessment as its serum level can be affected by gastrointestinal malabsorption syndromes, such as those seen with pernicious anemia, Crohn's disease, or gastric bypass surgery; by excessive alcohol intake; or by common medications, including antacids and antidiabetic agents, among others. Although none of our subjects showed vitamin B12 deficiencies in plasma on standard blood tests, we cannot exclude that intake may have been underestimated in some participants. Moreover, a significant component to vitamin D serum levels is sunlight exposure as well as dietary intake; residents of large cities have been shown to be particularly at risk for vitamin D deficiency due to a chronic lack of sunlight exposure. Malabsorption syndromes may also affect vitamin D bioavailability regardless of sunlight exposure or dietary intake, as well as conditions such as obesity or renal insufficiency, which may interfere with vitamin D extraction or conversion to its active form. A few

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

participants reported taking vitamin D on a seasonal basis. Overall, our preliminary results using SFFQs warrant replication by direct quantification of nutrient levels from plasma to better address these potential confounds, as well as confirmation on medical screening that medical conditions or medications that may interfere with vitamin bioavailability are not present. Finally, replication of these preliminary research findings in community-based populations with more diversified socio-economic and medical status is warranted and clinical application is not yet justified.

In conclusion, we identified specific nutrients associated with healthy brain aging, which provides support for further exploration of nutritional status in the prevention of AD.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Legends

Figure 1. Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats.

Nutrient values are age and caloric intake-adjusted residuals. Unadjusted values (minimum, mean, maximum) are provided for reference purposes. The mean value is in italic.

FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless].

Corresponding *p* values are found in Table 2

Figure 2. Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA).

Nutrient values are age and caloric intake-adjusted residuals. Unadjusted values (minimum, mean, maximum) are provided for reference purposes. The mean value is in italic.

PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless].

Corresponding *p* values are found in Table 2.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16).

Nutrient values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 1. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

β-carotene values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 1. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status

Nutrient values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 2. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Funding/support: This study was supported by NIH/NIA grants AG035137, AG13616, Alzheimer's Association IIRG-09-132030, and an Anonymous foundation.

Contributorship Statement: Study concept and design: Mosconi, McHugh, Marmar. Acquisition of data: Mosconi, Murray, Davies, Williams, Spector, Osorio, McHugh, Vallabhajosula. Analysis and interpretation of data: Mosconi, Pirraglia, Butler, Osorio, Glodzik, McHugh, Marmar. Drafting of the manuscript: Mosconi and McHugh. Critical revision of the manuscript for important intellectual content: Mosconi, Butler, Osorio, Glodzik, Vallabhajosula, McHugh, Marmar and de Leon. Statistical analysis: Mosconi and Pirraglia. Obtained funding: Mosconi and de Leon. Administrative, technical, and material support: Mosconi and de Leon. Study supervision: Mosconi.

Competing Interests

None

Data Sharing Statement

No additional data available

Financial disclosures: Drs. Mosconi, Tsui and de Leon have a patent on a technology that was licensed to Abiant Imaging Inc. by NYU and, as such, have a financial interest in this license agreement and hold stock and stock options on the company. Drs. Mosconi, Li and de Leon have received compensation for consulting services from Abiant Imaging. Dr. de Leon and Glodzik have received honoraria from the French Alzheimer Foundation. Drs. Murray, Spector, Davies, Pirraglia, Williams, Butler, Osorio, McHugh, Vallabhajosula and Marmar report no biomedical financial interests or potential conflicts of interest.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-483.
2. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545-1550.
3. Scarmeas N, Stern Y, Tang MX, et al. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912-921.
4. Scarmeas N, Stern Y, Mayeux R, et al. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216-225.
5. Rinaldi P, Polidori MC, Metastasio A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915-919.
6. Morris MC, Evans DA, Tangney CC, et al. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849-1853.
7. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230-3237.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
9. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469-1475.
10. Mosconi L, Rinne JO, Tsui WH, et al. Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci U S A 2010;107:5949-5954.
11. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009;106:6820-6825.
12. Langbaum JB, Fleisher AS, Chen K, et al. Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 2013;9:371-381.
13. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004;101:284-289.
14. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A 2007;104:19067-19072.
15. Hixson JE, Powers PK. Restriction isotyping of human apolipoprotein A-IV: rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. J Lipid Res 1991;32:1529-1535.
16. Hendrickson SJ, Willett WC, Rosner BA, et al. Food predictors of plasma carotenoids. Nutrients 2013;5:4051-4066.
17. Morris MC, Tangney CC, Bienias JL, et al. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. Am J Epidemiol 2003;158:1213-1217.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

18. Newby PK, Hu FB, Rimm EB, et al. Reproducibility and validity of the Diet Quality Index Revised as assessed by use of a food-frequency questionnaire. Am J Clin Nutr 2003;78:941-949.
19. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
20. Willett W, Stampfer M, Chu NF, et al. Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. Am J Epidemiol 2001;154:1107-1112.
21. Gu Y, Nieves JW, Stern Y, et al. Food combination and Alzheimer disease risk: a protective diet. Arch Neurol 2010;67:699-706.
22. Gu Y, Schupf N, Cosentino SA, et al. Nutrient intake and plasma beta-amyloid. Neurology 2012;78:1832-1840.
23. Luchsinger JA, Tang MX, Siddiqui M, et al. Alcohol intake and risk of dementia. J Am Geriatr Soc 2004;52:540-546.
24. Mosconi L, Rinne JO, Tsui WH, et al. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2013;34:22-34.
25. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154-159.
26. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821.
27. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology 2005;64:1860-1867.
28. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-2181.
29. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
30. Minoshima S, Frey KA, Foster NL, et al. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. J Comput Assist Tomogr 1995;19:541-547.
31. Willett WC, Stampfer MJ. Implications of total energy intake for epidemiological analyses. In: Willett WC, ed. Nutritional epidemiology. New York: Oxford University Press, 1998: 273-301.
32. Vina J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. J Alzheimers Dis 2010;20 Suppl 2:S527-533.
33. Farrer LA, O'Sullivan DM, Cupples LA, et al. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol 1989;25:485-493.
34. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001;32:181-184.
35. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. J Nutr Health Aging 2004;8:226-232.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

36. Corrada MM, Kawas CH, Hallfrisch J, et al. Reduced risk of Alzheimer's disease with high folate intake: the Baltimore Longitudinal Study of Aging. Alzheimers Dement 2005;1:11-18.
37. La Rue A, Koehler KM, Wayne SJ, et al. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am J Clin Nutr 1997;65:20-29.
38. Luchsinger JA, Tang MX, Miller J, Green R, et al. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. Arch Neurol 2007;64:86-92.
39. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641-645.
40. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 2007;369:208-216.
41. Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. J Alzheimers Dis 2006;9:429-433.
42. Kesse-Guyot E, Andreeva VA, Ducros V, et al. Carotenoid-rich dietary patterns during midlife and subsequent cognitive function. Br J Nutr 2013:1-9.
43. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids, alpha-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res 2013;2013:951786.
44. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5:56-65.
45. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. Eur J Nutr 2013;52:1553-1567.
46. de Oliveira BF, Veloso CA, Nogueira-Machado JA, et al. Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci 2012.
47. Takasaki J, Ono K, Yoshiike Y, et al. Vitamin A has anti-oligomerization effects on amyloid-beta in vitro. J Alzheimers Dis 2011;27:271-280.
48. Balion C, Griffith LE, Strifler L, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 2012;79:1397-1405.
49. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18-26.
50. Lu'o'ng KV, Nguyen LT. The role of vitamin D in Alzheimer's disease: possible genetic and cell signaling mechanisms. Am J Alzheimers Dis Other Demen 2013;28:126-136.
51. Kalmijn S, Launer LJ, Ott A, et al. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776-782.
52. Morris MC, Evans DA, Bienias JL, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194-200.
53. Bowman GL, Shannon J, Ho E, et al. Reliability and validity of food frequency questionnaire and nutrient biomarkers in elders with and without mild cognitive impairment. Alzheimer Dis Assoc Disord 2011;25:49-57.
54. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940-946.
55. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007;69:1921-1930.
56. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302:638-648.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

57. Gardener S, Gu Y, Rainey-Smith SR, et al. Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. Transl Psychiatry 2012;2:e164.
58. Gu Y, Scarmeas N. Dietary patterns in Alzheimer's disease and cognitive aging. Curr Alzheimer Res 2011;8:510-519.
59. Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599-2608.
60. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241-249.
61. Murphy DG, DeCarli C, McIntosh AR, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 1996;53:585-594.
62. Vellas BJ, Hunt WC, Romero LJ, et al. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. Nutrition 1997;13:515-519.
63. Trumbo P, Schlicker S, Yates AA, et al. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-1630.
64. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-520S.
65. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-2342.
66. de Roos B. Personalised nutrition: ready for practice? Proc Nutr Soc 2013;72:48-52.
67. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68:270-281.
68. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
69. Bayer-Carter JL, Green PS, Montine TJ, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Arch Neurol 2011;68:743-752.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 1. Demographic and clinical characteristics

