

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

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## ABSTRACT

**Objective:** 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 examinations, a neurocognitive test battery, positron emission tomography (PET) with <sup>11</sup>C-Pittsburgh Compound-B (PiB; a measure of amyloid- $\beta$  (A $\beta$ ) load) and <sup>18</sup>F-fluorodeoxyglucose (FDG; a proxy of neuronal activity), and completed semiquantitative food frequency questionnaires.

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

**Participants:** 49 NL individuals (age 25–72 years, 69% women) with dietary information, <sup>11</sup>C-PiB and <sup>18</sup>F-FDG PET scans were examined.

**Results:** Controlling for age and total caloric intake, higher intake of vitamin B<sub>12</sub>, vitamin D and  $\omega$ -3 polyunsaturated fatty acid (PUFA) was associated with lower A $\beta$  load in AD regions on PiB-PET, while higher intake of  $\beta$ -carotene and folate was associated with higher glucose metabolism on FDG-PET.  $\beta$ -carotene and folate were associated with reduced glucose metabolism for women, apolipoprotein E epsilon 4 (APOE4) carriers and participants with positive AD family history, but not for their risk-free counterparts. The associations of vitamin B<sub>12</sub>, vitamin D and  $\omega$ -3 PUFA 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.

**Conclusions:** Our data provide a potential pathophysiological mechanism for epidemiological

## Strengths and limitations of this study

- While the importance of nutrition in health is well understood, the specific effects of nutrition on brain ageing and cognitive decline are less so. This study shows a relationship between nutrients and Alzheimer's disease (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, apolipoprotein E genotype and family history need to be replicated with larger samples.

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.

## 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 on which our body and brain are built. While the importance of nutrition in health

is well understood, the specific effects of nutrition on brain ageing 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.<sup>1–7</sup> However, the biological mechanisms underlying the relationship between dietary nutrients, brain ageing and AD are largely unexplored. Understanding how diet and nutrition promote healthy brain ageing 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- $\beta$  (A $\beta$ ), 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 A $\beta$  load assessed using <sup>11</sup>C-Pittsburgh Compound-B (PiB) positron emission tomography (PET), and glucose metabolism assessed using <sup>18</sup>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 with controls,<sup>8</sup> as well as in non-demented elderly at risk for AD.<sup>9–11</sup> Additionally, FDG-PET metabolic reductions occur prior to dementia onset and correlate with clinical symptoms.<sup>12–14</sup> Our goals were to define which nutrients are associated with lower AD burden (as reflected in lower brain A $\beta$  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

From among a larger pool of participants who underwent PET examinations, 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-PET and FDG-PET examinations and dietary questionnaires within 6 months of each other. Participants were derived from multiple community sources, including individuals interested in research participation, family members and caregivers of impaired patients. All participants 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, that is, stroke, diabetes, head trauma, any neurodegenerative diseases, depression, hydrocephalus, intracranial mass and

infarcts on MRI, and those taking psychoactive medications were excluded. Participants were 25–72 years of age, with education  $\geq 12$  years, Clinical Dementia Rating=0, Global Deterioration Scale (GDS)  $\leq 2$ , Mini-Mental State Examination  $\geq 28$ , Hamilton depression scale  $< 16$ , Modified Hachinski Ischaemia Scale  $< 4$  and had a normal cognitive test performance for age and education.<sup>14</sup> A family history of late-onset AD that included at least one first-degree relative whose AD onset was after age 60 was elicited using standardised questionnaires.<sup>14</sup> *APOE* genotypes were determined using standard quantitative PCR.<sup>15</sup> Individuals with at least one copy of the  $\epsilon 4$  allele were grouped as *APOE*  $\epsilon 4$  carriers (*APOE4+*) and compared with  $\epsilon 4$  non-carriers (*APOE4-*).