N	49
Age, y, mean (SD)	54(11), range 25-72
Female gender, %	69%
Education, y, mean (SD)	16(2)
Family history of LOAD, % positive	69%
APOE ε4 status [a], % positive	39%
Ethnicity	
White	82%
Black	8%
Hispanic	6%
Other	4%
Body Mass Index [unitless], mean (SD)	26(4)
Hip to waist ratio [unitless], mean (SD)	1.2(0.2)
Blood pressure (mm/Hg), mean (SD)	
Systolic	118(15)
Diastolic	71(9)
Glucose (mg/dl)	78(12)
Cholesterol (mg/dl)	200(38)
HDL (mg/dl)	62(19)
LDL (mg/dl)	119(33)
Triglycerids (mg/dl)	91(40)
Homocystein (micromol/l)	10(2)
Neuropsychological tests, mean (SD)	
Mini Mental State Exam	29(1)
Digit symbol substitution	62(10)
Paired associates delayed recall	7(2)
Paragraph delayed recall	10(3)
Designs	8(2)
Object naming	55(12)
WAIS-vocabulary	65(14)
Nutrients levels, mean (SD), range	
β-carotene (mcg)	
Foods	6,556(8,593), 825-25,451
Foods and supplements	7,438(8,447), 1331-25,957
ω-3 polyunsaturated fatty acid (g)	
Foods	0.19(0.14), 0.05-0.82
Foods and supplements	0.23(0.18), 0.08-1.09
ω-6 polyunsaturated fatty acid (g)	
Foods	19.7(17.3), 5.2-75.5
Foods and supplements	20.1(17.2), 5.3-75.5
Folate (mcg)	
Foods	486.6(345.1), 181.4-1,674.2
Foods and supplements	648.1(335.9), 187.6-1,759.9

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Monounsaturated fats (g)	
Foods	38.7(31.5), 10.8-164.5
Foods and supplements	n.a.
Saturated fats (g)	
Foods	23.5(13.0), 8.6-61.3
Foods and supplements	n.a.
Vitamin B12 (mcg)	
Foods	4.5(2.7), 1.3-12.1
Foods and supplements	15.4(9.9), 2.6-58.3
Vitamin C (mg)	
Foods	129(100), 35-521
Foods and supplements	305(267), 69-1,565
Vitamin D (mcg)	
Foods	4.7(2.9), 1.1-16.3
Foods and supplements	729(445), 1.8-1,806
Vitamin E (mg)	
Foods	13.2(11.5), 3.6-57.4
Foods and supplements	697(37), 632-797

[a] *APOE* genotyping was obtained from 41/49 participants

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 2. Associations between nutrients and AD-biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG glucose metabolism							
β-carotene	β	0.31[a]	0.25[c]	0.33[a]	0.31[a]	0.32[a]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35[b]	0.33[a]	0.31[a]	0.31[a]	0.36[b]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20[c]	-0.16	-0.15	-0.24[c]	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B12	β	-0.32[a]	-0.37[b]	-0.29[a]	-0.33[a]	-0.37[a]	-0.27[c]
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26[c]	-0.21	-0.28[a]	-0.13	-0.28[a]	-0.27[c]
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω3-PUFA	β	-0.18[c]	-0.28[a]	-0.07	-0.24[c]	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω3-PUFA EPA	β	-0.33[a]	-0.37[b]	-0.32[a]	-0.20	-0.31[a]	-0.28[c]
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrients from food sources

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p = 0.06\text{-}0.10$.

Abbreviations: average cortical ROI (AVG), eicosapentaenoic acid 20:4 (EPA), inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), polyunsaturated fatty acids (PUFA), posterior cingulate cortex (PCC), prefrontal cortex (PFC).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3. Effects of gender and family history on the relationship between nutrients and brain glucose metabolism

		AVG_{FDG}	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs. men=16)							
β-carotene							
Women	β	0.33 [a]	0.29	0.32 [a]	0.33 [a]	0.34 [a]	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.01	-0.01	0.08	0.08	0.06	-0.02
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate							
Women	β	0.31 [a]	0.34 [a]	0.24	0.28	0.38	0.24
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.29	0.25	0.18	0.22	0.31	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
Women	β	-0.35 [a]	-0.30	-0.32 [a]	-0.38 [a]	-0.37 [a]	-0.34 [a]
	SE	0.008	0.009	0.007	0.01	0.01	0.009
Men	β	0.15	0.16	0.17	0.12	0.09	0.12
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Family history (FH+, n=33 vs. FH-=16)							
β-carotene							
FH+	β	0.42 [b]	0.35 [a]	0.42 [b]	0.43 [b]	0.45 [b]	0.42 [b]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
FH-	β	-0.10	-0.10	-0.17	0.12	0.06	-0.2
Gender × Family history (FH+ women, n=23 vs. other subjects=26)							
β-carotene							
FH+ women	β	0.48 [a]	0.43 [a]	0.46 [a]	0.49 [b]	0.50 [b]	0.47 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.04	-0.006	0.08	0.09	0.04	0.05
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Gender × APOE genotype (APOE4+ women, n=13 vs. other subjects=36)							
β-carotene							
*APOE*4+ women	β	0.52 [a]	0.46 [a]	0.48 [a]	0.54 [a]	0.53 [a]	0.48 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.10	0.10	0.10	-0.08	0.03	-0.08
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
*APOE*4+ women	β	-0.66 [b]	-0.60 [a]	-0.59 [b]	-0.57 [b]	-0.68 [b]	-0.57 [a]
	SE	0.02	0.03	0.02	0.03	0.03	0.03
Other	β	0.11	0.11	0.16	0.18	0.08	0.19

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrient values

[a] $p<0.05$, [b] $p\leq0.01$. See Legend to Table 2.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study

Lisa Mosconi[1*], PhD, John Murray[1], BA, Michelle Davies[1], BA, Schantel Williams[1], RN, Elizabeth Pirraglia[1], MA, Nicole Spector[1], BA, Wai H. Tsui[1], MS, Yi Li[1], MD, Tracy Butler[1], MD, Ricardo S. Osorio[1], MD, Lidia Glodzik[1], MD, PhD, Shankar Vallabhajosula[2], PhD, Pauline McHugh[1], MD, Charles Marmar[1,3], MD, Mony J. de Leon[1], EdD

[1]Department of Psychiatry, New York University School of Medicine, New York, NY 10016; [2]Department of Radiology, Citigroup Biomedical Imaging Center (CBIC), Weill Cornell Medical College, New York, NY 10021; [3]Steven and Alexandra Cohen Veterans Center for PTSD and TBI, New York, NY 10016

* Dr. Lisa Mosconi, Department of Psychiatry, NYU School of Medicine, 145 East 32nd St, 2nd Floor, New York NY, 10016. Tel: (212) 263-3255, Fax: (212) 263-3270 Email: lisa.mosconi@nyumc.org

Word count

Title: 137 characters (including spaces)

Manuscript Words: 3965

Abstract Words: 300

Tables: 3, Figures: 2, Supplemental files: 3 [eFigure 1; eFigure 2; eFigure 3]

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Abstract

Objectives. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD). This study examines the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal individuals (NL) with and without AD risk factors.

Design. As part of an ongoing brain imaging study, participants received clinical and laboratory exams, a neurocognitive test battery, Positron Emission Tomography (PET) with ^{11}C-Pittsburgh Compound B (PiB; a measure of amyloid-β (Aβ) load) and ^{18}F-fluorodeoxyglucose (FDG; a proxy of neuronal activity), and completed semi-quantitative food frequency questionnaires.

Setting. Research center affiliated with the Alzheimer's disease Core Center at New York University School of Medicine.

Participants. Forty-nine NL individuals (age 25-72 y, 69% female) with dietary information, ^{11}C-PiB and ^{18}F-FDG PET scans were examined.

Results. Controlling for age and total caloric intake, higher intake of vitamin B12, vitamin D and ω-3 polyunsaturated fatty acid (PUFA) associated with lower Aβ load in AD-regions on PiB-PET, while higher intake of β-carotene and folate associated with higher glucose metabolism on FDG-PET. β-carotene and folate were associated with reduced glucose metabolism for women, *APOE*4 carriers and subjects with positive AD family history, but not for their risk-free counterparts. The associations of vitamin B12, vitamin D and ω-3PUFA with PiB retention were independent of gender, APOE and family history. The identified nutrient combination was associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and lower intake of high-fat dairies, meat and sweets.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Conclusions. Our data provide a potential pathophysiological mechanism for epidemiological findings showing that dietary interventions may play a role in the prevention of AD. Longitudinal studies are needed to determine whether there is a direct link between nutrient intake, brain biomarkers and risk of AD.

Article focus

- To investigate the relationship between dietary nutrients and brain biomarkers of AD in cognitively normal (NL) adults with and without AD risk factors.
- To define which nutrients are protective against AD pathology (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals
- To test whether the effects would be stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype, a major susceptibility gene for late-onset AD.

Key messages

- Higher intake of vitamin B12, vitamin D, ω-3 polyunsaturated fatty acid (PUFA), β-carotene and folate was associated with lower amyloid-beta pathology and higher metabolic activity in AD-vulnerable brain regions.
- These associations were stronger for women, *APOE*4 carriers and subjects with positive AD family history, than for their risk-free counterparts.
- Nutrient-biomarkers associations were stronger using nutrients from foods only, which is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging and cognitive decline are less so. This study shows a relationship between nutrients and AD-biomarkers in cognitively intact persons at risk for AD, suggesting that dietary interventions may play a role in the prevention of AD
- This was a cross-sectional study whose purpose was to explore a possible link between dietary nutrients and brain biomarkers of AD in yet asymptomatic individuals; further longitudinal studies are necessary to show causal links.
- More studies with larger sample sizes and plasma nutrient measures are needed to further elucidate the inter-relationship between dietary choices, brain physiology and risk of future AD.
- Our preliminary findings of interaction effects between nutrients and AD risk factors such as gender, APOE genotype and family history need to be replicated with larger samples.

Funding/support: This study was supported by NIH/NIA grants AG035137, AG13616, Alzheimer's Association IIRG-09-132030, and an Anonymous foundation.

Financial disclosures: Drs. Mosconi, Tsui and de Leon have a patent on a technology that was licensed to Abiant Imaging Inc. by NYU and, as such, have a financial interest in this license agreement and hold stock and stock options on the company. Drs. Mosconi, Li and de Leon have received compensation for consulting services from Abiant Imaging. Dr. de Leon and Glodzik

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

have received honoraria from the French Alzheimer Foundation. Drs. Murray, Spector, Davies, Pirraglia, Williams, Butler, Osorio, McHugh, Vallabhajosula and Marmar report no biomedical financial interests or potential conflicts of interest.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Introduction

"We are what we eat": the nutritional content of what we eat determines the composition of our cell membranes, bone marrow, blood, hormones, and is therefore the foundation upon which our body and brain are built. While the importance of nutrition in health is well understood, the specific effects of nutrition on brain aging are less so. There is increasing evidence to suggest that diet, one of the most important modifiable environmental factors, may play a role in preventing or delaying cognitive decline and Alzheimer's disease (AD), a major public health problem[1-7]. However, the biological mechanisms underlying the relationship between dietary nutrients, brain aging and AD are largely unexplored. Understanding how diet and nutrition promote healthy brain aging in cognitively normal (NL) individuals, especially those at increased risk for AD, is critical prior to implementing dietary recommendations for prevention and treatment of disease.

While it is intuitive to assume a link between nutrient intake and overall brain functioning exists, to our knowledge there are no studies that examined the relationship between nutrient intake and brain amyloid-β (Aβ), a major hallmark of AD pathology, or glucose metabolism, a proxy for neuronal activity, in NL individuals.

The present brain imaging study examines the relationship between nutrient intake, brain Aβ load assessed using ^{11}C-Pittsburgh Compound-B (PiB) Positron Emission Tomography (PET), and glucose metabolism assessed using ^{18}F-fluorodeoxyglucose (FDG) PET in a cohort of young to late-middle aged NL individuals with or without risk factors for AD. Several studies demonstrated increased PiB-PET retention in AD and Mild Cognitive Impairment (MCI), often a prodromal AD phase, compared to controls[8], as well as in non-demented elderly at risk for AD[9-11]. Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

clinical symptoms[12-14]. Our goals were to define which nutrients are associated with lower AD-burden (as reflected in lower brain Aβ and higher glucose metabolic rates) among NL individuals, and to test whether the associations are stronger in those with AD-risk factors, including age, gender, education, family history of AD and Apolipoprotein E (*APOE*) genotype.

Methods

Participants

Among a larger pool of participants who underwent PET exams, this study included 49 prospectively recruited, clinically and cognitively normal (NL) individuals participating in longitudinal PET studies at NYU School of Medicine, who completed clinical, PiB- and FDG-PET exams and dietary questionnaires within 6 months of each other. Subjects were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All subjects provided written informed consent to participate in this IRB approved study.

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[14]. A family history of late-onset AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited using standardized questionnaires[14]. *APOE* genotypes were determined using standard

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

quantitative polymerase chain reaction (qPCR)[15]. Individuals with at least one copy of the ε4 allele were grouped as *APOE* ε4 carriers (APOE4+) and compared to ε4 non-carriers (APOE4-).

Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 61-item version of Willett's semi-quantitative food frequency questionnaire (SFFQ)[16-20]. The 61-food items were categorized into 30 food groups based on similarities in food and nutrient composition, and intake (g/day) of each food group was calculated by summing the intakes of member food items. The daily intake of nutrients was then computed by multiplying the consumption frequency of each portion of every food by the nutrient content of the specified portion[16].

Similarly to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function[21, 22], including saturated fatty acids, monounsaturated fatty acids, ω-3 polyunsaturated fatty acid (PUFA), ω-6 PUFA, β-carotene, vitamin B12, vitamin C, vitamin D, vitamin E, and folate. As moderate alcohol drinking may be protective against dementia[23], alcohol intake (g/day) was also calculated.

The nutrient intakes from foods and from supplements were separately estimated. The nutrient intake from foods was the main focus of this analysis. A total of 29/49 (59%) participants reported taking no supplements for >1 year prior to PET, and the remaining 20/49 (41%) subjects reported taking a multivitamin at least 3 times a week, for >1 year prior to PET. Of these 20 subjects, 8 (16%) reported taking additional fish oil supplements, 5 (10%) reported taking vitamin D (300-1000 IU) and/or vitamin E (>600 IU), and 2 (4%) reported taking vitamin B12 (500 mcg) regularly. Twelve subjects reported taking vitamin C (1000 mg) and vitamin D (300-

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1000 IU) on a seasonal basis only, and mostly occasionally. None of the subjects were taking saturated fats or monounsaturated fatty acids supplementation. As some subjects reported taking supplements consistently, we additionally looked at nutrient intake from supplements, and the combined nutrient intake from food and supplements.

Brain imaging

All subjects received PiB- and FDG-PET scans using standardized procedures[10, 14, 24]. Briefly, subjects were positioned in the scanner 60 min after injection of 15 mCi of ^{11}C-PiB , and scanned for 30 min in 3D-mode on an LS Discovery or BioGraph PET/CT scanner. The FDG scan was performed 30 min after completion of the PiB scan or on a separate day. After an overnight fast, subjects were injected with 5 mCi of ^{18}F-FDG, positioned in the scanner 35 min after injection, and scanned for 20 min. All images were corrected for photon attenuation, scatter, and radioactive decay and smoothed for uniform resolution[25].

For each subject, summed PET images corresponding to 40-60 min of FDG data and 60-90 min of PiB data were coregistered to corresponding MRI using the Normalized Mutual Information routine implemented in Statistical Parametric Mapping (SPM8)[26]. MRI were spatially normalized to Montreal Neurological Institute space by high-dimensional warping (DARTEL)[26]. MRI-coregistered FDG and PiB scans were spatially normalized using subject-specific transformation matrixes from MRI and sampled using automated regions-of-interest (ROI)[27, 28]. We focused on pre-selected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), posterior cingulate cortex (PCC) and prefrontal cortex (PFC). Standardized uptake value ratios (SUVR) were generated for each ROI by normalizing PiB uptake by cerebellar grey matter uptake[29] and FDG by pons activity[30]. A

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

composite cortical PiB retention mask (AVG_{PiB}) was created by combining parieto-temporal, frontal and PCC regions, and an FDG mask (AVG_{FDG}) by combining parieto-temporal and PCC regions[24].

Statistical Analysis

Clinical and demographical measures were examined with descriptive statistics. Stepwise forward and backward linear regressions were used to evaluate the associations between FDG and PiB ROI measures (independent variables), nutrients (dependent variables), and covariates. We first focused on nutrients from foods only. As nutrient levels are also affected by subjects' intake of supplements, analyses were repeated for the combined nutrient intake from foods and supplements, and for nutrients from supplements only. All regression models were tested for violations of the model assumptions. Variables that were not normally distributed were log-transformed which normalized the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age- and caloric intake-adjusted residuals[31]. We then examined gender[32], education, ethnicity, body mass index (BMI), alcohol consumption, family history[33] and *APOE* status[34] as covariates. Education and BMI were modeled as continuous variables. Gender (male vs. female), FH (positive vs. negative), and *APOE* status (*APOE*4+ vs. *APOE*4-) were examined as dichotomous variables. Ethnic group was based on self-report using the format of the 1990 census. Ethnicity was used as a dichotomous variable (White/non-Hispanic vs. other ethnic groups). Alcohol intake was used as a dichotomous variable (mild-moderate (0-30 g/day) vs. no (0 g/day) or more than moderate ($\geq$30 g/day) consumption).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

If one or more covariates showed significant effects on the association between nutrients and biomarkers, those covariates were separately examined for interaction effects in adjusted models by first entering the main effects, then the 2-way interactions between each nutrient and the covariate(s) in the next step. Only significant interaction terms were retained in the models.

The food sources of nutrients associated with PiB retention and FDG uptake were examined by testing for correlations between food sources (g/day) and nutrient intake[3, 4, 21, 22].

Results were considered significant at $p<0.05$ (2-sided tests). SPSS v.21 (SPPS Inc., 2013) was used for all analyses.

Results

Subjects' characteristics are found in **Table 1**. None of the participants were diabetics, smokers, or met criteria for obesity.

FDG-PET

Among all nutrients examined, β-carotene and folate from food sources were positively associated with brain glucose metabolism in several ROIs ($p\leq0.05$; **Figure 1, Table 2**). Results remained ~~substantially~~ unchanged using the combined nutrient intake from food and supplements. There were no associations between nutrients from supplements only and glucose metabolism in any ROI. As supplements did not appear to have significant effects, and less than half of our subjects were taking multivitamins at the time of the exam, results from β-carotene and folate from food sources are presented in what follows.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Adjustment for education, ethnicity, BMI, and alcohol consumption did not attenuate the relationships between β-carotene, folate and glucose metabolism, while gender, family history and *APOE* status showed significant interaction effects with β-carotene and folate.