### Dietary assessments

Dietary data regarding average food consumption over the prior year were obtained using the 116-item version of Willett's semiquantitative food frequency questionnaire (SFFQ).<sup>16–20</sup> The 116-food items were categorised 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.<sup>19</sup>

Similar to previous reports, we focused on 10 nutrients that have been related to AD or cognitive function<sup>21–22</sup> including saturated fatty acids, monounsaturated fatty acids,  $\omega$ -3 polyunsaturated fatty acid (PUFA),  $\omega$ -6 PUFA,  $\beta$ -carotene, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E and folate. As moderate alcohol drinking may be protective against dementia,<sup>23</sup> 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%) participants reported taking a multivitamin at least three times a week, for  $> 1$  year prior to PET. Of these 20 participants, 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 B<sub>12</sub> (500  $\mu$ g) regularly. Twelve participants reported taking vitamin C (1000 mg) and vitamin D (300–1000 IU) on a seasonal basis only, and mostly occasionally. None of the participants were taking saturated fats or monounsaturated fatty acid supplementation. As some participants reported taking supplements consistently, we additionally looked at nutrient intake from supplements, and the combined nutrient intake from food and supplements.

### Brain imaging

All participants received PiB-PET and FDG-PET scans using standardised procedures.<sup>10 14 24</sup> Briefly,

participants were positioned in the scanner 60 min after injection of 15 mCi of  $^{11}\text{C}$ -PiB, and scanned for 30 min in three-dimensional 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, participants were injected with 5 mCi of  $^{18}\text{F}$ -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.<sup>25</sup>

For each participant, 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 Normalised Mutual Information routine implemented in Statistical Parametric Mapping.<sup>26</sup> MRI were spatially normalised to Montreal Neurological Institute space by high-dimensional warping (DARTEL).<sup>26</sup> MRI-coregistered FDG and PiB scans were spatially normalised using participant-specific transformation matrixes from MRI and sampled using automated regions-of-interest ROI.<sup>27–28</sup> We focused on preselected, AD-vulnerable ROI including inferior parietal lobe (IPL), lateral temporal lobe (LTL), medial frontal gyrus, posterior cingulate cortex (PCC) and prefrontal cortex. Standardised uptake value ratios were generated for each ROI by normalising PiB uptake by cerebellar grey matter uptake<sup>29</sup> and FDG by pons activity.<sup>30</sup> A composite cortical PiB retention mask ( $\text{AVG}_{\text{PiB}}$ ) was created by combining parietotemporal, frontal and PCC regions, and an FDG mask ( $\text{AVG}_{\text{FDG}}$ ) by combining parietotemporal and PCC regions.<sup>24</sup>

### 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 participants' 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 normalised the distributions.

For all analyses, we regressed each nutrient by age (years) and caloric intake (kilocalories) to calculate age-adjusted and caloric intake-adjusted residuals.<sup>31</sup> We then examined gender,<sup>32</sup> education, ethnicity, body mass index (BMI), alcohol consumption, family history<sup>33</sup> and *APOE* status<sup>34</sup> as covariates. Education and BMI were modelled as continuous variables. Gender (male vs female), FH (positive vs negative) and *APOE* status (*APOE4+* vs *APOE4-*) 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).<sup>23</sup>

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 two-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.<sup>3–4 21 22</sup>

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

## RESULTS

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

### FDG-PET

Among all nutrients examined,  $\beta$ -carotene and folate from food sources were positively associated with brain glucose metabolism in several ROIs ( $p \leq 0.05$ ; 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 participants were taking multivitamins at the time of the examination, results from  $\beta$ -carotene and folate from food sources are presented in what follows.

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

Gender  $\times$   $\beta$ -carotene interaction effects on brain glucose metabolism were observed in PCC and  $\text{AVG}_{\text{FDG}}$  ( $p \leq 0.03$ , table 3). In these regions, women showed significant positive associations between  $\beta$ -carotene and glucose metabolism ( $p < 0.05$ ) while men showed no associations (table 3), resulting in steeper regression slopes of glucose metabolism on  $\beta$ -carotene in women compared with men (efigure 1). Interaction effects of gender  $\times$  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, efigure 1). Additionally, marginally significant gender  $\times$  saturated fats interaction effects were observed on PCC and  $\text{AVG}_{\text{FDG}}$  glucose metabolism ( $p \leq 0.08$ ), as women

**Table 1** Demographic and clinical characteristics

N	49
Age, year, mean (SD)	54 (11), range 25–72
Female gender, %	69
Education, year, mean (SD)	16 (2)
Family history of LOAD, % positive	69
APOE ε4 status*, % 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)
Triglycerides (mg/dL)	91 (40)
Homocystein (μmol/L)	10 (2)
Neuropsychological tests, mean (SD)	
Mini-Mental State Examination	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 (μg)	
Foods	6556 (8593), 825–25 451
Foods and supplements	7438 (8447), 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 (μg)	
Foods	486.6 (345.1), 181.4–1674.2
Foods and supplements	648.1 (335.9), 187.6–1759.9
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 B <sub>12</sub> (μg)	
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 (μg)	
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

\*APOE genotyping was obtained from 41/49 participants. APOE, apolipoprotein E; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

showed significant negative associations between saturated fats and glucose metabolism ( $p < 0.05$ ) while men showed no associations (table 3).