Gender × β-carotene interaction effects on brain glucose metabolism were observed in PCC and AVG_{FDG} ($p \leq 0.03$, **Table 3**). In these regions, women showed significant positive associations between β-carotene and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**), resulting in steeper regression slopes of glucose metabolism on β-carotene in women compared to men (e**Figure 1**). Interaction effects of gender × folate on glucose metabolism were observed in all ROIs ($p \leq 0.05$), with women showing significant positive associations between folate and glucose metabolism ($p<0.05$) and men showing no associations (**Table 3,** e**Figure 1**). Additionally, marginally significant gender × saturated fats interaction effects were observed on PCC and AVG_{FDG} glucose metabolism ($p \leq 0.08$), as women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$) while men showed no associations (**Table 3**).

Family history × β-carotene interaction effects on glucose metabolism were observed for all ROIs ($p \leq 0.05$). In these regions, subjects with positive family history showed significant associations between β-carotene and glucose metabolism ($p<0.05$), while those with negative family history showed no associations (**Table 3**). Interaction effects of gender × family history × β-carotene on glucose metabolism were observed ($p<0.05$), as women with a positive family history showed significant associations between β-carotene and glucose metabolism in PCC, LTL and AVG_{FDG} ($p<0.05$) whereas the other groups (i.e., men with positive family history, men and women with negative family history) did not show significant associations (**Table 3**), resulting in significant interaction effects ($p<0.05$, **eFigure 2**).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Interaction effects of *APOE* × β-carotene were marginally significant ($p \leq 0.08$), with *APOE*4+ subjects showing steeper regression slopes vs. *APOE*4-. Nonetheless, there were significant gender × *APOE* × β-carotene interaction effects, as women *APOE*4+ had the steepest regression slopes of PCC and AVG_{FDG} glucose metabolism on β-carotene as compared to the other groups (i.e., APOE4+ men, APOE4- men and women) ($p<0.05$; **Table 3, eFigure 2**). Additionally, there were significant gender × *APOE* × saturated fat interactions in glucose metabolism, as *APOE*4+ women showed significant negative associations between saturated fats and glucose metabolism ($p<0.05$), whereas the other groups (i.e., APOE4+ men, APOE4- men and women) did not (**Table 3**).

Correlations between nutrients associated with FDG uptake and food groups showed that β-carotene was mainly from dark leaf, green leafy and cruciferous vegetables and fresh fruit, with correlation coefficients of 0.82, 0.77, 0.69 and 0.53, respectively (p's$\leq$0.001). Folate was from grains, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32 and 0.30; p's$\leq$0.04), and saturated fats were from high fat dairies, salad dressing, fried potatoes, sweets and processed meat (0.65, 0.52, 0.5, 0.45, 0.41, p's$\leq$0.003).

PiB-PET

Among all nutrients examined, vitamin B12 and vitamin D levels from food sources were negatively associated with PiB retention in all ROIs ($p<0.05$; **Table 2**, **Figure 2**). There were marginally significant negative associations between PiB retention and ω-3 PUFA ($p \leq 0.10$), which prompted exploratory analyses of 3 major ω-3 PUFAs subtypes (i.e., eicosapentaenoic acid 20:5, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:3, ALA). PiB retention

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

was negatively associated with EPA (β range -0.37 in IPL to -0.31 in PCC, SE 0.49-0.43, $p<0.04$; **Figure 2**), but not with DHA or ALA ($p>0.20$, n.s.).

Analysis of nutrients from food and supplements attenuated the relationships between PiB retention and vitamin D, while those with vitamin B12 and ω-3PUFA EPA remained significant ($p\leq0.05$). There were no associations between nutrients from supplements only and PiB retention in any ROI. Since supplements did not appear to have significant effects other than to attenuate the relationship between nutrients and PiB retention, and only 41% of our subjects were taking supplements at the time of the exam, results using nutrients from food sources only are presented in what follows.

Including education, ethnicity, BMI, alcohol consumption, gender, family history and *APOE* status as covariates in the model did not attenuate the relationships between vitamin B12, vitamin D, ω-3PUFA EPA and PiB retention. There were no interaction effects between possible AD-risk factors and nutrient intake on PiB retention in any ROI (**eFigure 3**).

Correlations between nutrients associated with PiB and food groups showed that vitamin B12 was mainly from meat, eggs and butter with correlation coefficients of 0.35, 0.31 and 0.36, respectively ($p<0.04$). Vitamin D was mostly from low-fat dairies and fish (0.64 and 0.55, $p<0.001$), and ω-3PUFA EPA from fish and other vegetables (0.36 and 0.31, $p<0.01$).

Discussion

This brain imaging study shows that higher intake of vitamin B12, vitamin D, and ω3-PUFA EPA from food sources was associated with lower Aβ load, and higher β-carotene and folate

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

intake was associated with higher brain glucose metabolism in NL individuals. Higher consumption of saturated fats was associated with lower brain glucose metabolism, albeit weakly. This data indicates that a healthy diet rich in natural folate, β-carotene, ω3-PUFA, vitamin B12 and vitamin D might be particularly useful to support healthy brain aging. The identified 'AD-protective' nutrients were associated with higher intake of vegetables, fruit, whole grains, fish and legumes, and with lower intake of high-fat dairies, processed meat and sweets. A significant impact of risk factors such as gender, *APOE* status, and family history of late-onset AD was noted on the associations between nutrients and glucose metabolism in AD-regions. Specifically, women, individuals with positive family history and APOE ε4 carriers showed the strongest associations between β-carotene, folate and saturated fats on glucose metabolism as compared to their risk-free counterparts. These effects were not evident on PiB retention, as the associations between Aβ load and intake of vitamin B12, vitamin D and ω3-PUFA EPA were independent of these risk factors for late-onset AD.

Some studies have shown that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly[35-41], possibly due to their ability to reduce homocysteine levels, although results are not conclusive[41]. β-carotene (a major precursor to vitamin A) might have beneficial effects via its antioxidant or Aβ anti-oligomerization effects[42-47]. Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculo-protective and synaptic plasticity-enhancing effects, modulation of vascular endothelial factor expression, angiogenin, and advanced glycation end products[48-50]. On the other hand, higher intake of saturated fats is known to have negative effects on cognitive functions[51, 52], while intake of polyunsaturated fatty acids, especially ω3-PUFA, is known to decrease risk of decline[21, 22, 53, 54].

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

A distinction must be made between diet and nutrition, where nutrition refers to the components of the foods which one may absorb while diet refers to patterns of foods eaten. Knowledge of AD-protective nutrients is important to identify the food sources of these nutrients in order to develop and implement dietary recommendations. Several epidemiological studies have identified dietary patterns (i.e., food combinations) that are protective against AD. Despite differences in the analytic approaches, high adherence to dietary patterns characterized by higher intakes of fruits, vegetables, fish, nuts and legumes, and lower intake of meat, high-fat dairies and sweets, is consistently associated with reduced risk for AD[3, 4, 21, 55-59]. The food sources associated with the nutrients identified as being AD-protective using brain PET in this study are consistent with prior epidemiological findings. Other studies are needed to test for specific associations between PET biomarkers and specific food groups.

A community-based study of NL elderly showed that ω-3PUFA was associated with lower peripheral plasma Aβ levels [22]. Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3PUFA EPA, further reinforcing the connection between ω-3PUFA and AD pathology. Other studies with larger samples and a wider age range are needed to assess whether other ω-3PUFA markers are also associated with brain Aβ load in the elderly.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time[21], the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman et al[60] showed that higher levels of plasma anti-oxidants, vitamin B, D and ω3-PUFA were associated with more favorable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 y). Analysis of plasma nutrients would be very valuable in younger NL at risk for AD. In our data set, none of the nutrients were associated with neuropsychological measures, possibly because our subjects were

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

relatively young and all high-school graduates, which resulted in a "ceiling-effect". Longitudinal studies with larger samples are warranted to assess whether the relationship between "protective" nutrients, AD-biomarkers and cognitive performance varies with age and disease.

In our data-set, gender, family history and *APOE* status affected the relationships between brain glucose metabolism, β-carotene and to a lesser extent folate levels. To our knowledge, there are no previous studies that examined the interaction between nutrients and AD-risk factors on glucose metabolism in normal aging. Our results were in the expected direction, as at-risk individuals showed stronger effects of nutrient levels on glucose metabolism. Several studies have shown that female gender, a 1st-degree family history of late-onset AD, and the *APOE* ε4 genotype are all associated with reduced glucose metabolism in NL individuals[13, 14, 61]. Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs[62, 63], particularly of vitamin D/calcium and folic acid, as women are susceptible to hormonal changes associated with menstruation, child-bearing and osteoporosis. While limited by the small sample, APOE4+ women with a positive family history showed the strongest associations between glucose metabolism and β-carotene ($p<0.04$). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarkers associations were stronger using nutrients from foods only, which reinforces the general recommendations of a lifelong diversified diet that includes an abundance of nutrient-rich foods, and is consistent with findings that nutritional supplementation is not equivalent to obtaining nutrients from whole foods[64], especially for women[65]. While both family history and *APOE* status have been associated with increased Aβ load in NL[10, 11], the effects of vitamin B12, vitamin D12 and ω3-PUFA EPA on PiB retention were not exacerbated in presence of these risk factors or female gender. Overall, our data suggests that nutritional interventions aimed at

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

preserving brain activity might be particularly useful if instituted in young adulthood in NL at risk for AD, before irreversible neuronal loss. Second, these results indicate a genetic component to dietary needs, as genetic risk in conjunction with unhealthy eating habits may boost genetic predisposition[66], and support the view that AD is a multi-factorial disease resulting from genetic, life-style and environmental interactions[67]. Third, nutrient effects on Aβ load were independent of AD-risk factors, but this may differ in older populations with more substantial Aβ deposition. Nutrients may have an impact on Aß oligomers, which are not detectable with PiB-PET, prior to plaque formation[68]. A low saturated fat/glycemic index diet was shown to improve Aβ composition in cerebrospinal fluid (CSF) in MCI [69], suggesting that diet is a powerful environmental factor that modulates AD-risk through its effects on brain Aβ and associated neuronal injury.

This study has several limitations. The NL population selected in our study consists of a group with a high a priori risk of preclinical AD-changes, and results were found in small numbers of carefully screened subjects under controlled clinical conditions. While a sample of 49 subjects with FDG and PiB-PET scans is quite sizeable from a neuroimaging perspective, larger samples are warranted to replicate these preliminary studies. The small sample led us to examine predictors and confounds in separate analyses rather than simultaneously so as to avoid over-fitting. Simultaneous statistical control of potential confounds would be informative because of conjoint confounding that stepwise approaches do not capture. Nonetheless, only gender, family history and APOE genotype had a significant impact on the associations between nutrient levels and glucose metabolism, yielding significant interaction effects even in this small sample. The sample size also may have decreased the likelihood of a wide distribution of nutrient intake to fully test for potential associations. For instance, we did not report associations between

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

biomarkers and vitamin E, possibly because vitamin E intake in our healthy population did not include the level below or above which an association may be observed. ~~However, while there is evidence for an association between higher intakes of vitamin E or α-tocopherol equivalents and reduced incidence of AD[70], a clinical trial showed no clinical benefits of vitamin E supplementation (2000 IU/day) in MCI[71]~~. Likewise, we observed positive associations between folate and brain glucose metabolism in a NL, albeit at risk population, although folate levels were generally moderate. The literature indicates that whereas higher food intake of folate may be beneficial, high intake levels of folic acid (the synthetic form of folate) may be harmful, particularly in individuals with low B12 status [41], as high dosages of folic acid may mask vitamin B12 deficiencies.

Second, the sample size was not large enough to detect non-linear associations that may be present for nutrients and imaging biomarker measures. An interesting approach would be to analyze indicator variables of quantiles (i.e., tertiles, quartiles) of the nutrients to test for quadratic, rather than linear associations. Third, dietary intake assessments may lead to incorrect quantification of bioavailable nutrient levels. For instance, vitamin B12 intake may be challenging to characterize by dietary intake assessment as its serum level can be affected by gastrointestinal malabsorption syndromes, such as those seen with pernicious anemia, Crohn's disease, or gastric bypass surgery; by excessive alcohol intake; or by common medications, including antacids and antidiabetic agents, among others. Although none of our subjects showed vitamin B12 deficiencies in plasma on standard blood tests, we cannot exclude that intake may have been underestimated in some participants. Moreover, a significant component to vitamin D serum levels is sunlight exposure as well as dietary intake; residents of large cities have been shown to be particularly at risk for vitamin D deficiency due to a chronic lack of sunlight

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

exposure. Malabsorption syndromes may also affect vitamin D bioavailability regardless of sunlight exposure or dietary intake, as well as conditions such as obesity or renal insufficiency, which may interfere with vitamin D extraction or conversion to its active form. A few participants reported taking vitamin D on a seasonal basis. Overall, our preliminary results using SFFQs warrant replication by direct quantification of nutrient levels from plasma to better address these potential confounds, as well as confirmation on medical screening that medical conditions or medications that may interfere with vitamin bioavailability are not present. Finally, replication of these preliminary research findings in community-based populations with more diversified socio-economic and medical status is warranted and clinical application is not yet justified.

In conclusion, we identified specific nutrients associated with healthy brain aging, which provides support for further exploration of nutritional status in the prevention of AD.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Legends

Figure 1. Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats.

Nutrient values are age and caloric intake-adjusted residuals. Unadjusted values (minimum, mean, maximum) are provided for reference purposes. The mean value is in italic.

FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2

Figure 2. Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (eicosapentaenoic acid 20:4, EPA).

Nutrient values are age and caloric intake-adjusted residuals. Unadjusted values (minimum, mean, maximum) are provided for reference purposes. The mean value is in italic.

PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. Corresponding *p* values are found in Table 2.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16).

Nutrient values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 1. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

β-carotene values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 1. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status

Nutrient values are age and caloric intake-adjusted residuals. Corresponding unadjusted values are shown in Fig. 2. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

References

1. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-483.
2. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545-1550.
3. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912-921.
4. Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. Arch Neurol 2009;66:216-225.
5. Rinaldi P, Polidori MC, Metastasio A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915-919.
6. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849-1853.
7. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230-3237.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
9. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469-1475.
10. Mosconi L, Rinne JO, Tsui WH, et al. Increased fibrillar amyloid-{beta} burden in normal individuals with a family history of late-onset Alzheimer's. Proc Natl Acad Sci U S A 2010;107:5949-5954.
11. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 2009;106:6820-6825.
12. Langbaum JB, Fleisher AS, Chen K, et al. Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 2013;9:371-381.
13. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 2004;101:284-289.
14. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A 2007;104:19067-19072.
15. Hixson JE, Powers PK. Restriction isotyping of human apolipoprotein A-IV: rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. J Lipid Res 1991;32:1529-1535.
16. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51-65.
17. Hendrickson SJ, Willett WC, Rosner BA, Eliassen AH. Food predictors of plasma carotenoids. Nutrients 2013;5:4051-4066.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

18. Morris MC, Tangney CC, Bienias JL, Evans DA, Wilson RS. Validity and reproducibility of a food frequency questionnaire by cognition in an older biracial sample. Am J Epidemiol 2003;158:1213-1217.
19. Newby PK, Hu FB, Rimm EB, et al. Reproducibility and validity of the Diet Quality Index Revised as assessed by use of a food-frequency questionnaire. Am J Clin Nutr 2003;78:941-949.
20. Willett W, Stampfer M, Chu NF, Spiegelman D, Holmes M, Rimm E. Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. Am J Epidemiol 2001;154:1107-1112.
21. Gu Y, Nieves JW, Stern Y, Luchsinger JA, Scarmeas N. Food combination and Alzheimer disease risk: a protective diet. Arch Neurol 2010;67:699-706.
22. Gu Y, Schupf N, Cosentino SA, Luchsinger JA, Scarmeas N. Nutrient intake and plasma beta-amyloid. Neurology 2012;78:1832-1840.
23. Luchsinger JA, Tang MX, Siddiqui M, Shea S, Mayeux R. Alcohol intake and risk of dementia. J Am Geriatr Soc 2004;52:540-546.
24. Mosconi L, Rinne JO, Tsui WH, et al. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. Neurobiol Aging 2013;34:22-34.
25. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154-159.
26. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11:805-821.
27. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology 2005;64:1860-1867.
28. Li Y, Rinne JO, Mosconi L, et al. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. Eur J Nucl Med Mol Imaging 2008;35:2169-2181.
29. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 2005;25:1528-1547.
30. Minoshima S, Frey KA, Foster NL, Kuhl DE. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. J Comput Assist Tomogr 1995;19:541-547.
31. Willett WC, Stampfer MJ. Implications of total energy intake for epidemiological analyses. In: Willett WC, ed. Nutritional epidemiology. New York: Oxford University Press, 1998: 273-301.
32. Vina J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. J Alzheimers Dis 2010;20 Suppl 2:S527-533.
33. Farrer LA, O'Sullivan DM, Cupples LA, Growdon JH, Myers RH. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol 1989;25:485-493.
34. Tanzi RE, Bertram L. New frontiers in Alzheimer's disease genetics. Neuron 2001;32:181-184.
35. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. J Nutr Health Aging 2004;8:226-232.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

36. Corrada MM, Kawas CH, Hallfrisch J, Muller D, Brookmeyer R. Reduced risk of Alzheimer's disease with high folate intake: the Baltimore Longitudinal Study of Aging. Alzheimers Dement 2005;1:11-18.
37. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am J Clin Nutr 1997;65:20-29.
38. Luchsinger JA, Tang MX, Miller J, Green R, Mayeux R. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. Arch Neurol 2007;64:86-92.
39. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol 2005;62:641-645.
40. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 2007;369:208-216.
41. Morris MC, Schneider JA, Tangney CC. Thoughts on B-vitamins and dementia. J Alzheimers Dis 2006;9:429-433.
42. Kesse-Guyot E, Andreeva VA, Ducros V, et al. Carotenoid-rich dietary patterns during midlife and subsequent cognitive function. Br J Nutr 2013:1-9.
43. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids, alpha-Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. J Aging Res 2013;2013:951786.
44. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002;5:56-65.
45. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. Eur J Nutr 2013;52:1553-1567.
46. de Oliveira BF, Veloso CA, Nogueira-Machado JA, et al. Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients. Nutr Neurosci 2012.
47. Takasaki J, Ono K, Yoshiike Y, et al. Vitamin A has anti-oligomerization effects on amyloid-beta in vitro. J Alzheimers Dis 2011;27:271-280.
48. Balion C, Griffith LE, Strifler L, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 2012;79:1397-1405.
49. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18-26.
50. Lu'o'ng KV, Nguyen LT. The role of vitamin D in Alzheimer's disease: possible genetic and cell signaling mechanisms. Am J Alzheimers Dis Other Demen 2013;28:126-136.
51. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776-782.
52. Morris MC, Evans DA, Bienias JL, et al. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194-200.
53. Bowman GL, Shannon J, Ho E, et al. Reliability and validity of food frequency questionnaire and nutrient biomarkers in elders with and without mild cognitive impairment. Alzheimer Dis Assoc Disord 2011;25:49-57.
54. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940-946.
55. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007;69:1921-1930.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

56. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302:638-648.
57. Gardener S, Gu Y, Rainey-Smith SR, et al. Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. Transl Psychiatry 2012;2:e164.
58. Gu Y, Scarmeas N. Dietary patterns in Alzheimer's disease and cognitive aging. Curr Alzheimer Res 2011;8:510-519.
59. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599-2608.
60. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. Neurology 2012;78:241-249.
61. Murphy DG, DeCarli C, McIntosh AR, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 1996;53:585-594.
62. Vellas BJ, Hunt WC, Romero LJ, Koehler KM, Baumgartner RN, Garry PJ. Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. Nutrition 1997;13:515-519.
63. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-1630.
64. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003;78:517S-520S.
65. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-2342.
66. de Roos B. Personalised nutrition: ready for practice? Proc Nutr Soc 2013;72:48-52.
67. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68:270-281.
68. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
69. Bayer-Carter JL, Green PS, Montine TJ, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Arch Neurol 2011;68:743-752.
70. Morris MC, Evans DA, Tangney CC, et al. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. Am J Clin Nutr 2005;81:508-514.
71. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005;352:2379-2388.

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Table 1. Demographic and clinical characteristics

N	49
Age, y, mean (SD)	54(11), range 25-72
Female gender, %	69%
Education, y, mean (SD)	16(2)
Family history of LOAD, % positive	69%
APOE ε4 status [a], % positive	39%
Ethnicity	
White	82%
Black	8%
Hispanic	6%
Other	4%
Body Mass Index [unitless], mean (SD)	26(4)
Hip to waist ratio [unitless], mean (SD)	1.2(0.2)
Blood pressure (mm/Hg), mean (SD)	
Systolic	118(15)
Diastolic	71(9)
Glucose (mg/dl)	78(12)
Cholesterol (mg/dl)	200(38)
HDL (mg/dl)	62(19)
LDL (mg/dl)	119(33)
Triglycerids (mg/dl)	91(40)
Homocystein (micromol/l)	10(2)
Neuropsychological tests, mean (SD)	
Mini Mental State Exam	29(1)
Digit symbol substitution	62(10)
Paired associates delayed recall	7(2)
Paragraph delayed recall	10(3)
Designs	8(2)
Object naming	55(12)
WAIS-vocabulary	65(14)
Nutrients levels, mean (SD), range	
β-carotene (mcg)	
Foods	6556(8593), 825-35451
Foods and supplements	7438(8447), 1331-35957
ω-3 polyunsaturated fatty acid (g)	
Foods	1.9(1.4), 0.5-8.2
Foods and supplements	2.3(1.8), 0.8-10.9
ω-6 polyunsaturated fatty acid (g)	
Foods	19.7(17.3), 5.2-75.5
Foods and supplements	20.1(17.2), 5.3-75.5
Folate (mcg)	
Foods	486.6(345.1), 181.4-1674.2

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Foods and supplements	648.1(335.9), 187.6-1759.9
Monounsaturated fatty acids (g)	
Foods	38.7(31.5), 10.8-164.5
Foods and supplements	n.a.
Saturated fatty acids (g)	
Foods	23.5(13.0), 8.6-61.3
Foods and supplements	n.a.
Vitamin B12 (mcg)	
Foods	4.5(2.7), 1.3-12.1
Foods and supplements	15.4(9.9), 2.6-58.3
Vitamin C (mg)	
Foods	129(100), 35-521
Foods and supplements	305(267), 69-1565
Vitamin D (mcg)	
Foods	4.7(2.9), 1.1-16.3
Foods and supplements	729(445), 1.8-1806
Vitamin E (mg)	
Foods	13.2(11.5), 3.6-57.4
Foods and supplements	697(37), 632-797

[a] *APOE* genotyping was obtained from 41/49 participants

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 2. Associations between nutrients and AD-biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG glucose metabolism							
β-carotene	β	0.31[a]	0.25[c]	0.33[a]	0.31[a]	0.32[a]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35[b]	0.33[a]	0.31[a]	0.31[a]	0.36[b]	0.27[c]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20[c]	-0.16	-0.15	-0.24[c]	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B12	β	-0.32[a]	-0.37[b]	-0.29[a]	-0.33[a]	-0.37[a]	-0.27[c]
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26[c]	-0.21	-0.28[a]	-0.13	-0.28[a]	-0.27[c]
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω3-PUFA	β	-0.18[c]	-0.28[a]	-0.07	-0.24[c]	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω3-PUFA EPA	β	-0.33[a]	-0.37[b]	-0.32[a]	-0.20	-0.31[a]	-0.28[c]
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrients from food sources

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p = 0.06\text{-}0.10$.

Abbreviations: average cortical ROI (AVG), eicosapentaenoic acid 20:4 (EPA), inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus (MFG), polyunsaturated fatty acids (PUFA), posterior cingulate cortex (PCC), prefrontal cortex (PFC).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3. Effects of gender and family history on the relationship between nutrients and brain glucose metabolism

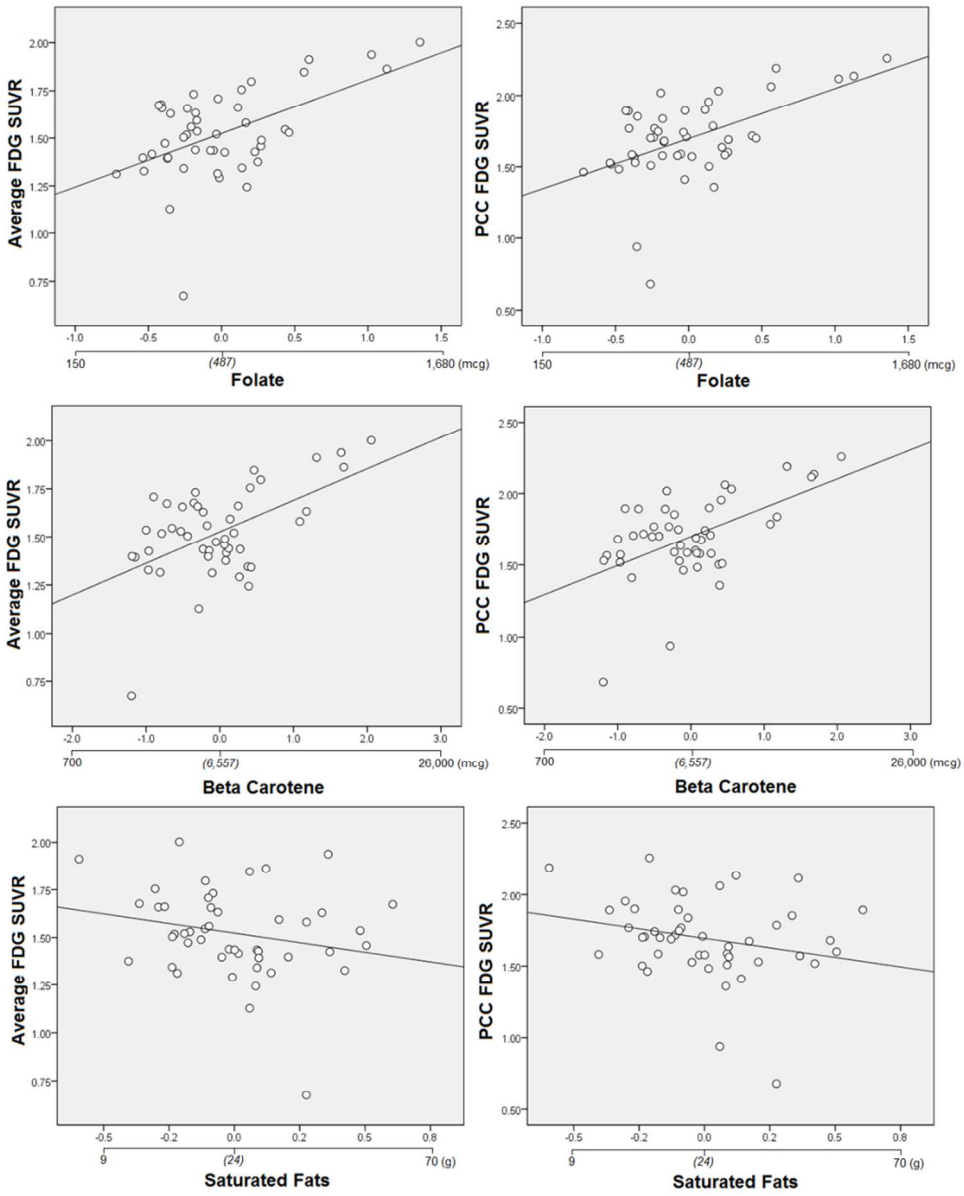
		AVG_{FDG}	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs. men=16)							
β-carotene							
Women	β	0.33 [a]	0.29	0.32 [a]	0.33 [a]	0.34 [a]	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.01	-0.01	0.08	0.08	0.06	-0.02
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate							
Women	β	0.31 [a]	0.34 [a]	0.24	0.28	0.38	0.24
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Men	β	0.29	0.25	0.18	0.22	0.31	0.28
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
Women	β	-0.35 [a]	-0.30	-0.32 [a]	-0.38 [a]	-0.37 [a]	-0.34 [a]
	SE	0.008	0.009	0.007	0.01	0.01	0.009
Men	β	0.15	0.16	0.17	0.12	0.09	0.12
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Family history (FH+, n=33 vs. FH-=16)							
β-carotene							
FH+	β	0.42 [b]	0.35 [a]	0.42 [b]	0.43 [b]	0.45 [b]	0.42 [b]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
FH-	β	-0.10	-0.10	-0.17	0.12	0.06	-0.2
Gender × Family history (FH+ women, n=23 vs. other subjects=26)							
β-carotene							
FH+ women	β	0.48 [a]	0.43 [a]	0.46 [a]	0.49 [b]	0.50 [b]	0.47 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.04	-0.006	0.08	0.09	0.04	0.05
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Gender × APOE genotype (APOE4+ women, n=13 vs. other subjects=36)							
β-carotene							
*APOE*4+ women	β	0.52 [a]	0.46 [a]	0.48 [a]	0.54 [a]	0.53 [a]	0.48 [a]
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Other	β	0.10	0.10	0.10	-0.08	0.03	-0.08
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats							
*APOE*4+ women	β	-0.66 [b]	-0.60 [a]	-0.59 [b]	-0.57 [b]	-0.68 [b]	-0.57 [a]
	SE	0.02	0.03	0.02	0.03	0.03	0.03
Other	β	0.11	0.11	0.16	0.18	0.08	0.19