Family history  $\times$   $\beta$ -carotene interaction effects on glucose metabolism were observed for all ROIs ( $p \leq 0.05$ ). In these regions, participants with positive family history showed significant associations between  $\beta$ -carotene and glucose metabolism ( $p < 0.05$ ), while those with negative family history showed no associations (table 3). Interaction effects of gender  $\times$  family history  $\times$   $\beta$ -carotene on glucose metabolism were observed ( $p < 0.05$ ), as women with a positive family history showed significant associations between  $\beta$ -carotene and glucose metabolism in PCC, LTL and AVGF<sub>FDG</sub> ( $p < 0.05$ ) whereas the other groups (ie, 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).

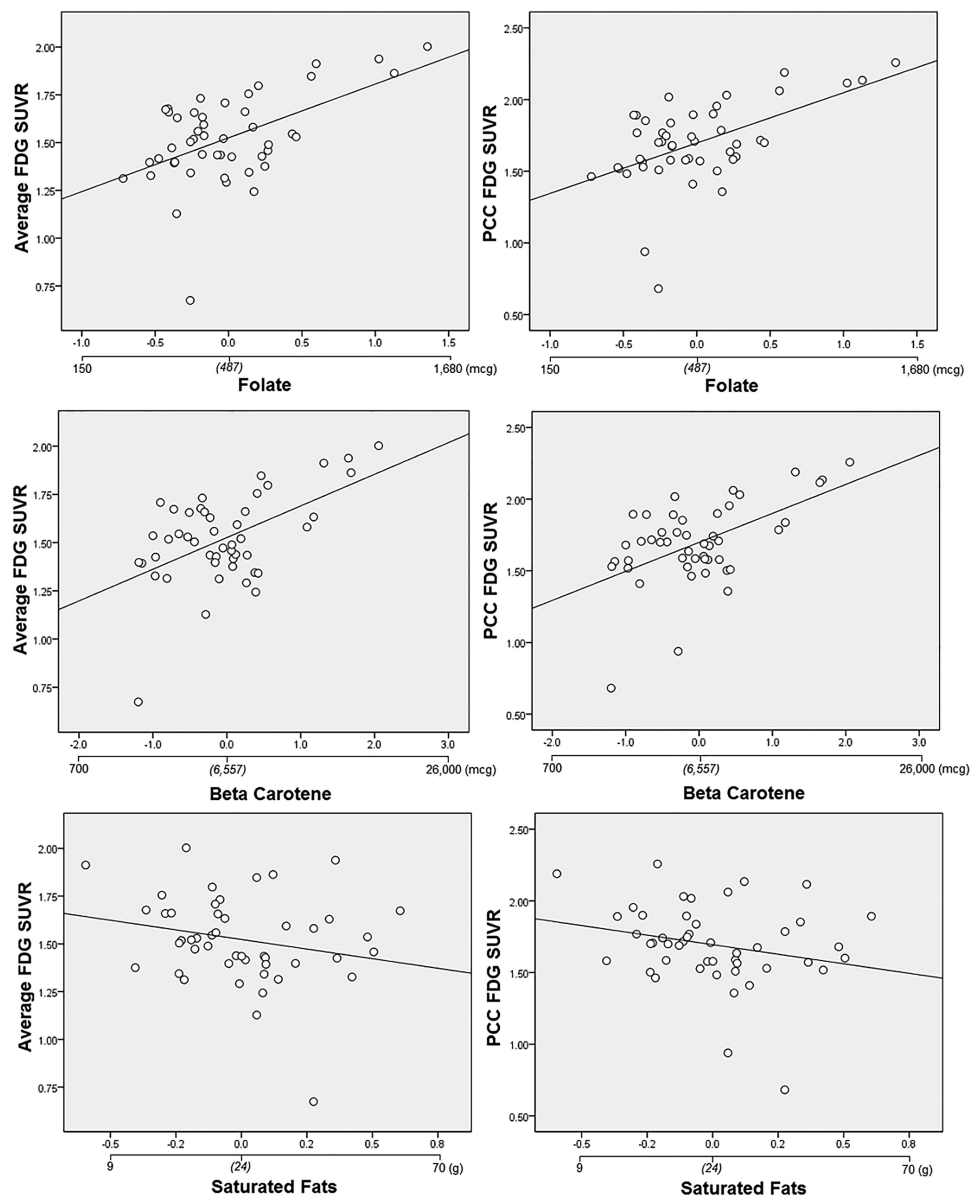
Interaction effects of APOE  $\times$   $\beta$  carotene were marginally significant ( $p \leq 0.08$ ), with APOE4+ participants showing steeper regression slopes vs APOE4-. Nonetheless, there were significant gender  $\times$  APOE  $\times$   $\beta$  carotene interaction effects, as women APOE4+ had the steepest regression slopes of PCC and AVGF<sub>FDG</sub> glucose metabolism on  $\beta$ -carotene as compared with the other groups (ie, APOE4+ men, APOE4- men and women;  $p < 0.05$ ; table 3, efigure 2). Additionally, there were significant gender  $\times$  APOE  $\times$  saturated fat interactions in glucose metabolism, as APOE4+ women showed significant negative associations between saturated fats and glucose metabolism ( $p < 0.05$ ), whereas the other groups (ie, APOE4+ men, APOE4- men and women) did not (table 3).

Correlations between nutrients associated with FDG uptake and food groups showed that  $\beta$ -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, ( $p \leq 0.001$ ). Folate was from grains, legumes, cruciferous vegetables and fresh fruit (0.44, 0.34, 0.32 and 0.30;  $p \leq 0.04$ ), 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,  $p \leq 0.003$ ).

### PiB-PET

Among all nutrients examined, vitamin B<sub>12</sub> 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 three major ω-3 PUFAs subtypes (ie, eicosapentaenoic acid 20:5, EPA; docosahexaenoic acid 22:6, DHA; α-linolenic acid 18:3, ALA). PiB retention was negatively associated with EPA ( $\beta$  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,



**Figure 1** Associations between brain glucose metabolism in AD regions,  $\beta$ -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 standardised uptake value ratios to pons activity (SUVR, unitless). Corresponding p values are found in [table 2](#). AD, Alzheimer's disease; FDG,  $^{18}\text{F}$ -fluorodeoxyglucose; PCC, posterior cingulate cortex; SUVR, standardised uptake value ratios.

while those with vitamin B<sub>12</sub> and  $\omega$ -3 PUFA EPA remained significant ( $p \leq 0.05$ ). 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 participants were taking supplements at the time of the examination, 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 B<sub>12</sub>, vitamin D,  $\omega$ -3 PUFA 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 B<sub>12</sub> 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  $\omega$ -3 PUFA 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 B<sub>12</sub>, vitamin D and  $\omega$ -3 PUFA EPA from food

**Table 2** Associations between nutrients and AD biomarkers

		AVG	IPL	LTL	MFG	PCC	PFC
FDG glucose metabolism							
β-carotene	β	0.31 <sup>a</sup>	0.25 <sup>c</sup>	0.33 <sup>a</sup>	0.31 <sup>a</sup>	0.32 <sup>a</sup>	0.27 <sup>c</sup>
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Folate	β	0.35 <sup>b</sup>	0.33 <sup>a</sup>	0.31 <sup>a</sup>	0.31 <sup>a</sup>	0.36 <sup>b</sup>	0.27 <sup>c</sup>
	SE	0.001	0.001	0.001	0.001	0.001	0.001
Saturated fats	β	-0.20 <sup>c</sup>	-0.16	-0.15	-0.24 <sup>c</sup>	-0.13	-0.16
	SE	0.006	0.006	0.007	0.007	0.006	0.005
PiB retention							
Vitamin B <sub>12</sub>	β	-0.32 <sup>a</sup>	-0.37 <sup>b</sup>	-0.29 <sup>a</sup>	-0.33 <sup>a</sup>	-0.37 <sup>a</sup>	-0.27 <sup>c</sup>
	SE	0.006	0.01	0.009	0.008	0.001	0.008
Vitamin D	β	-0.26 <sup>c</sup>	-0.21	-0.28 <sup>a</sup>	-0.13	-0.28 <sup>a</sup>	-0.27 <sup>c</sup>
	SE	0.006	0.009	0.009	0.007	0.006	0.007
ω-3 PUFA	β	-0.18 <sup>c</sup>	-0.28 <sup>a</sup>	-0.07	-0.24 <sup>c</sup>	-0.09	-0.16
	SE	0.01	0.04	0.04	0.01	0.03	0.01
ω-3 PUFA EPA	β	-0.33 <sup>a</sup>	-0.37 <sup>b</sup>	-0.32 <sup>a</sup>	-0.20	-0.31 <sup>a</sup>	-0.28 <sup>c</sup>
	SE	0.32	0.49	0.47	0.40	0.42	0.39

Standardised β and SE estimates from linear regression using age-residual nutrients and caloric intake-residual nutrients from food sources. <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p=0.06–0.10.

AVG, average cortical ROI; EPA, eicosapentaenoic acid 20 : 4; FDG, <sup>18</sup>F-fluorodeoxyglucose; IPL, inferior parietal lobe; LTL, lateral temporal lobe; MFG, medial frontal gyrus; PUFA, polyunsaturated fatty acids; PCC, posterior cingulate cortex; PFC, prefrontal cortex.

sources was associated with lower Aβ load, and higher β-carotene and folate 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. These data indicate that a healthy diet rich in natural folate, β-carotene, ω-3 PUFA, vitamin B<sub>12</sub> and vitamin D might be particularly useful to support healthy brain ageing. 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 with their risk-free counterparts. These effects were not evident on PiB retention, as the associations between Aβ load and intake of vitamin B<sub>12</sub>, vitamin D and ω-3 PUFA EPA were independent of these risk factors for late-onset AD.

Some studies have shown that higher intakes of vitamin B<sub>12</sub> and folate are related to better cognitive functioning or lower AD risk in the elderly,<sup>35–41</sup> possibly due to their ability to reduce homocysteine levels, although results are not conclusive.<sup>41</sup> β-Carotene (a major precursor to vitamin A) might have beneficial effects via its antioxidant or Aβ antioligomerisation effects.<sup>42–47</sup> Vitamin D has been associated with reduced risk of AD and cerebrovascular disease through several mechanisms including vasculoprotective and synaptic plasticity-enhancing effects, modulation of vascular

endothelial factor expression, angiogenin and advanced glycation end products.<sup>48–50</sup> On the other hand, higher intake of saturated fats is known to have negative effects on cognitive functions,<sup>51 52</sup> while intake of polyunsaturated fatty acids, especially ω-3 PUFA, is known to decrease risk of decline.<sup>21 22 53 54</sup>

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 (ie, food combinations) that are protective against AD. Despite differences in the analytic approaches, high adherence to dietary patterns characterised 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.<sup>3 4 21 55–59</sup> 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-3 PUFA was associated with lower peripheral plasma Aβ levels.<sup>22</sup> Our PiB-PET results in a younger NL cohort show associations between brain Aβ and ω-3 PUFA EPA, further reinforcing the connection between ω-3 PUFA and AD pathology. Other studies with larger samples and a wider age range are needed to assess whether other ω-3 PUFA markers are also associated with brain Aβ load in the elderly.

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

		AVG <sub>FDG</sub>	IPL	LTL	MFG	PCC	PFC
Gender (women, n=33 vs men=16)							
β-carotene							
Women	β	0.33 <sup>a</sup>	0.29	0.32 <sup>a</sup>	0.33 <sup>a</sup>	0.34 <sup>a</sup>	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 <sup>a</sup>	0.34 <sup>a</sup>	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 <sup>a</sup>	-0.30	-0.32 <sup>a</sup>	-0.38 <sup>a</sup>	-0.37 <sup>a</sup>	-0.34 <sup>a</sup>
	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 <sup>b</sup>	0.35 <sup>a</sup>	0.42 <sup>b</sup>	0.43 <sup>b</sup>	0.45 <sup>b</sup>	0.42 <sup>b</sup>
	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
	SE	0.004	0.005	0.004	0.008	0.005	0.007
Gender×family history (FH+ women, n=23 vs other participants=26)							
β-carotene							
FH+ women	β	0.48 <sup>a</sup>	0.43 <sup>a</sup>	0.46 <sup>a</sup>	0.49 <sup>b</sup>	0.50 <sup>b</sup>	0.47 <sup>a</sup>
	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 participants=36)							
β-carotene							
APOE4+ women	β	0.52 <sup>a</sup>	0.46 <sup>a</sup>	0.48 <sup>a</sup>	0.54 <sup>a</sup>	0.53 <sup>a</sup>	0.48 <sup>a</sup>
	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							
APOE4+ women	β	-0.66 <sup>b</sup>	-0.60 <sup>a</sup>	-0.59 <sup>b</sup>	-0.57 <sup>b</sup>	-0.68 <sup>b</sup>	-0.57 <sup>a</sup>
	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
	SE	0.001	0.001	0.001	0.001	0.001	0.001

Standardised β and SE estimates from linear regression using age-residual nutrient values and caloric intake-residual nutrient values.

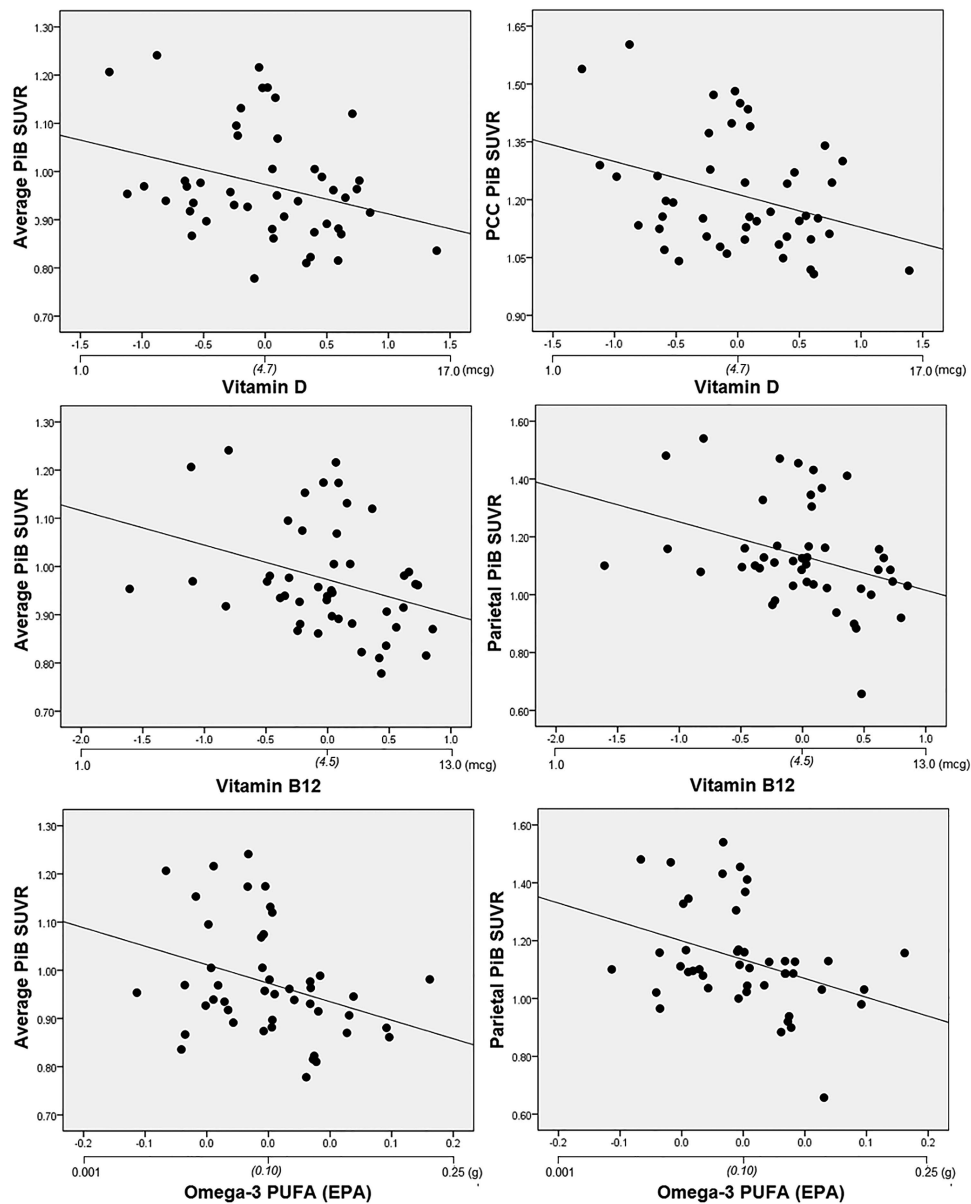
<sup>a</sup>p<0.05, <sup>b</sup>p≤0.01.

APOE, apolipoprotein E; AVG, average cortical ROI; IPL, inferior parietal lobe; LTL, lateral temporal lobe; MFG, medial frontal gyrus; PCC, posterior cingulate cortex; PFC, prefrontal cortex.

While SFFQs are fairly comprehensive and SFFQ-derived dietary patterns remain broadly stable over time,<sup>21</sup> the method may be subject to faulty recall of dietary intake. Plasma nutrient studies might obviate this issue. Bowman *et al*<sup>60</sup> showed that higher levels of plasma antioxidants, vitamin B, D and ω-3 PUFA were associated with more favourable cognitive and MRI white matter hyperintensity measures in an elderly population (mean age 87 years). 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 participants 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 ageing. 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 first-degree family history of late-onset AD and the APOE ε4 genotype are all associated with reduced glucose metabolism in NL individuals.<sup>13 14 61</sup>



**Figure 2** Associations between brain amyloid- $\beta$  load in AD regions, as measured with  $^{11}\text{C}$ -PiB, vitamin B $_{12}$ , vitamin D and  $\omega$ -3 PUFA (EPA 20 : 4). 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 standardised uptake value ratios to cerebellar uptake (SUVR, unitless). Corresponding p values are found in [table 2](#). AD, Alzheimer's disease;  $^{11}\text{C}$ -PiB,  $^{11}\text{C}$ -Pittsburgh Compound-B; EPA, eicosapentaenoic acid; PCC, posterior cingulate cortex; PUFA, polyunsaturated fatty acid; SUVR, standardised uptake value ratios.

Nutritional studies have shown that women need fewer calories than men, but in many cases, they have higher vitamin and mineral needs.<sup>62 63</sup> 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  $\beta$ -carotene ( $p < 0.04$ ). These descriptive observations warrant replication in larger samples. Additionally, nutrient-biomarker 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,<sup>64</sup> especially for women.<sup>65</sup> While family history and APOE status have been associated with increased A $\beta$  load in NL,<sup>10 11</sup> the effects of vitamin B $_{12}$ , vitamin D $_{12}$  and  $\omega$ -3 PUFA EPA on PiB retention were not exacerbated in the presence of these risk factors or female gender. Overall, our data suggest 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,<sup>66</sup> and support the view that AD is a multifactorial disease resulting from genetic, lifestyle and environmental interactions.<sup>67</sup> Third, nutrient effects on A $\beta$  load were independent of AD-risk factors, but this may differ in older populations with more substantial A $\beta$  deposition. Nutrients may have an impact on A $\beta$  oligomers, which are not detectable with PiB-PET, prior to plaque formation.<sup>68</sup> A low saturated fat/glycaemic index diet was shown to improve A $\beta$  composition in cerebrospinal fluid in MCI,<sup>69</sup> suggesting that diet is a powerful environmental factor that modulates AD risk through its effects on brain A $\beta$  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 participants under controlled clinical conditions. While a sample of 49 participants 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 overfitting. 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 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 an 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 vitamin B<sub>12</sub> status,<sup>41</sup> as high dosages of folic acid may mask vitamin B<sub>12</sub> 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 analyse indicator variables of quantiles (ie, tertiles and 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 B<sub>12</sub> intake may be challenging to characterise by dietary intake assessment as its serum level can be affected by gastrointestinal malabsorption syndromes, such as those seen with

pernicious anaemia, 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 participants showed vitamin B<sub>12</sub> deficiencies in plasma on standard blood tests, we cannot exclude that intake may have been underestimated in some participants. Moreover, a significant component of 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 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 socioeconomic and medical status is warranted and clinical application is not yet justified.

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

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**Contributors** LM, PMcH and CM participated in study concept and design. LM, JM, MD, SW, NS, RSO, PMcH and SV participated in acquisition of data. LM, EP, TB, RSO, LG, PMcH and CM participated in analysis and interpretation of data. LM and PMcH participated in drafting of the manuscript. LM, TB, RSO, LG, SV, PMcH, CM and MJdL participated in critical revision of the manuscript for important intellectual content. LM and EP participated in statistical analysis. LM and MJdL obtained funding. LM and MJdL participated in administrative, technical and material support. LM participated in study supervision.

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