Standardized β and standard error (SE) estimates from linear regression using age- and caloric intake-residual nutrient values

[a] $p<0.05$, [b] $p\leq0.01$. See Legend to Table 2.

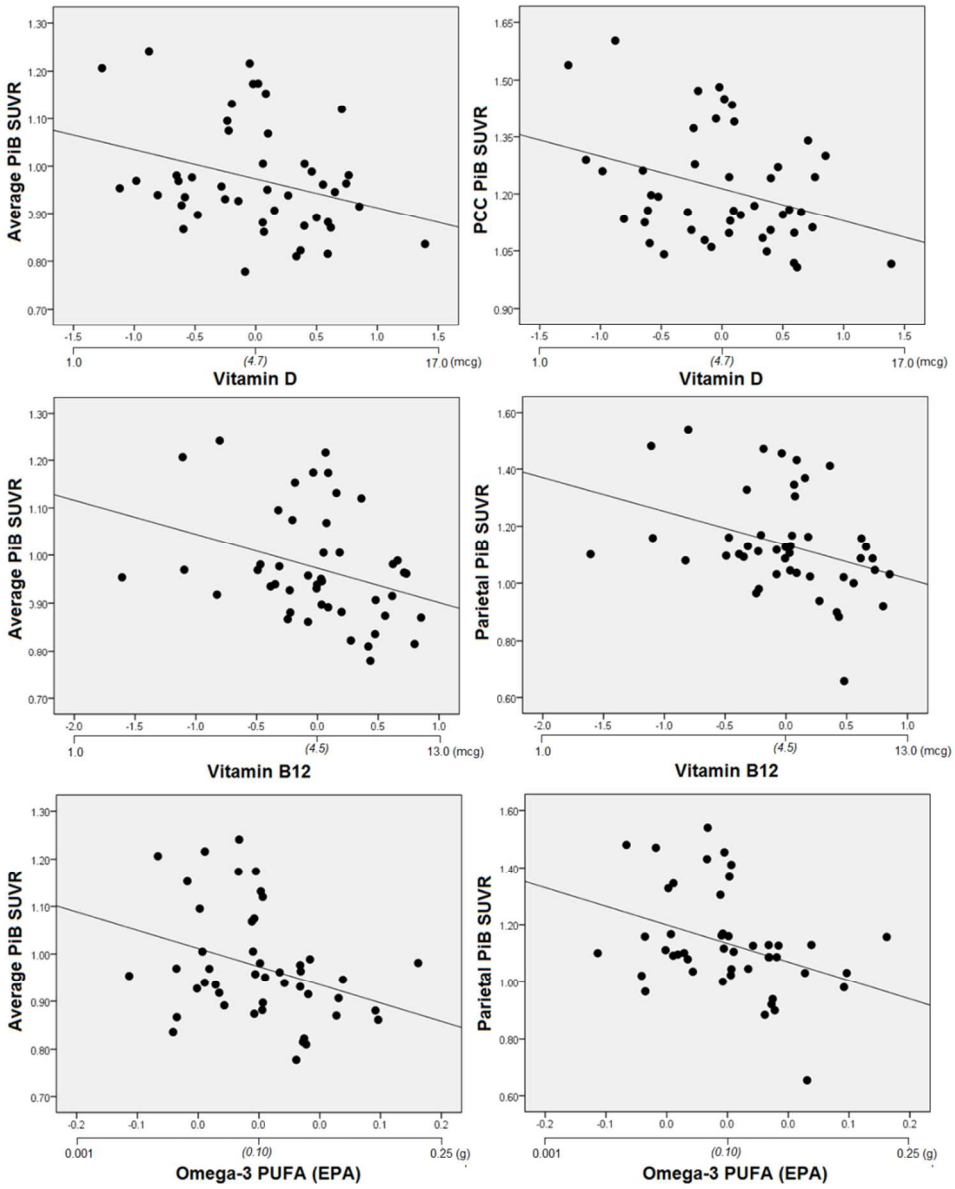
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain glucose metabolism in AD-regions, β-carotene, folate and saturated fats
90x110mm (300 x 300 DPI)

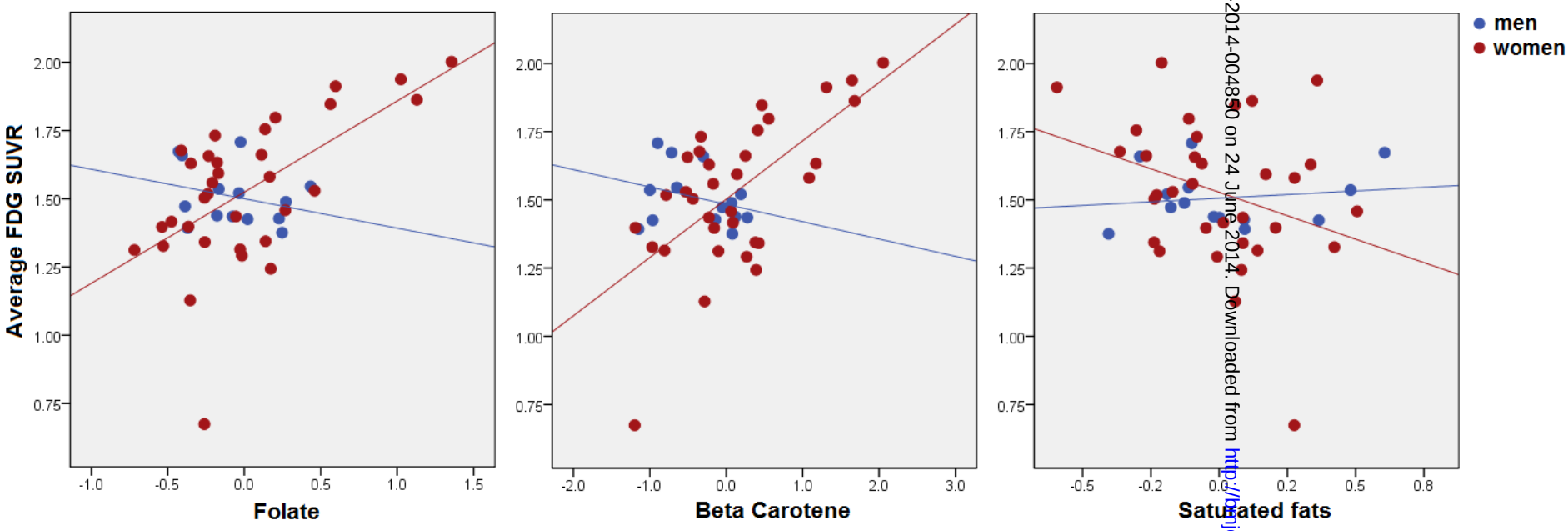
BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Associations between brain amyloid-β load in AD-regions, as measured with 11C-PiB, vitamin B12, vitamin D and ω3-PUFA (EPA)
90x111mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 1. Associations between brain glucose metabolism in AD-regions, folate, β-carotene and saturated fats in women (n=33) vs. men (n=16)

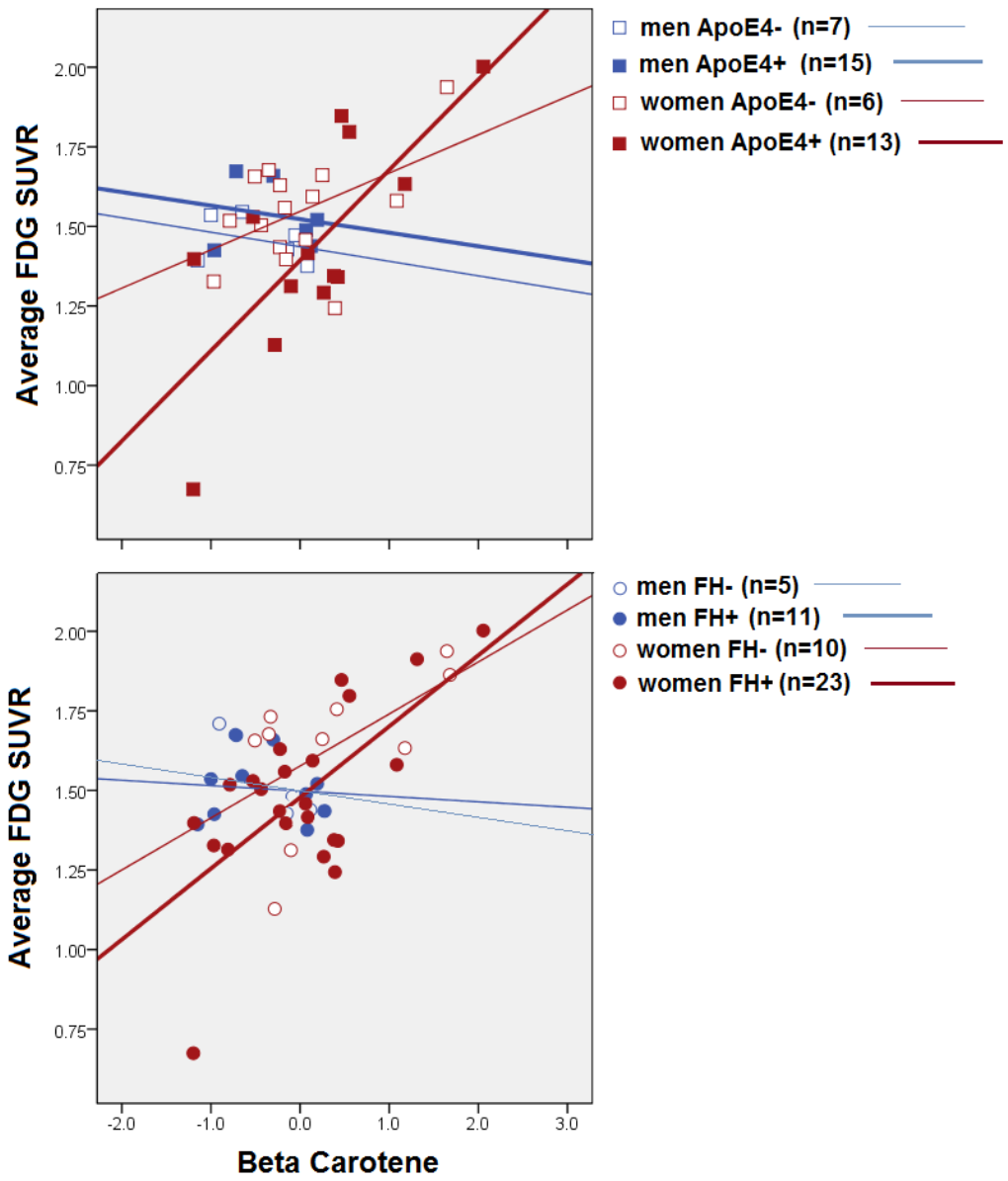


Nutrient values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 2. Associations between brain glucose metabolism in AD-regions and β-carotene as a function of gender × *APOE* × family history of AD (FH)

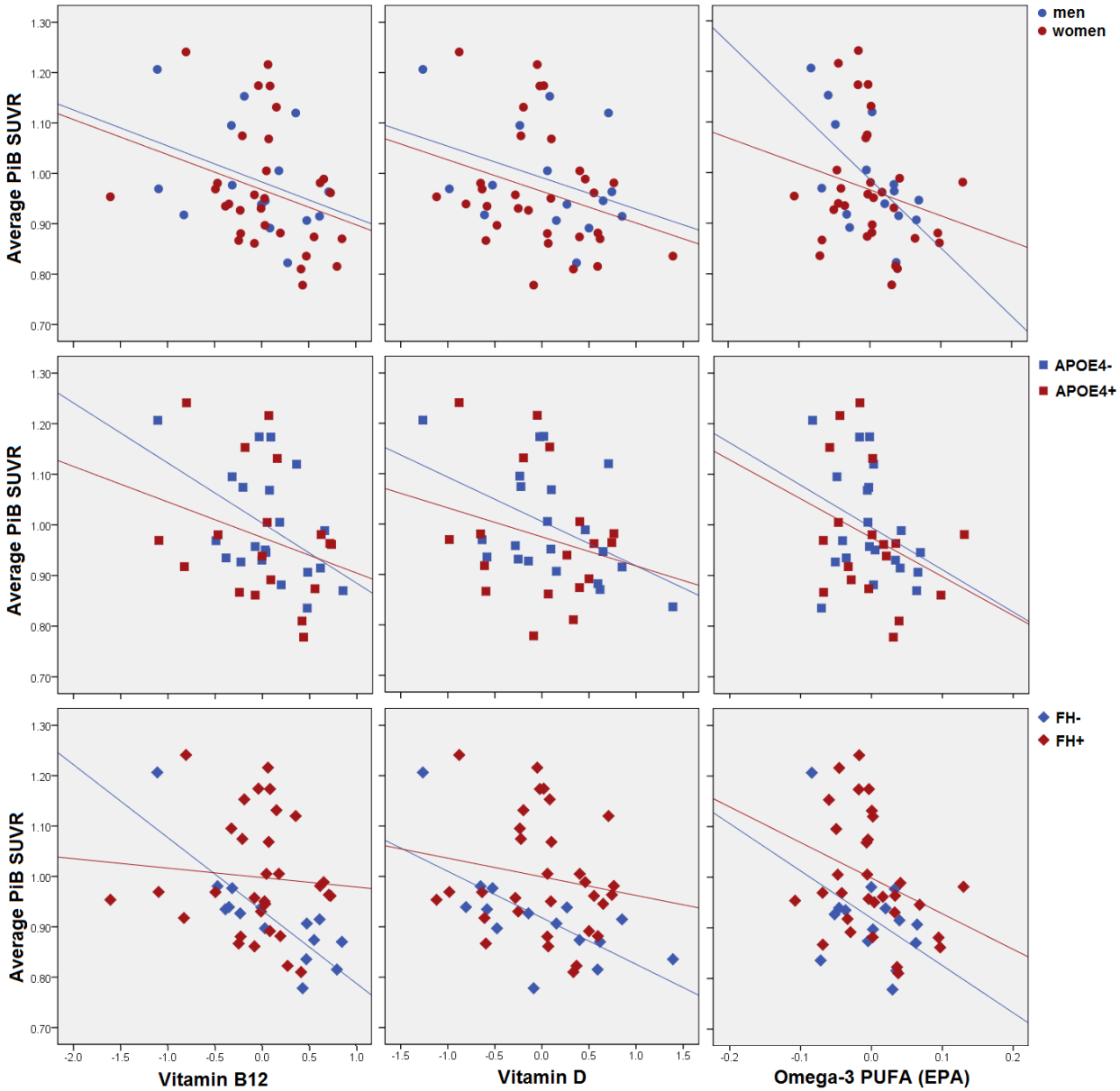


β-carotene values are age and caloric intake-adjusted residuals. FDG values are regional standardized uptake value ratios to pons activity [SUVR, unitless]. Corresponding *p* values are found in Table 2. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-)

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

eFigure 3. Associations of PiB retention in AD-regions with vitamin B12, vitamin D and omega3-PUFA (EPA) as a function of gender, family history of late-onset AD (FH) and *APOE* status



Nutrient values are age and caloric intake-adjusted residuals. PiB values are regional standardized uptake value ratios to cerebellar uptake [SUVR, unitless]. There are no significant nutrient×group interaction effects at $p<0.05$. Abbreviations: *APOE* ε4 allele carriers (*APOE*4+), *APOE* ε4 allele non-carriers (*APOE*4-), positive family history of late-onset AD (FH+), negative family history of late-onset AD (FH-).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract DONE p.1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found DONE p.2-3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported DONE p.6
Objectives	3	State specific objectives, including any prespecified hypotheses DONE p.7
Methods		
Study design	4	Present key elements of study design early in the paper DONE
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection DONE p.7-9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants DONE p.7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable DONE p.10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group DONE p.8,9 and 10-11
Bias	9	Describe any efforts to address potential sources of bias DONE p.8,9 and 10-11
Study size	10	Explain how the study size was arrived at DONE p.7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why DONE p.10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding DONE p.10-11
		(*b*) Describe any methods used to examine subgroups and interactions DONE p.10-11
		(*c*) Explain how missing data were addressed n.a.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Continued on next page

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed N.A.
		(b) Give reasons for non-participation at each stage N.A.
		(c) Consider use of a flow diagram N.A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders DONE p.7-8
		(b) Indicate number of participants with missing data for each variable of interest N.A.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) N.A.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures DONE p.11-13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included DONE p.11-13
		(*b*) Report category boundaries when continuous variables were categorized DONE p.11-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives DONE p. 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias DONE p.15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence DONE
Generalisability	21	Discuss the generalisability (external validity) of the study results DONE
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based DONE p.4

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-004850 on 24 June 2014. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml