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## Effects of lifestyle and vascular risk on Alzheimer's brain biomarker changes during middle age: a 3-year longitudinal study in the broader New York City area

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Effects of lifestyle and vascular risk on Alzheimer’s brain biomarker changes during middle age: a 3-year longitudinal study in the broader New York City area

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### Abstract

**Objective:** To investigate the effect of lifestyle and vascular risk factors on Alzheimer’s disease (AD) biomarker changes [beta-amyloid load via <sup>11</sup>C-PiB PET, and neurodegeneration via <sup>18</sup>F-FDG PET and structural MRI] and cognition in middle-aged asymptomatic participants at risk for AD.

**Design:** Prospective, longitudinal.

**Setting:** The study was conducted at New York University Langone/Weill Cornell Medical Centers in New York City.

**Participants:** Seventy cognitively normal participants from multiple community sources, aged 30-60 years with lifestyle measures (diet, intellectual and physical activity), vascular risk measures, and two imaging biomarkers visits over at least two years, were included in the study.

**Outcome measures:** We examined MRI-based cortical thickness, FDG glucose metabolism, and PiB beta-amyloid in AD-vulnerable regions. A global cognitive Z-score served as our summary cognition measure. We used regression change models to investigate the associations of clinical, lifestyle, and vascular risk measures with changes in AD biomarkers and global cognition.

**Results:** Diet influenced neurodegeneration changes detected via FDG-PET, but not amyloid or MRI changes. With and without accounting for demographic measures, vascular risk, and baseline FDG measures, lower adherence to a Mediterranean-style diet was associated with faster rates of FDG declines in posterior cingulate cortex (p<0.05) and marginally in frontal cortex (p=0.07). None of the other lifestyle variables and none of the vascular measures showed associations with AD biomarker changes. Higher baseline plasma homocysteine was associated with faster rates of decline in global cognition, with and without accounting for lifestyle and biomarker measures (p<0.05). None of the lifestyle variables were associated with cognition.

**Conclusions:** Diet influenced neurodegeneration in middle-aged participants, while plasma homocysteine explained variability in cognitive performance. These findings

suggest that these modifiable risk factors affect AD risk through different pathways and support further investigation of AD-risk reduction strategies in midlife.

### Strengths and limitations of this study

- A key strength of our study is the availability of healthy, cognitively intact middle-aged individuals with multiple lifestyle and vascular risk measures, as well as neuroimaging scans at least two years apart.
- Another strength of our study was our ability to look at diet, intellectual and physical activity, and vascular risk factors, and our statistical model enabled us to simultaneously assess these risk factors.
- Another limitation of our study is that we obtained information regarding lifestyle habits via self-reported questionnaires, which are vulnerable to error.
- We caution that the participants included in our study were carefully screened, healthy individuals without severe cardiac or cerebrovascular disease, which limits the generalizability of our results to the entire population.

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## INTRODUCTION

Unless effective strategies for prevention are found, the prevalence of Alzheimer’s disease (AD), the most common form of dementia affecting nearly 34 million people worldwide, is expected to triple by 2050.<sup>1</sup>

Leading a healthy lifestyle in combination with strategies to reduce vascular disease risk is increasingly viewed as preventative against cognitive decline and dementia.<sup>2</sup> Findings from population-attributable risk models estimate that one in every three AD cases may be accounted for by *modifiable* risk factors, such as midlife hypertension, obesity, diabetes, and several lifestyle factors.<sup>3 4</sup>

Most studies in this field have focused on the effects of lifestyle and cardiovascular factors on either cognitive decline or incidence of dementia as outcome measures.<sup>5</sup> However, there is evidence that AD pathophysiology develops up to 20 years upstream of cognitive symptoms,<sup>6 7</sup> namely in midlife. As such, biological markers of AD are needed to determine whether lifestyle and vascular risk impact emergence and progression of brain AD-endophenotype.

Previous investigations into the effects of modifiable risk factors on AD biomarkers have yielded mixed results.<sup>8-14</sup> For example, studies have shown minimal to null effects of intellectual enrichment and physical activity on AD biomarker changes.<sup>11-13</sup> However, most previous studies did not take diet or vascular risk into account, and focused on elderly populations, rather than asymptomatic middle-aged individuals.<sup>8-14</sup>

We recently showed that, at cross-section, adherence to a Mediterranean diet (MeDi) was positively associated with MRI-based cortical thickness in middle-aged persons, after accounting for intellectual activity, physical activity, and vascular measures.<sup>15</sup>

Herein, we present a 3-year multi-modality brain imaging study aimed at assessing the impact of multiple lifestyle and vascular risk factors on rate of decline in AD biomarkers, as measured by amyloid-beta (Aβ) deposition on <sup>11</sup>C-PiB PET, and neurodegeneration via <sup>18</sup>F-FDG PET and MRI, in a cohort of cognitively normal, middle-aged individuals at risk for AD.

## METHODS

### Participants

Study participants were recruited from a longitudinal brain imaging study conducted at New York University (NYU) Langone School of Medicine and Weill Cornell Medical College (WCMC) between 2010-2016. The study aimed at examining risk factors for AD among clinically and cognitively normal young to late middle-aged adults.

Details about the study design have previously been published.<sup>16 17</sup> Briefly, participants were derived from multiple community sources, including individuals interested in research participation and family members and caregivers of impaired patients.

All participants received clinical, laboratory, neuropsychological, and brain imaging exams including MRI and FDG- and PiB-PET at baseline and at least two years later. To be included in this study, participants had to be 30-60 years old at baseline, with education  $\geq 12$  years, Clinical Dementia Rating = 0, Global Deterioration Scale  $\leq 2$ , Mini Mental State Examination  $\geq 27$ , Hamilton depression scale  $< 16$ , and normal cognitive test performance for age and education.<sup>18</sup> Those 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.

A family history of AD that included at least one first degree relative whose AD onset was after age 60 was elicited using standardized questionnaires.<sup>17</sup>

Apolipoprotein E (APOE) genotypes were determined using standard qPCR procedures.<sup>17</sup>

### Standard protocol approvals, registrations, and patient consents.

All participants provided informed consent to participate in this NYU School of



Medicine/WCMC IRB-approved study.

### Global cognition measure

The neuropsychological battery of tests was previously described.<sup>18</sup> At both time points, we assessed three cognitive domains from the following tests: memory (immediate and delayed recall of a paragraph, immediate and delayed recall of paired associates), executive function (Wechsler Adult Intelligence Scale [WAIS] digit symbol substitution), and language (WAIS vocabulary).

We computed a *global cognitive summary score* by first z-scoring each measure within each domain, and then averaging each component of the three domains listed above. Global cognition, calculated as the average of the composite memory, executive function, and language variables at each time point, was used as an outcome variable.

### Vascular risk-related measures

Vascular risk factors included in the model were: a) body mass index (BMI); b) *presence of hypertension*, conservatively determined based on either current antihypertensive treatment or blood pressure assessments; c) *plasma cholesterol and/or homocysteine*, as obtained after overnight fasting using standard laboratory procedures; and d) *insulin resistance*, measured with the Quantitative Insulin Sensitivity Check Index (QUICKI),<sup>19</sup> where lower scores reflect greater insulin resistance.

### Lifestyle variables

Dietary data regarding average food consumption over the prior year were obtained using the Harvard semi-quantitative food frequency questionnaire.<sup>15 20</sup> Briefly, 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 food group items. For the construction of MeDi scores, we first regressed caloric intake and calculated the derived residuals of daily intake for each of the following categories: dairy, meat, fruits, vegetables, legumes, cereals, and fish. Individuals were assigned a value of one for each beneficial component (fruits, vegetables, legumes, cereals, and fish) whose consumption was at or above the sex-specific median; a value of one for each harmful component (meat and dairy products) whose consumption was below the median; a value of one for a ratio of monounsaturated fats to saturated fats above the median; and a value of one for mild to moderate alcohol consumption. These values were summed to generate a MeDi score, with a greater score indicating greater MeDi adherence.

The Minnesota Leisure Time Physical Activity questionnaire was used to estimate physical activity.<sup>21</sup> For each activity, information was collected on the frequency and duration of engagement, which were multiplied with an activity-specific intensity code indicating calorie expenditure. The activity-dependent scores were summed to obtain the overall intensity of physical activity per person during the last 12 months and converted to metabolic equivalents.

Intellectual activity throughout life was assessed using a validated 25-item interview in which participants were asked to report how often they engaged in common cognitively demanding activities with minimal dependence on socioeconomic status, such as reading books or newspapers, writing letters or e-mails, going to the library, and playing games, at different ages.<sup>8 22</sup> Previous studies described this instrument in detail and reported high internal consistency and positive associations of intellectual activity with educational and cognitive performance.<sup>22</sup>

## AD biomarkers

All subjects received MRI, PiB-PET and FDG-PET scans at least two years apart, following standardized procedures.<sup>16 23 24</sup>

Participants received 3T volumetric T1-MPRAGE scans at both time points. Freesurfer v. 5.3 with a longitudinal processing pipeline was used to obtain entorhinal (EC) and

posterior cingulate cortex (PCC) thickness on longitudinal MRI scans.<sup>25</sup> Total intracranial volumes (TIV) were also estimated.

PET images were acquired with PET/CT scanners operating in 3D mode and analyzed using a fully automated image processing pipeline.<sup>26 27</sup> Statistics on image voxel values were extracted from automatically labeled cortical regions of interest using the automated anatomic labeling atlas.<sup>28</sup> We selected PCC/precuneus as the target AD-related region-of-interest, and frontal cortex (including prefrontal and medial frontal regions) as the target aging-related region.<sup>29</sup> For each region of interest, PiB uptake was divided by cerebellar gray matter uptake, and FDG uptake was normalized by the global activity.

Statistical analysis

Statistical analyses were performed using Stata, version 13. We conducted two types of analyses to examine the associations of age, sex, APOE4 genotype, lifestyle variables (diet, intellectual activity, and physical activity), and vascular risk variables (BMI, plasma cholesterol, plasma homocysteine, hypertension, and insulin resistance) with AD biomarkers and cognition.

The first analysis consisted of a partial correlation analysis to evaluate the direct associations among the predictors and dependent variables at baseline and over time. We estimated these associations using partial Pearson correlations ( $r_s$ ) and adjusted for the effects of age, gender, and APOE status. The PIB variables had skewed distributions and were log transformed, and MRI measures were adjusted by TIV.

In the second analysis, we used regressed change models<sup>30</sup> to evaluate lifestyle and vascular measures as predictors of change in biomarker values and global cognition. Regressed change models, as opposed to difference score models, overcome several of the disadvantages of difference score measures.<sup>30</sup> For example, difference scores are often negatively correlated with baseline values and are doubly affected by unreliability and missing data points in the measures.

We considered continuous variables including age (years), education (years), diet, exercise, and intellectual activity scores, BMI, QUICKI scores, and lab measures. Sex (female versus male) and APOE genotype (presence of either 1 or 2 versus absence of  $\epsilon 4$  alleles) were used as dichotomous variables with female sex and APOE4 positivity as the reference. Presence or absence of hypertension was used as a dichotomous variable with absence of the condition used as the reference.

In each model, we predicted regressed change in each outcome measure by running a series of regression models that isolated the effect of our predictors on the outcome measure at follow-up while holding the baseline measures constant. Additionally, the baseline biomarker measures were examined as predictors of change in cognition in addition to the other predictors. For each model, we used a backwards elimination procedure to remove non-significant covariates and form the most parsimonious model.

We fit four separate models for each outcome measure:

- Model 1: the full model with all predictor variables included;
- Model 2: the full model with non-significant adjustment variables removed;
- Model 3: reduced model with lifestyle predictors only, and non-significant adjustment variables removed;
- Model 4: reduced model with vascular predictors only, and non-significant adjustment variables removed.

The resulting unstandardized beta coefficients ( $\beta_s$ ) can be interpreted as partial correlations. All results were considered significant at  $p < 0.05$ .

## RESULTS

### Participants

A total of 86 participants complete baseline imaging evaluations. Six participants did not complete the follow-up evaluations. Of the remaining 80 participants, 10 had incomplete lifestyle questionnaires and were excluded. The remaining 70 participants

were examined in this study.

Participants' characteristics are shown in **Table 1**. The average age was 49 years, ranging from 30-60 years, with 69% women. A family history of AD was reported by 67% of participants, and 39% had at least one copy of the APOE4 allele.

**Table 1.** Clinical and demographic characteristic at baseline.

Characteristics	N = 70
Age (years)	49 (8), range 30-60
Female, No. (%)	48 (69)
Education (years)	16 (2)
Caucasians, No. (%)	56 (80)
Positive family history of AD, No. (%)	47 (67)
APOE4 carriers, No. (%)	27 (39)
Positive subjective complaints, No. (%)	48 (69)
Time to follow-up (years)	3 (1), range 2-3.5
<b>Lifestyle measures</b>	
Mediterranean diet scores	4 (2), range 1-9
Physical activity scores	9 (5), range 1-37
Intellectual activity scores	4 (1), range 2-5
<b>Vascular measures</b>	
Body mass index (kg/m <sup>2</sup> )	25 (4)
Presence of hypertension, No. (%)	10 (14)
QUICKI score	0.32 (0.03)
Plasma cholesterol/HDL ratio	3.3 (0.8)
Plasma homocysteine (micromol/L)	7.9 (6.2)

Neuropsychological measures	
Mini Mental State Examination	29 (1)
Paragraph Recall, Immediate	7.2 (2.5)
Paragraph Recall, Delayed	9.8 (2.8)
Paired Associates Recall, Immediate	6.4 (2.5)
Paired Associates Recall, Delayed	7.3 (2.6)
Object naming	55 (9)
Design test	8.1 (2.3)
Digit Symbol Substitution	66 (13)
WAIS Vocabulary	68 (8)

Values are presented as mean (SD), unless otherwise specified.

Abbreviations: AD, Alzheimer's disease; QUICKI, Quantitative Insulin Sensitivity Check Index; WAIS, Wechsler Adult Intelligence Scale.

### Models for Prediction of Cognitive Changes

None of the adjustment variables were associated with cognition at baseline or longitudinally. At baseline, higher intellectual activity was associated with better cognition, with and without adjusting for age, sex, and APOE status ( $r_s \geq 0.401$ ,  $p < 0.01$ ; **eTables 1-3**).

Longitudinal results are summarized in **Table 2**. Accounting for baseline cognition, higher baseline plasma homocysteine was associated with faster rates of decline in global cognition scores ( $p = 0.048$ ). None of the lifestyle variables were directly associated with cognitive changes.

The baseline biomarkers did not predict cognitive changes, except for a negative non-significant association between baseline FDG uptake in frontal cortex and faster rates of declines in global cognition ( $p = 0.11$ ). Therefore, including the baseline

biomarkers in the models did not significantly shift the relationships between cognition and the lifestyle or vascular variables.

**Table 2.** Prediction of changes in global cognition.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
Mediterranean diet	-0.012 (0.046)	-0.013 (0.048)	-0.029 (0.046)	
Physical activity	0.001 (0.008)	-0.001 (0.008)	-0.004 (0.008)	
Intellectual activity	0.089 (0.128)	0.044 (0.128)	0.103 (0.128)	
Plasma homocysteine	-0.068 (0.034) <sup>†</sup>	-0.063 (0.032) <sup>†</sup>		-0.066 (0.032) <sup>*</sup>
Plasma cholesterol	0.001 (0.002)	0.001 (0.002)		0.001 (0.002)
Body mass index	0.007 (0.014)	0.006 (0.014)		0.006 (0.014)
QUICKI scores	0.343 (3.965)	0.537 (3.813)		0.798 (3.837)
Hypertension	-0.080 (0.117)	-0.056 (0.118)		-0.055 (0.117)
Global cognition at baseline	0.872 (0.132) <sup>***</sup>	0.827 (0.143) <sup>***</sup>	0.801 (0.146) <sup>***</sup>	0.842 (0.132) <sup>***</sup>
Sex	-0.023 (0.078)			
APOE status	0.104 (0.079)			
Age	0.006 (0.012)			
Time to follow-up	-0.000 (0.000)			
Constant	-0.097 (0.654)	-0.125 (0.620)	0.005 (0.068)	-0.167 (0.621)

<sup>\*</sup>p<0.05; <sup>\*\*</sup>p<0.01; <sup>\*\*\*</sup>p<0.001; <sup>†</sup>0.05<p<0.10

Values are presented as unstandardized beta coefficients (standard error).

<sup>a</sup>Model 1: full model with all variables examined

<sup>b</sup>Model 2: full model without non-significant adjustment variables

<sup>c</sup>Model 3: model with lifestyle variables only, and without non-significant adjustment variables

<sup>d</sup>Model 4: model with vascular variables only, and without non-significant adjustment variables

Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index.



## Models for Prediction of Amyloid Accumulation

Baseline results are summarized in **eTable 1**. None of the clinical, lifestyle, and vascular risk variables were associated with baseline PiB uptake.

Longitudinal results are summarized in **Table 3**. APOE4 status was positively, though not significantly associated with faster rates of amyloid deposition in frontal cortex ( $p=0.084$ ). None of the vascular variables were associated with change in amyloid measures.

**Table 3.** Prediction of PiB-PET amyloid deposition.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
<b>PCC</b>				
Mediterranean diet	-0.017 (0.016)	-0.018 (0.017)	-0.011 (0.016)	
Physical activity	-0.005 (0.003) <sup>†</sup>	-0.005 (0.003) <sup>†</sup>	-0.005 (0.003) <sup>†</sup>	
Intellectual activity	0.053 (0.053)	0.021 (0.052)	0.016 (0.051)	
Plasma homocysteine	-0.013 (0.012)	-0.006 (0.012)		-0.010 (0.012)
Plasma cholesterol	-0.002 (0.001)	-0.001 (0.001)		-0.001 (0.001)
Body mass index	-0.004 (0.005)	-0.005 (0.005)		-0.006 (0.005)
QUICKI scores	-1.720 (1.550)	-2.155 (1.581)		-2.016 (1.575)
Hypertension	-0.048 (0.041)	-0.026 (0.037)		-0.019 (0.036)
PCC PiB uptake at baseline	0.094 (0.238)	0.194 (0.259)	0.156 (0.261)	0.232 (0.252)
Sex	0.020 (0.031)			
APOE status	0.042 (0.029)			
Age	0.007 (0.004)			
Time to follow-up	0.000 (0.000)			
Constant	0.394 (0.256)	0.465 (0.264) <sup>†</sup>	0.126 (0.028) <sup>***</sup>	0.447 (0.263) <sup>†</sup>



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<i>Frontal cortex</i>				
Mediterranean diet	-0.016 (0.011) <sup>†</sup>	-0.018 (0.011) <sup>†</sup>	-0.011 (0.011)	
Physical activity	-0.000 (0.002)	-0.001 (0.002)	-0.001 (0.002)	
Intellectual activity	0.050 (0.036)	0.031 (0.036)	0.019 (0.036)	
Plasma homocysteine	-0.001 (0.008)	-0.001 (0.008)		-0.002 (0.008)
Plasma cholesterol	-0.001 (0.001)	-0.001 (0.001)		-0.001 (0.001)
Body mass index	-0.001 (0.003)	-0.001 (0.003)		-0.001 (0.003)
QUICKI scores	-1.293 (1.008)	-1.497 (1.012)		-1.184 (1.001)
Hypertension	-0.005 (0.029)	-0.005 (0.026)		0.001 (0.025)
Frontal PiB uptake at baseline	0.000 (0.143)	0.003 (0.145)	0.001 (0.144)	0.026 (0.140)
Sex	0.017 (0.019)			
APOE status	0.033 (0.019) <sup>†</sup>			
Age	0.000 (0.002)			
Time to follow-up	-0.000 (0.000)			
Constant	0.234 (0.167)	0.256 (0.168)	0.010 (0.018)	0.208 (0.166)

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; <sup>†</sup>  $0.05 < p < 0.10$   
See Legend to Table 2.  
Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index; PCC, posterior cingulate cortex.

Models for Prediction of FDG Changes

Baseline results are summarized in **eTable 2**. Among lifestyle variables, frontal FDG uptake was positively associated with intellectual activity ( $r_s=0.27$ ,  $p=0.042$ ). None of the vascular variables showed associations with baseline FDG uptake.

Longitudinal results are summarized in **Table 4**. Older age was marginally associated with increased rates of FDG declines in frontal cortex ( $p=0.091$ ), though not in PCC.

None of the other clinical variables were associated with changes in FDG uptake. With and without accounting for baseline FDG uptake, lower MeDi adherence was associated with faster rates of FDG declines in PCC ( $p \leq 0.048$ ) and marginally in frontal cortex ( $p \leq 0.072$ ). None of the vascular variables were associated with FDG changes.

**Table 4.** Prediction of FDG-PET metabolic changes.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
<b><i>PCC</i></b>				
Mediterranean diet	0.010 (0.005) <sup>†</sup>	0.010 (0.005) <sup>*</sup>	0.010 (0.005) <sup>*</sup>	
Physical activity	-0.000 (0.001)	-0.000 (0.001)	-0.000 (0.001)	
Intellectual activity	0.005 (0.015)	0.004 (0.015)	0.004 (0.014)	
Plasma homocysteine	0.004 (0.004)	0.004 (0.003)		0.004 (0.004)
Plasma cholesterol	0.000 (0.000)	0.000 (0.000)		-0.000 (0.000)
Body mass index	-0.001 (0.001)	-0.001 (0.001)		-0.001 (0.001)
QUICKI scores	0.165 (0.480)	0.184 (0.438)		0.049 (0.459)
Hypertension	0.001 (0.013)	0.003 (0.011)		-0.000 (0.011)
PCC FDG uptake at baseline	0.573 (0.194) <sup>**</sup>	0.587 (0.183) <sup>**</sup>	0.566 (0.184) <sup>**</sup>	0.515 (0.184) <sup>**</sup>
Sex	-0.003 (0.010)			
APOE status	0.001 (0.008)			
Age	0.000 (0.001)			
Time to follow-up	-0.000 (0.000)			
Constant	1.154 (0.080) <sup>***</sup>	1.154 (0.083) <sup>***</sup>	1.182 (0.007) <sup>***</sup>	1.173 (0.076) <sup>***</sup>
<b><i>Frontal cortex</i></b>				

Mediterranean diet	-0.011 (0.007) <sup>†</sup>	-0.012 (0.007) <sup>†</sup>	-0.012 (0.007) <sup>†</sup>	
Physical activity	0.001 (0.001)	0.000 (0.001)	0.001 (0.001)	
Intellectual activity	0.010 (0.020)	0.008 (0.019)	0.005 (0.019)	
Plasma homocysteine	0.003 (0.005)	0.002 (0.005)		0.002 (0.005)
Plasma cholesterol	0.001 (0.000)	0.000 (0.000)		0.001 (0.000)
Body mass index	0.002 (0.002)	0.003 (0.002)		0.003 (0.002)
QUICKI scores	-0.116 (0.690)	-0.100 (0.669)		0.102 (0.668)
Hypertension	0.017 (0.018)	0.009 (0.015)		0.013 (0.015)
Frontal FDG uptake at baseline	0.202 (0.112) <sup>†</sup>	0.239 (0.107) <sup>*</sup>	0.196 (0.104) <sup>†</sup>	0.235 (0.101) <sup>*</sup>
Sex	0.007 (0.011)			
APOE status	-0.006 (0.012)			
Age	-0.001 (0.001) <sup>†</sup>			
Time to follow-up	-0.000 (0.000)			
Constant	1.209 (0.114) <sup>***</sup>	1.197 (0.111) <sup>***</sup>	1.174 (0.010) <sup>***</sup>	1.166 (0.110) <sup>***</sup>

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; <sup>†</sup>  $0.05 < p < 0.10$

See Legend to Table 2.

Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index; PCC, posterior cingulate cortex; FDG, <sup>18</sup>F-Fluorodeoxyglucose.

### Models for Prediction of MRI Changes

Baseline results are summarized in **eTable 3**. None of the clinical, lifestyle, and vascular risk variables were associated with baseline MRI measures.

Longitudinal results are summarized in **Table 5**. APOE4 status was positively, though not significantly, associated with faster rates of EC thickness reduction ( $p = 0.149$ ).

None of the other variables were associated with change in MRI measures.

**Table 5.** Prediction of MRI-based cortical thickness change.

	<b>Model 1<sup>a</sup></b>	<b>Model 2<sup>b</sup></b>	<b>Model 3<sup>c</sup></b>	<b>Model 4<sup>d</sup></b>
<b><i>Entorhinal cortex</i></b>				
Mediterranean diet	0.061 (0.042)	0.049 (0.042)	0.061 (0.040)	
Physical activity	-0.010 (0.008)	-0.009 (0.008)	-0.008 (0.008)	
Intellectual activity	-0.180 (0.112)	-0.110 (0.111)	-0.120 (0.106)	
Plasma homocysteine	0.012 (0.029)	0.012 (0.029)		0.006 (0.029)
Plasma cholesterol	-0.001 (0.002)	-0.002 (0.002)		-0.003 (0.002)
Body mass index	-0.000 (0.011)	0.001 (0.012)		0.003 (0.012)
QUICKI scores	3.542 (4.008)	2.844 (4.075)		1.726 (4.345)
Hypertension	-0.035 (0.093)	-0.072 (0.083)		-0.088 (0.085)
EC thickness at baseline	0.407 (0.167)*	0.382 (0.168)*	0.361 (0.162)*	0.400 (0.157)*
Sex	0.096 (0.079)			
APOE status	-0.092 (0.064)			
Age	-0.009 (0.008)			
Time to follow-up	-0.0007 (0.0002)			
Constant	2.710 (0.654)***	2.784 (0.667)***	3.303 (0.059)***	2.956 (0.710)***
<b><i>PCC</i></b>				
Mediterranean diet	0.003 (0.016)	0.002 (0.015)	0.002 (0.015)	
Physical activity	-0.002 (0.003)	-0.002 (0.003)	-0.001 (0.003)	
Intellectual activity	-0.034 (0.047)	-0.015 (0.045)	-0.019 (0.041)	
Plasma homocysteine	0.009 (0.012)	0.008 (0.011)		0.009 (0.010)
Plasma cholesterol	-0.000 (0.001)	-0.000 (0.001)		-0.000 (0.001)

Body mass index	-0.002 (0.005)	-0.001 (0.005)		-0.001 (0.005)
QUICKI scores	0.801 (1.643)	0.794 (1.577)		0.698 (1.581)
Hypertension	0.052 (0.038)	0.037 (0.033)		0.035 (0.033)
PCC thickness at baseline	0.390 (0.205) <sup>†</sup>	0.459 (0.209) <sup>*</sup>	0.508 (0.205) <sup>*</sup>	0.506 (0.182) <sup>**</sup>
Sex	0.012 (0.032)			
APOE status	-0.020 (0.028)			
Age	-0.003 (0.004)			
Time to follow-up	0.000 (0.000)			
Constant	2.321 (0.267) <sup>***</sup>	2.311 (0.260) <sup>***</sup>	2.418 (0.024) <sup>***</sup>	2.326 (0.260) <sup>***</sup>

<sup>\*</sup>*p*<0.05; <sup>\*\*</sup>*p*<0.01; <sup>\*\*\*</sup>*p*<0.001; <sup>†</sup>0.05<*p*<0.10

Values are presented as unstandardized beta coefficients (standard error). MRI models are also adjusted for total intracranial volume.

See Legend to Table 2.

Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index; PCC, posterior cingulate cortex; EC, entorhinal cortex.

DISCUSSION

The main findings from this study are as follows: 1) during middle age, MeDi adherence influences neurodegenerative changes detected via FDG-PET, but not amyloid or MRI changes; 2) baseline plasma homocysteine was the only predictor of decline in cognition; and 3) exercise and intellectual activity did not predict changes in AD biomarkers or cognition.

A previous longitudinal study investigated the effects of physical and intellectual activity on brain AD biomarker changes, and reported absent to minimal associations among non-demented elderly.<sup>11</sup> This study, however, did not examine the effects of diet or vascular risk factors. The present results confirm a lack of association between physical and intellectual activity and brain AD biomarker

changes in middle-aged participants and demonstrate that diet and vascular risk play a role instead.

MeDi adherence predicted changes in FDG-PET neurodegeneration biomarkers while accounting for possible risk factors such as age, sex, APOE status, vascular risks, and physical and intellectual activity. Lower MeDi adherence was associated with faster rates of FDG declines in PCC, an early site of cerebral glucose utilization declines in AD.<sup>29</sup>

Progressive PCC hypometabolism is a well-established finding in at-risk individuals,<sup>16</sup> <sup>31</sup> and accurately predicts decline from mild cognitive impairment to AD.<sup>29</sup> Our data suggests that the neuroprotective effects of the MeDi may lie in its ability to preserve brain metabolic activity, which may in turn help delay the onset of cognitive impairment.<sup>32</sup>

Increased plasma homocysteine, a well-known risk factor for AD,<sup>33 34</sup> predicted declines in cognition, and also showed borderline associations with increased rates of metabolic decline.

Altogether, these data suggest that diet and homocysteine-related vascular risk may influence brain aging and AD through different, yet to some extent interconnected pathways. Hence, adopting a healthy diet, particularly the MeDi, in combination with vascular risk management in midlife might be protective against future AD.

Neurodegenerative changes observed with FDG are believed to emerge downstream to A $\beta$  accumulation.<sup>35</sup> While we did not observe direct effects of lifestyle or vascular risk on A $\beta$  pathology, lower MeDi adherence was marginally, though non-significantly, associated with faster rates of A $\beta$  deposition. This suggests that the associations between lower MeDi adherence and increased metabolic declines may be related to emerging A $\beta$  plaque pathology and/or increasing soluble A $\beta$  (undetectable with PET).

A $\beta$  deposition in plaques is an age-dependent phenomenon, with 0% of cognitively normal individuals between 45-49 years old testing positive for A $\beta$ , and just under 6% between 50-59 years old testing positive for A $\beta$ .<sup>36</sup> Considering that all our

participants were cognitively normal and between 30-60 years of age, very few (if any) would have had substantial amyloid burden, making this cohort an ideal population for testing primary prevention strategies.

As with other studies in asymptomatic at-risk individuals,<sup>26 27 37</sup> imaging biomarkers were not associated with cognitive measures, most likely because our participants were all cognitively normal and younger than 60 years old at baseline. Previous studies have demonstrated that associations between brain biomarkers and cognition are evident in clinical AD patients, such as those with clear brain pathology, but not among normal populations.<sup>38</sup>

Among the limitations of our study is that lifestyle habits were self-reported, and as self-reported lifestyle questionnaires are vulnerable to error, this may have reduced our ability to detect additional associations of lifestyle factors and AD risk.

Since we did not find evidence for significant effects of physical activity and intellectual activity on biomarker change, we tested to see if we had adequate power to detect important associations (see **e-appendix**). Based on the sensitivity analysis, we had adequate power to detect associations of interest, indicating that null effects are not necessarily attributable to methodology or sample size concerns. Additionally, null effects in our cohort are consistent with findings from large-scale, community-based studies in the elderly.<sup>11 12</sup> Therefore, we offer that the strongest arguments of the study are the significant findings that manifest themselves despite the above limitations. That said, clinical trials are needed to test whether these lifestyle interventions may alter the rate of AD biomarkers and cognition. Recent clinical trials provided encouraging evidence that multi-modal lifestyle and vascular risk interventions improve cognition in the elderly.<sup>39</sup>

Additionally, we assumed linearity in the rate of change in biomarker and cognition, which is reasonable for short time frames as in this 3-year study, but possibly different for longer periods of observation. For instance, increasing pathological burden with age may cause an acceleration in cognitive decline and neurodegenerative biomarkers. Also, due to the relatively small sample, we did not

examine possible interactions between different biomarkers, which may be influenced by lifestyle and vascular risk.

Lastly, while our results are pertinent to healthy, middle-aged research participants without severe cardiac or cerebrovascular disease, results may differ in the elderly, in demented patients, and in those with vascular or metabolic disease. Studies with larger samples and longer follow-ups are needed to assess the generalizability of these findings in community-based populations with higher variability in socio-economic and medical status.

Our study has a number of strengths. While previous studies focused on non-demented elderly, including those with cognitive impairment, this longitudinal biomarker study focused on cognitively intact middle-aged individuals. There is consensus that lifestyle interventions have the highest chances of success when implemented well before old age,<sup>5</sup> making our results particularly relevant to efforts aimed at preventing AD.

The majority of previous studies looked at intellectual and physical activity, but not at diet. We demonstrated that diet does in fact influence the rate of change in metabolic AD biomarkers, whereas intellectual and physical activity do not appear to do so.

Lastly, our statistical model enabled us to simultaneously assess multiple lifestyle and vascular risk factors, yielding a more comprehensive understanding of the associations between these modifiable risk factors and AD risk. Furthermore, a combined reduction in several modifiable AD risk factors is projected to have a far greater impact than any one factor alone.<sup>4</sup>



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**Contributors**

MW, JS, CG, CQ, REA, DCM, and LM: analysis and interpretation. CQ, RSO, SV, MJDL, and LM: acquisition of data. RI and LM: study concept and design. All authors: critical revision of the manuscript for important intellectual content. LM: study supervision.

**Competing Interests**

The authors declare no disclosures.

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**Patient consent**

Obtained.

**Ethical approval**

This study was approved by NYU School of Medicine and Weill Cornell Medical College Institutional Review Board.

**Data sharing**

All relevant data have been included in the paper. Technical appendix, statistical code, and dataset will be made available on request.

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**eTable 1. Associations between regional PiB uptake, lifestyle, vascular risk and global cognition at baseline.**

	PiB-amyloid deposition		Lifestyle variables			Vascular risk-related measures						Cognition
	PCC	FC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hypertension	Global cognition
PCC	1	<b>.37**</b>	-.02	-.07	-.12	-.09	.08	-.13	.14	-.13	-.01	-.12
FC		1	-.04	.08	.14	-.25	-.00	.05	-.09	.08	.09	.06
MeDi adherence			1	.03	<b>.39**</b>	.08	-.17	-.27	.04	-.12	-.11	.16
Physical activity				1	.05	.12	-.07	.10	.17	.04	-.10	-.04
Intellectual activity					1	-.04	.02	-.02	-.16	.09	-.13	<b>.40**</b>
Plasma HCY						1	-.14	-.18	.07	-.04	-.05	.04
Plasma cholesterol							1	.22	-.06	-.15	<b>-.28*</b>	-.13
Hip-to-waist								1	<b>-.68***</b>	<b>.37*</b>	-.09	.22
Body Mass Index									1	<b>-.39**</b>	-.03	-.24
QUICKI scores										1	-.10	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001  
Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)

**eTable 2. Associations between regional FDG uptake, lifestyle, vascular risk and global cognition at baseline.**

	FDG metabolism		Lifestyle variables			Vascular risk-related measures						Cognition
	PCC	FC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hypertension	Global cognition
PCC	1	<b>.32**</b>	.14	-.05	-.14	-.06	.00	-.17	.01	-.08	.06	-.00
FC		1	.11	.12	<b>.27*</b>	-.14	-.04	.01	-.23	-.03	.03	-.06
MeDi adherence			1	.03	<b>.39**</b>	.08	-.17	-.27	.04	-.12	-.11	.16
Physical activity				1	.05	.12	-.07	.10	.17	.04	-.10	-.04
Intellectual activity					1	-.04	.02	-.02	-.16	.09	-.13	<b>.40**</b>
Plasma HCY						1	-.14	-.18	.07	-.04	-.05	.04
Plasma cholesterol							1	.22	-.06	-.15	<b>-.28*</b>	-.13
Hip-to-waist								1	<b>-.68***</b>	<b>.37*</b>	-.09	.22
Body Mass Index									1	<b>-.39**</b>	-.03	-.24
QUICKI scores										1	-.10	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001

Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)



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**eTable 3. Associations between MRI cortical thickness, lifestyle, vascular risk and global cognition at baseline.**

	MRI thickness		Lifestyle variables			Vascular risk-related measures						Cognition
	EC	PCC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hyper-tension	Global cognition
EC	1	<b>.36**</b>	.00	-.33	-.02	-.20	.00	.11	-.06	-.11	.16	.05
PCC		1	-.05	-.31	-.16	-.08	-.11	.08	-.12	-.06	.23	.11
MeDi adherence			1	.02	<b>.37**</b>	.15	-.17	-.27	.12	-.14	-.14	.15
Physical activity				1	-.01	.11	-.06	.06	.22	.00	-.11	-.02
Intellectual activity					1	-.03	.05	-.07	-.08	.05	-.18	<b>.41**</b>
Plasma HCY						1	-.14	-.22	.02	-.06	-.04	.06
Plasma cholesterol							1	.27	-.11	-.12	<b>-.27*</b>	-.13
Hip-to-waist								1	<b>-.69***</b>	.34	-.10	.23
Body Mass Index									1	<b>-.38**</b>	.02	-.23
QUICKI scores										1	-.03	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001  
Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)

## eAppendix

### Sensitivity analysis: power calculations

Since we generally did not find significant associations of physical activity and intellectual activity with biomarker values, we wanted to ensure that our null findings were not primarily attributable to our sample size. To do so, we utilized a series of simulations to estimate minimum sample sizes required to detect our observed effect sizes at an alpha level of 0.05 (two-tailed).

Holding the observed variability constant as well as the influence of all other variables in our full model, we found that physical activity only predicted .12% of unique variance in FDG changes overtime. This corresponded to an  $f^2$  effect size of 0.0012. Based on the observed effect size, we estimated that 6,535 participants would be needed to obtain 80% power to detect significant associations between physical activity and FDG changes.

Additionally, with the same model specifications described above, we found that intellectual activity only accounted for .09% of unique variance in FDG changes overtime. This corresponded to an  $f^2$  effect size of 0.0009. Based on the observed effect size, we estimated that 8,716 participants would be needed to obtain 80% power to detect significant associations between intellectual activity and FDG changes over 3 years.

In contrast to these results, MeDi scores uniquely predicted 8.83% of the variance in FDG changes over time. This corresponded to an  $f^2$  effect size of 0.097. Based on the observed effect size, we estimated that as few as 84 participants are needed to obtain 80% power to detect significant associations between MeDi adherence and FDG changes over 3 years.

Given the small magnitude of the observed effect sizes of physical and intellectual activity, as well as the unrealistically large sample sizes required to obtain satisfactory power of detecting significant differences, we conclude that our null results are not attributable to sample size concerns. Lack of associations between physical activity, intellectual activity, and AD biomarker changes are also consistent with previous studies in the elderly.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
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	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6-7
		(b) Describe any methods used to examine subgroups and interactions	6-7
		(c) Explain how missing data were addressed	6-7
		(d) If applicable, explain how loss to follow-up was addressed	6-7
		(e) Describe any sensitivity analyses	6-7
Results			6-7

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	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8-9
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	9-14
		(b) Report category boundaries when continuous variables were categorized	9-14
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9-14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15, eappendix, etables
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	14-15
<b>Limitations</b>			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
<b>Other information</b>			
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	17

\*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](http://www.strobe-statement.org).

# BMJ Open

## Associations of lifestyle and vascular risk factors with Alzheimer's brain biomarker changes during middle age: a 3-year longitudinal study in the broader New York City area

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Manuscripts

**Associations of lifestyle and vascular risk factors with Alzheimer’s brain biomarker changes during middle age: a 3-year longitudinal study in the broader New York City area**

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## Abstract

**Objective:** To investigate the associations between lifestyle and vascular risk factors and changes in Alzheimer’s disease (AD) biomarkers (beta-amyloid load via <sup>11</sup>C-PiB PET, glucose metabolism via <sup>18</sup>F-FDG PET, and neurodegeneration via structural MRI) and global cognition in middle-aged asymptomatic participants at risk for AD.

**Design:** Prospective, longitudinal.

**Setting:** The study was conducted at New York University Langone/Weill Cornell Medical Centers in New York City.

**Participants:** Seventy cognitively normal participants from multiple community sources, aged 30-60 years with lifestyle measures (diet, intellectual activity, and physical activity), vascular risk measures, and two imaging biomarkers visits over at least two years, were included in the study.

**Outcome measures:** We examined MRI-based cortical thickness, FDG glucose metabolism, and PiB beta-amyloid in AD-vulnerable regions. A global cognitive z-score served as our summary cognition measure. We used regression change models to investigate the associations of clinical, lifestyle, and vascular risk measures with changes in AD biomarkers and global cognition.

**Results:** Diet influenced changes in glucose metabolism, but not amyloid or cortical thickness changes. With and without accounting for demographic measures, vascular risk, and baseline FDG measures, lower adherence to a Mediterranean-style diet was associated with faster rates of FDG decline in the posterior cingulate cortex ( $p\leq0.05$ ) and marginally in the frontal cortex ( $p=0.07$ ). None of the other lifestyle variables or vascular measures showed associations with AD biomarker changes. Higher baseline plasma homocysteine was associated with faster rates of decline in global cognition, with and without accounting for lifestyle and biomarker measures ( $p=0.048$ ). None of the lifestyle variables were associated with cognition.

**Conclusions:** Diet influenced brain glucose metabolism in middle-aged participants, while plasma homocysteine explained variability in cognitive performance. These



findings suggest that these modifiable risk factors affect AD risk through different pathways and support further investigation of risk reduction strategies in midlife.

### Strengths and limitations of this study

- A key strength of this study is the availability of healthy, cognitively normal middle-aged individuals with multiple lifestyle and vascular risk measures and neuroimaging scans at least two years apart.
- Another strength of our study was our ability to look at diet, intellectual and physical activity, and vascular risk factors and our statistical model enabled us to assess these risk factors simultaneously.
- Given the participants were cognitively normal and between ages 30-60, it is unlikely that we would see substantial changes in amyloid burden, an age-dependent phenomenon, and associated cognitive changes without lifestyle enrichment strategies this early in life.
- We caution that we carefully screened the study participants to include healthy individuals without severe cardiac or cerebrovascular disease, which limits the generalizability of our results to the entire population.

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## INTRODUCTION

Unless effective strategies for prevention are found, the prevalence of Alzheimer’s disease (AD), the most common form of dementia that affects nearly 34 million people worldwide, is expected to triple by 2050.<sup>1</sup>

Leading a healthy lifestyle in combination with strategies to reduce vascular disease risk is increasingly viewed as preventative against cognitive decline and dementia.<sup>2</sup> Findings from population-attributable risk models estimate that one in every three AD cases may be accounted for by modifiable risk factors, such as midlife hypertension, obesity, diabetes, and several lifestyle factors.<sup>3 4</sup>

Most studies in this field have focused on the effects of lifestyle and cardiovascular factors on cognitive decline or incidence of dementia as outcome measures.<sup>5</sup> However, there is evidence that AD pathophysiology develops up to 20 years upstream of cognitive symptoms,<sup>6 7</sup> namely in midlife. As such, studies investigating biological markers of AD are needed to determine whether lifestyle and vascular risk impact the emergence and progression of brain AD-endophenotype.

Studies involving older adults, with or without mild cognitive impairment, have found both positive and null associations between intellectual and physical activity and AD biomarkers.<sup>8-14</sup> The mixed results of these studies may be due to not taking diet or vascular risk into account, and focusing on elderly populations. Of those that included cognitively normal, middle-aged individuals, physical activity has been shown to attenuate age-related brain biomarker changes,<sup>15</sup> and insulin resistance has been positively associated with cerebral hypometabolism, atrophy, and amyloid deposition<sup>16-18</sup> and negatively related to regional cerebral blood flow.<sup>19</sup> We observed that adherence to a Mediterranean diet (MeDi) was positively associated with MRI-based cortical thickness in middle-aged individuals at cross-section, after accounting for intellectual activity, physical activity, and vascular risk measures.<sup>20</sup>

Herein, we present a 3-year multi-modality brain imaging study aimed at assessing the association of multiple lifestyle and vascular risk factors with change in AD biomarkers, as measured by amyloid-beta (Aβ) deposition on <sup>11</sup>C-PiB PET, glucose

metabolism via  $^{18}\text{F}$ -FDG PET, and neurodegeneration via MRI, in a cohort of cognitively normal, middle-aged individuals at risk for AD.

## METHODS

### Participants

Study participants were recruited from a longitudinal brain imaging study conducted at New York University (NYU) Langone School of Medicine and Weill Cornell Medical College (WCMC) between 2010-2016. The study aimed to examine risk factors for AD among clinically and cognitively normal middle-aged adults. Details about the study design have previously been published.<sup>21 22</sup> Briefly, participants were derived from multiple community sources, including individuals interested in research participation and family members and caregivers of impaired patients.

All participants received clinical, laboratory, neuropsychological, and brain imaging exams including MRI and FDG- and PiB-PET at baseline and at least two years later. To be included in this study, participants had to be 30-60 years old at baseline, with education  $\geq 12$  years, Clinical Dementia Rating = 0, Global Deterioration Scale  $\leq 2$ , Mini Mental State Examination  $\geq 27$ , Hamilton depression scale  $< 16$ , and normal cognitive test performance for age and education.<sup>23</sup> Those with past or current medical conditions that can affect brain structure or function (i.e., cardiovascular disease, stroke, diabetes, head trauma, any neurodegenerative diseases, depression, hydrocephalus, intracranial mass and infarcts on MRI) and those taking psychoactive medications were excluded.

A family history of AD that included at least one first degree relative whose AD onset was after age 60 was elicited using standardized questionnaires.<sup>22</sup>

Apolipoprotein E (APOE) genotypes were determined using standard qPCR procedures.<sup>22</sup> Participants were grouped as positive versus negative family history, and as APOE4 carriers versus non-carriers. Participants with a family history of AD and/or APOE4 positivity were considered at increased risk for AD.

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**Standard protocol approvals, registrations, and patient consents**

All participants provided informed consent to participate in this NYU School of Medicine/WCMC IRB-approved study.

**Patient and Public Involvement**

Patients and or public were not involved in the planning or execution of the study. Results were not disseminated to study participants

**Global cognition measure**

The neuropsychological battery of tests was previously described.<sup>23</sup> At both time points, we assessed three cognitive domains from the following tests: memory (immediate and delayed recall of a paragraph and immediate and delayed recall of paired associates), executive function (Wechsler Adult Intelligence Scale [WAIS] digit symbol substitution), and language (WAIS vocabulary).

We computed a global cognitive summary score by first z-scoring each measure within each domain and averaging each component of the three domains listed above. Global cognition, calculated as the average of the composite memory, executive function, and language variables at each time point, was used as an outcome variable.

**Vascular risk-related measures**

Vascular risk factors included in the model were: a) body mass index (BMI); b) presence of hypertension, conservatively based on either current antihypertensive treatment or blood pressure assessments; c) plasma cholesterol and/or homocysteine, obtained after overnight fasting using standard laboratory procedures; and d) insulin resistance, measured with the Quantitative Insulin Sensitivity Check Index (QUICKI)<sup>24</sup> derived from fasting plasma insulin (via enzyme-

linked immunosorbent assay kit) and fasting plasma glucose, where lower QUICKI scores reflect greater insulin resistance.

### Lifestyle variables

Dietary data regarding average food consumption over the prior year were obtained using the Harvard Willett semi-quantitative food frequency questionnaire.<sup>20 25</sup> Briefly, 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 food group items. For the construction of MeDi scores, we first regressed caloric intake and calculated the derived residuals of daily intake for each of the following categories: dairy, meat, fruits, vegetables, legumes, cereals, and fish. Individuals were assigned a value of one for each beneficial component (fruits, vegetables, legumes, cereals, and fish) whose consumption was at or above the sex-specific median; a value of one for each harmful component (meat and dairy products) whose consumption was below the median; a value of one for a ratio of monounsaturated fats to saturated fats above the median; and a value of one for mild to moderate alcohol consumption. These values were summed to generate a MeDi score, with a higher score indicating higher MeDi adherence.

The Minnesota Leisure Time Physical Activity questionnaire was used to estimate physical activity.<sup>26</sup> For each activity, information was collected on the frequency and duration of engagement, which was multiplied by an activity-specific intensity code indicating calorie expenditure. The activity-dependent scores were summed to obtain the overall intensity of physical activity per person during the last 12 months and converted to metabolic equivalents.

Intellectual activity throughout life was assessed using a validated 25-item interview in which participants were asked to report how often they engaged in common cognitively demanding activities with minimal dependence on socioeconomic statuses, such as reading books or newspapers, writing letters or e-mails, going to the library, and playing games, at different ages.<sup>14 27</sup> Previous studies have described

this instrument in detail and reported high internal consistency and positive associations of intellectual activity with educational and cognitive performance.<sup>27</sup>

AD biomarkers

All subjects received MRI, PiB-PET and FDG-PET scans at least two years apart following standardized procedures.<sup>21 28 29</sup>

Participants received 3T volumetric T1-MPRAGE scans at both time points. Freesurfer v. 5.3 with a longitudinal processing pipeline was used to obtain entorhinal (EC) and posterior cingulate cortex (PCC) thickness on longitudinal MRI scans.<sup>30</sup> These ROIs were chosen based on previous reports of AD- and lifestyle-related changes at the preclinical AD stages.<sup>20 31</sup> Total intracranial volumes (TIV) were also estimated and used as a covariate.

PET images were acquired with PET/CT scanners operating in 3D mode and analyzed using a fully automated image-processing pipeline.<sup>32 33</sup> Statistics on image voxel values were extracted from automatically labeled cortical regions of interest using the automated anatomic labeling atlas.<sup>34</sup> We selected PCC/precuneus as the target AD-related region of interest and frontal cortex (including prefrontal and medial frontal regions) as the target aging-related region.<sup>35</sup> For each region of interest, PiB uptake was divided by cerebellar gray matter uptake, and FDG uptake was normalized by the global activity.

Statistical analysis

Statistical analyses were performed using Stata, version 13. We conducted two types of analyses to examine the associations of age, sex, APOE4 genotype, lifestyle variables (diet, intellectual activity, and physical activity), and vascular risk variables (BMI, plasma cholesterol, plasma homocysteine, hypertension, and insulin resistance) with AD biomarkers and cognition.

The first analysis consisted of a partial correlation analysis to evaluate the direct associations between the predictors and dependent variables at baseline and over time. We estimated these associations using partial Pearson correlations ( $r_s$ ) and adjusted for the effects of age, gender, and APOE status. The PIB variables had skewed distributions and were log transformed, and MRI measures were adjusted by TIV.

In the second analysis, we used regressed change models<sup>36</sup> to evaluate lifestyle and vascular measures as predictors of change in biomarker values and global cognition. Regressed change models, as opposed to difference score models, overcome several of the disadvantages of difference score measures.<sup>36</sup> For example, difference scores are often negatively correlated with baseline values and are doubly affected by unreliability and missing data points in the measures.

We considered continuous variables including age (years), education (years), diet, exercise, and intellectual activity scores, BMI, QUICKI scores, and lab measures. Sex (female versus male) and APOE genotype (presence of either 1 or 2 versus absence of  $\epsilon 4$  alleles) were used as dichotomous variables with female sex and APOE4 positivity as the reference. Presence or absence of hypertension was used as a dichotomous variable with the absence of the condition used as the reference.

In each model, we predicted regressed change in each outcome measure by running a series of regression models that isolated the effect of our predictors on the outcome measure at follow-up while holding the baseline measures constant. Additionally, the baseline biomarker measures were examined as predictors of change in cognition in addition to the other predictors. For each model, we used a backward elimination procedure to remove non-significant covariates and form the most parsimonious model.

We fit four separate models for each outcome measure:

- Model 1: full model with all predictor variables included;
- Model 2: full model with non-significant adjustment variables removed;



- Model 3: reduced model with lifestyle predictors only, and non-significant adjustment variables removed;
- Model 4: reduced model with vascular predictors only, and non-significant adjustment variables removed.

The resulting unstandardized beta coefficients ( $\beta_s$ ) can be interpreted as partial correlations. All results were considered significant at  $p<0.05$ .

## RESULTS

### Participants

A total of 86 participants completed baseline imaging evaluations. Six participants did not complete the follow-up evaluations. Of the remaining 80 participants, 10 had incomplete lifestyle questionnaires and were excluded. The remaining 70 participants were examined in this study.

Participants' characteristics are shown in **Table 1**. The average age was 49 years, ranging from 30-60 years, with 69% of participants being women. While none of the participants had cognitive impairment, 69% reported subjective memory complaints, as determined using the Global Deterioration Scale.<sup>37</sup> Sixty-seven percent of participants reported a family history of AD and 39% had at least one copy of the APOE4 allele.

**Table 1.** Clinical and demographic characteristic at baseline.

Sample size (n)	70
Age (years)	49 (8), range 30-60
Female, No. (%)	48 (69)
Education (years)	16 (2)
Caucasians, No. (%)	56 (80)



Positive family history of AD, No. (%)	47 (67)
APOE4 carriers, No. (%)	27 (39)
Positive subjective complaints, No. (%)	48 (69)
Time to follow-up (years)	3 (1), range 2-3.5
<i>Lifestyle measures</i>	
Mediterranean diet scores	4 (2), range 1-9
Physical activity scores	9 (5), range 1-37
Intellectual activity scores	4 (1), range 2-5
<i>Vascular risk measures</i>	
Body mass index (kg/m <sup>2</sup> )	25 (4)
Presence of hypertension, No. (%)	10 (14)
QUICKI scores	0.32 (0.03)
Plasma cholesterol/HDL ratio	3.3 (0.8)
Plasma homocysteine (micromol/L)	7.9 (6.2)
<i>Neuropsychological measures</i>	
Mini Mental State Examination	29 (1)
Paragraph Recall, Immediate	7.2 (2.5)
Paragraph Recall, Delayed	9.8 (2.8)
Paired Associates Recall, Immediate	6.4 (2.5)
Paired Associates Recall, Delayed	7.3 (2.6)
Object naming	55 (9)
Design test	8.1 (2.3)
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WAIS Vocabulary	68 (8)
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Values are presented as mean (SD), unless otherwise specified.

Abbreviations: AD, Alzheimer’s disease; QUICKI, Quantitative Insulin Sensitivity Check Index; WAIS, Wechsler Adult Intelligence Scale.

Models for Prediction of Cognitive Changes

None of the adjustment variables were associated with cognition at baseline or longitudinally. At baseline, higher intellectual activity was associated with better cognition, with and without adjusting for age, sex, and APOE status ( $r_s \geq 0.401$ ,  $p < 0.01$ ; **eTables 1-3**).

Longitudinal results are summarized in **Table 2**. After accounting for baseline cognition, higher baseline plasma homocysteine was associated with faster rates of decline in global cognition scores ( $p = 0.048$ ). None of the lifestyle variables were directly associated with cognitive changes.

The baseline biomarkers did not predict cognitive changes, except for a negative non-significant association between baseline FDG uptake in the frontal cortex and faster rates of declines in global cognition ( $p = 0.11$ ). Therefore, including the baseline biomarkers in the models did not significantly shift the relationships between cognition and the lifestyle or vascular variables.

Table 2. Prediction of changes in global cognition.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
Mediterranean diet	-0.012 (0.046)	-0.013 (0.048)	-0.029 (0.046)	
Physical activity	0.001 (0.008)	-0.001 (0.008)	-0.004 (0.008)	
Intellectual activity	0.089 (0.128)	0.044 (0.128)	0.103 (0.128)	
Plasma homocysteine	-0.068 (0.034) p=0.061	-0.063 (0.032) p=0.066		-0.066 (0.032) p=0.048
Plasma cholesterol	0.001 (0.002)	0.001 (0.002)		0.001 (0.002)

Body mass index	0.007 (0.014)	0.006 (0.014)		0.006 (0.014)
QUICKI scores	0.343 (3.965)	0.537 (3.813)		0.798 (3.837)
Hypertension	-0.080 (0.117)	-0.056 (0.118)		-0.055 (0.117)
Global cognition at baseline	0.872 (0.132) p<0.001	0.827 (0.143) p<0.001	0.801 (0.146) p<0.001	0.842 (0.132) p<0.001
Sex	-0.023 (0.078)			
APOE status	0.104 (0.079)			
Age	0.006 (0.012)			
Time to follow-up	-0.000 (0.000)			
Constant	-0.097 (0.654)	-0.125 (0.620)	0.005 (0.068)	-0.167 (0.621)

Values are presented as unstandardized beta coefficients (standard error). Only significant and marginally significant p values are reported in the table.

<sup>a</sup>Model 1: full model with all variables examined

<sup>b</sup>Model 2: full model without non-significant adjustment variables

<sup>c</sup>Model 3: model with lifestyle variables only, and without non-significant adjustment variables

<sup>d</sup>Model 4: model with vascular variables only, and without non-significant adjustment variables

Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index.

### Models for Prediction of Amyloid Accumulation

Baseline results are summarized in **eTable 1**. None of the clinical, lifestyle, or vascular risk variables were associated with baseline PiB uptake.

Longitudinal results are summarized in **Table 3**. APOE4 status was marginally positively associated with faster rates of amyloid deposition in the frontal cortex (p=0.084). None of the other vascular variables were associated with changes in amyloid measures.

Physical activity was marginally negatively associated with faster rates of amyloid changes in the PCC ( $p \leq .106$ ), while MeDi adherence was marginally negatively associated with amyloid changes in the frontal cortex in models accounting for lifestyle and vascular factors ( $p \leq .104$ ).

**Table 3.** Prediction of PiB-PET amyloid deposition.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
<b>PCC</b>				
Mediterranean diet	-0.017 (0.016)	-0.018 (0.017)	-0.011 (0.016)	
Physical activity	-0.005 (0.003) p=0.106	-0.005 (0.003)	-0.005 (0.003) p=0.096	
Intellectual activity	0.053 (0.053)	0.021 (0.052)	0.016 (0.051)	
Plasma homocysteine	-0.013 (0.012)	-0.006 (0.012)		-0.010 (0.012)
Plasma cholesterol	-0.002 (0.001)	-0.001 (0.001)		-0.001 (0.001)
Body mass index	-0.004 (0.005)	-0.005 (0.005)		-0.006 (0.005)
QUICKI scores	-1.720 (1.550)	-2.155 (1.581)		-2.016 (1.575)
Hypertension	-0.048 (0.041)	-0.026 (0.037)		-0.019 (0.036)
PCC PiB uptake at baseline	0.094 (0.238)	0.194 (0.259)	0.156 (0.261)	0.232 (0.252)
Sex	0.020 (0.031)			
APOE status	0.042 (0.029)			
Age	0.007 (0.004)			
Time to follow-up	0.000 (0.000)			
Constant	0.394 (0.256)	0.465 (0.264) p=0.088	0.126 (0.028) p<0.001	0.447 (0.263) p=0.090
<b>Frontal cortex</b>				
Mediterranean diet	-0.016 (0.011) p=0.104	-0.018 (0.011) p=0.102	-0.011 (0.011)	
Physical activity	-0.000 (0.002)	-0.001 (0.002)	-0.001 (0.002)	

Intellectual activity	0.050 (0.036)	0.031 (0.036)	0.019 (0.036)	
Plasma homocysteine	-0.001 (0.008)	-0.001 (0.008)		-0.002 (0.008)
Plasma cholesterol	-0.001 (0.001)	-0.001 (0.001)		-0.001 (0.001)
Body mass index	-0.001 (0.003)	-0.001 (0.003)		-0.001 (0.003)
QUICKI scores	-1.293 (1.008)	-1.497 (1.012)		-1.184 (1.001)
Hypertension	-0.005 (0.029)	-0.005 (0.026)		0.001 (0.025)
Frontal PiB uptake at baseline	0.000 (0.143)	0.003 (0.145)	0.001 (0.144)	0.026 (0.140)
Sex	0.017 (0.019)			
APOE status	0.033 (0.019) p=0.084			
Age	0.000 (0.002)			
Time to follow-up	-0.000 (0.000)			
Constant	0.234 (0.167)	0.256 (0.168)	0.010 (0.018) p<0.001	0.208 (0.166)

See Legend to Table 2. Only significant and marginally significant p values are reported in the table.

Abbreviations: PCC, posterior cingulate cortex.

### Models for Prediction of FDG Changes

Baseline results are summarized in **eTable 2**. Among the lifestyle variables, frontal FDG uptake was positively associated with intellectual activity ( $r_s=0.27$ ,  $p=0.042$ ). None of the vascular variables showed associations with baseline FDG uptake.

Longitudinal results are summarized in **Table 4**. Older age was marginally associated with increased rates of FDG declines in the frontal cortex ( $p=0.091$ ), though not in the PCC. None of the other clinical variables were associated with changes in FDG uptake. With and without accounting for baseline FDG uptake, lower MeDi adherence was associated with faster rates of FDG declines in the PCC ( $p\leq 0.048$ ) and

marginally in the frontal cortex ( $p \leq 0.106$ ). None of the vascular variables were associated with FDG changes.

**Table 4.** Prediction of FDG-PET metabolic changes.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
<b>PCC</b>				
Mediterranean diet	0.010 (0.005) p=0.050	0.010 (0.005) p=0.048	0.010 (0.005) p=0.043	
Physical activity	-0.000 (0.001)	-0.000 (0.001)	-0.000 (0.001)	
Intellectual activity	0.005 (0.015)	0.004 (0.015)	0.004 (0.014)	
Plasma homocysteine	0.004 (0.004)	0.004 (0.003)		0.004 (0.004)
Plasma cholesterol	0.000 (0.000)	0.000 (0.000)		-0.000 (0.000)
Body mass index	-0.001 (0.001)	-0.001 (0.001)		-0.001 (0.001)
QUICKI scores	0.165 (0.480)	0.184 (0.438)		0.049 (0.459)
Hypertension	0.001 (0.013)	0.003 (0.011)		-0.000 (0.011)
PCC FDG uptake at baseline	0.573 (0.194) p=0.006	0.587 (0.183) p=0.002	0.566 (0.184) p=0.003	0.515 (0.184) p=0.004
Sex	-0.003 (0.010)			
APOE status	0.001 (0.008)			
Age	0.000 (0.001)			
Time to follow-up	-0.000 (0.000)			
Constant	1.154 (0.080) p<0.001	1.154 (0.083) p<0.001	1.182 (0.007) p<0.001	1.173 (0.076) p<0.001
<b>Frontal cortex</b>				
Mediterranean diet	-0.011 (0.007)	-0.012 (0.007) p=0.106	-0.012 (0.007) p=0.072	
Physical activity	0.001 (0.001)	0.000 (0.001)	0.001 (0.001)	
Intellectual activity	0.010 (0.020)	0.008 (0.019)	0.005 (0.019)	
Plasma homocysteine	0.003 (0.005)	0.002 (0.005)		0.002 (0.005)
Plasma cholesterol	0.001 (0.000)	0.000 (0.000)		0.001 (0.000)

Body mass index	0.002 (0.002)	0.003 (0.002)		0.003 (0.002)
QUICKI scores	-0.116 (0.690)	-0.100 (0.669)		0.102 (0.668)
Hypertension	0.017 (0.018)	0.009 (0.015)		0.013 (0.015)
Frontal FDG uptake at baseline	0.202 (0.112) p=0.093	0.239 (0.107) p=0.040	0.196 (0.104) p=0.085	0.235 (0.101) p=0.036
Sex	0.007 (0.011)			
APOE status	-0.006 (0.012)			
Age	-0.001 (0.001) p=0.091			
Time to follow-up	-0.000 (0.000)			
Constant	1.209 (0.114) p<0.001	1.197 (0.111) p<0.001	1.174 (0.010) p<0.001	1.166 (0.110) p<0.001

See Legend to Table 2. Only significant and marginally significant p values are reported in the table.

### Models for Prediction of MRI Changes

Baseline results are summarized in **eTable 3**. None of the clinical, lifestyle, and vascular risk variables were associated with baseline MRI measures.

Longitudinal results are summarized in **Table 5**. APOE4 status showed a positive, non-significant association with faster EC thickness reduction (p=0.149). None of the other variables were associated with changes in MRI measures.

**Table 5.** Prediction of MRI-based cortical thickness change.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
<b>Entorhinal cortex</b>				
Mediterranean diet	0.061 (0.042)	0.049 (0.042)	0.061 (0.040)	
Physical activity	-0.010 (0.008)	-0.009 (0.008)	-0.008 (0.008)	
Intellectual activity	-0.180 (0.112)	-0.110 (0.111)	-0.120 (0.106)	

Plasma homocysteine	0.012 (0.029)	0.012 (0.029)		0.006 (0.029)
Plasma cholesterol	-0.001 (0.002)	-0.002 (0.002)		-0.003 (0.002)
Body mass index	-0.000 (0.011)	0.001 (0.012)		0.003 (0.012)
QUICKI scores	3.542 (4.008)	2.844 (4.075)		1.726 (4.345)
Hypertension	-0.035 (0.093)	-0.072 (0.083)		-0.088 (0.085)
EC thickness at baseline	0.407 (0.167) p=0.018	0.382 (0.168) p=0.025	0.361 (0.162) p=0.031	0.400 (0.157) p=0.012
Sex	0.096 (0.079)			
APOE status	-0.092 (0.064)			
Age	-0.009 (0.008)			
Time to follow-up	-0.0007 (0.0002)			
Constant	2.710 (0.654) p=0.001	2.784 (0.667) p=0.001	3.303 (0.059) p<0.001	2.956 (0.710) p<0.001
<b>PCC</b>				
Mediterranean diet	0.003 (0.016)	0.002 (0.015)	0.002 (0.015)	
Physical activity	-0.002 (0.003)	-0.002 (0.003)	-0.001 (0.003)	
Intellectual activity	-0.034 (0.047)	-0.015 (0.045)	-0.019 (0.041)	
Plasma homocysteine	0.009 (0.012)	0.008 (0.011)		0.009 (0.010)
Plasma cholesterol	-0.000 (0.001)	-0.000 (0.001)		-0.000 (0.001)
Body mass index	-0.002 (0.005)	-0.001 (0.005)		-0.001 (0.005)
QUICKI scores	0.801 (1.643)	0.794 (1.577)		0.698 (1.581)
Hypertension	0.052 (0.038)	0.037 (0.033)		0.035 (0.033)
PCC thickness at baseline	0.390 (0.205) p=0.099	0.459 (0.209) p=0.050	0.508 (0.205) p=0.023	0.506 (0.182) p=0.011
Sex	0.012 (0.032)			
APOE status	-0.020 (0.028)			
Age	-0.003 (0.004)			
Time to follow-up	0.000 (0.000)			



Constant	2.321 (0.267) p<0.001	2.311 (0.260) p<0.001	2.418 (0.024) p<0.001	2.326 (0.260) p<0.001
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See Legend to Table 2. Only significant and marginally significant p values are reported in the table. All MRI models are adjusted for total intracranial volume (data not shown).

## DISCUSSION

The main findings of this study are as follows: 1) during middle age, MeDi adherence predicts changes in glucose metabolism detected via FDG-PET, but not changes in amyloid deposition or cortical thickness, 2) baseline plasma homocysteine was the only predictor of cognitive changes, and 3) exercise and intellectual activity did not predict changes in AD biomarkers or cognition. As such, the present findings do not support an association between physical or intellectual activity and brain AD biomarker changes in middle-aged participants, but do contribute support to diet and vascular risk factors playing a role instead.

MeDi adherence predicted changes in FDG-PET hypometabolic biomarkers while accounting for possible risk factors such as age, sex, APOE status, vascular measures, and physical and intellectual activity. Lower MeDi adherence was associated with faster rates of FDG declines in the PCC, an early site of cerebral glucose utilization decline in AD.<sup>35</sup> Progressive PCC hypometabolism is a well-established finding in at-risk individuals,<sup>21 38</sup> and accurately predicts the decline from mild cognitive impairment to AD.<sup>35</sup> Our data suggest that the neuroprotective effects of the MeDi may lie in its ability to preserve brain metabolic activity, which may in turn help delay the onset of cognitive impairment.<sup>39</sup> Additionally, higher plasma homocysteine, a well-known risk factor for AD,<sup>40 41</sup> predicted declines in cognition and also showed borderline associations with increased rates of metabolic decline.

Altogether, these data suggest that diet and homocysteine-related vascular risk may influence brain aging and AD through different, yet to some extent interconnected pathways. Hence, adopting a healthy diet, particularly the MeDi, in combination with vascular risk management in midlife might be protective against future AD.

Hypometabolic changes observed with FDG are believed to emerge downstream to A $\beta$  accumulation.<sup>31</sup> While we did not find direct effects of lifestyle or vascular risk factors on A $\beta$  pathology, lower MeDi adherence was marginally, though non-significantly, associated with faster rates of A $\beta$  deposition. This suggests that the associations between lower MeDi adherence and increased metabolic declines may be related to emerging A $\beta$  plaque pathology and/or increasing soluble A $\beta$  (which is undetectable with PET).

Further, A $\beta$  deposition in plaques is an age-dependent phenomenon, with 0% of cognitively normal individuals between 45-49 years old testing positive for A $\beta$ , and just under 6% between 50-59 years old testing positive for A $\beta$ .<sup>42</sup> Considering that all our participants were cognitively normal and between 30-60 years of age, very few (if any) would have had substantial amyloid burden, making this cohort an ideal population for testing primary prevention strategies.

As with other studies in asymptomatic at-risk individuals,<sup>32 33 43</sup> imaging biomarkers were not associated with cognitive measures, most likely because our participants were all cognitively normal and younger than 60 years old at baseline. Previous studies have demonstrated that associations between brain biomarkers and cognition are evident in clinical AD patients, such as those with clear brain pathology, but not among normal populations.<sup>44</sup> A longer follow-up duration may allow for additional pathological and cognitive changes to manifest. We performed an additional sensitivity analysis to test for associations between biomarkers and domain-specific changes in memory, attention, and language, which left our conclusions substantially unchanged (see **e-appendix**).

We did not find a significant statistical relationship between intellectual and physical activity and biomarker changes. There is mixed evidence for the role of physical activity on brain aging. A recent randomized controlled trial showed that aerobic exercise interventions resulted in improved cardiorespiratory fitness, which in turn improved memory and reduced brain atrophy in the elderly.<sup>45</sup> Animal models have also suggested that physical activity has the potential to alleviate tau hyperphosphorylation.<sup>46</sup> However, a previous longitudinal study in non-demented

elderly reported absent to minimal associations between physical and intellectual activity and brain AD biomarker changes.<sup>8</sup> In our study, physical activity was only marginally negatively associated with PCC amyloid deposition. Since we did not find significant relationships between intellectual and physical activity and biomarker changes, we tested to see if we had adequate power to detect these associations (see **e-appendix**). Based on the sensitivity analysis, we had adequate power to detect associations of interest, indicating that the null results are not necessarily attributable to methodology or sample size concerns.

Additionally, the null results in our cohort are consistent with findings from large-scale, community-based studies in the elderly.<sup>8 9</sup> Therefore, we offer that the strongest arguments of the study are the significant findings that manifest themselves despite the above limitations. That said, clinical trials are needed to test whether these lifestyle interventions may alter the rate of change in AD biomarkers and cognition. Recent clinical trials provided encouraging evidence that multi-modal lifestyle and vascular risk interventions improve cognition in the elderly.<sup>47</sup>

Worth noting is that we assumed linearity in the rate of change in biomarkers and cognition, which is reasonable for short time frames, as in this 3-year study, but possibly different for more extended periods of observation. For instance, increasing pathological burden with age may cause acceleration in cognitive and biomarker changes. Also, due to the relatively small sample size, we did not examine interactions between the different biomarkers, which may be influenced by lifestyle and vascular risk factors.

We caution that lifestyle habits were self-reported, and as self-reported lifestyle questionnaires are vulnerable to error, this may have reduced our ability to detect additional associations between lifestyle factors and AD risk.

Lastly, while our results are pertinent to healthy, middle-aged research participants without severe cardiac or cerebrovascular disease, results may differ in the elderly, in demented patients, and in those with vascular or metabolic diseases. Studies with larger samples and longer follow-up times are needed to assess the generalizability

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of these findings in community-based populations with a higher variability in socio-economic and medical status.

Our study has a number of strengths. While previous studies focused on non-demented elderly, including those with cognitive impairment, this longitudinal biomarker study focused on cognitively intact, middle-aged individuals. There is a consensus that lifestyle interventions have the highest chances of success when implemented well before old age,<sup>5</sup> making our results particularly relevant to efforts aimed at preventing AD.

Further, the majority of previous studies looked at intellectual and physical activity, but not at diet. We demonstrated that diet does, in fact, influence the rate of change in metabolic AD biomarkers, whereas intellectual and physical activity do not appear to do so.

Lastly, our statistical model enabled us to simultaneously assess multiple lifestyle and vascular risk factors, yielding a more comprehensive understanding of the associations between these modifiable risk factors and AD risk. A combined reduction in several modifiable AD risk factors is projected to have a more significant impact than any one factor alone.<sup>4</sup>

## Contributors

MW, JS, CG, CQ, RDA, DCM, and LM: analysis and interpretation. CQ, RSO, SV, MJDL, and LM: acquisition of data. RSI and LM: study concept and design. All authors: critical revision of the manuscript for important intellectual content. LM: study supervision.

## Competing interests

The authors declare no disclosures.

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## Patient consent

Obtained.

## Ethical approval

This study was approved by NYU School of Medicine and WCMC Institutional Review Boards.

## Data sharing

All relevant data have been included in the paper. Technical appendix, statistical code, and dataset will be made available on request.

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**eTable 1. Associations between regional PiB uptake, lifestyle, vascular risk and global cognition at baseline.**

	PiB-amyloid deposition		Lifestyle variables			Vascular risk-related measures						Cognition
	PCC	FC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hypertension	Global cognition
PCC	1	<b>.37**</b>	-.02	-.07	-.12	-.09	.08	-.13	.14	-.13	-.01	-.12
FC		1	-.04	.08	.14	-.25	-.00	.05	-.09	.08	.09	.06
MeDi adherence			1	.03	<b>.39**</b>	.08	-.17	-.27	.04	-.12	-.11	.16
Physical activity				1	.05	.12	-.07	.10	.17	.04	-.10	-.04
Intellectual activity					1	-.04	.02	-.02	-.16	.09	-.13	<b>.40**</b>
Plasma HCY						1	-.14	-.18	.07	-.14	-.05	.04
Plasma cholesterol							1	.22	-.06	-.15	<b>-.28*</b>	-.13
Hip-to-waist								1	<b>-.68***</b>	<b>.57**</b>	-.09	.22
Body Mass Index									1	<b>-.38**</b>	-.03	-.24
QUICKI scores										1	-.10	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001

Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)

**eTable 2. Associations between regional FDG uptake, lifestyle, vascular risk and global cognition at baseline.**

	FDG metabolism		Lifestyle variables			Vascular risk-related measures						Cognition
	PCC	FC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hypertension	Global cognition
PCC	1	<b>.32**</b>	.14	-.05	-.14	-.06	.00	-.17	.01	-.08	.06	-.00
FC		1	.11	.12	<b>.27*</b>	-.14	-.04	.01	-.23	-.03	.03	-.06
MeDi adherence			1	.03	<b>.39**</b>	.08	-.17	-.27	.04	-.02	-.11	.16
Physical activity				1	.05	.12	-.07	.10	.17	.04	-.10	-.04
Intellectual activity					1	-.04	.02	-.02	-.16	.09	-.13	<b>.40**</b>
Plasma HCY						1	-.14	-.18	.07	-.04	-.05	.04
Plasma cholesterol							1	.22	-.06	-.05	<b>-.28*</b>	-.13
Hip-to-waist								1	<b>-.68***</b>	<b>-.37*</b>	-.09	.22
Body Mass Index									1	<b>-.39**</b>	-.03	-.24
QUICKI scores										1	-.10	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001

Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)

**eTable 3. Associations between MRI cortical thickness, lifestyle, vascular risk and global cognition at baseline.**

	MRI thickness		Lifestyle variables			Vascular risk-related measures						Cognition
	EC	PCC	MeDi adherence	Physical activity	Intellectual activity	Plasma HCY	Plasma Cholesterol	Hip/waist	BMI	QUICKI scores	Hypertension	Global cognition
EC	1	<b>.36**</b>	.00	-.33	-.02	-.20	.00	.11	-.06	-.11	.16	.05
PCC		1	-.05	-.31	-.16	-.08	-.11	.08	-.12	-.06	.23	.11
MeDi adherence			1	.02	<b>.37**</b>	.15	-.17	-.27	.12	-.14	-.14	.15
Physical activity				1	-.01	.11	-.06	.06	.22	.00	-.11	-.02
Intellectual activity					1	-.03	.05	-.07	-.08	.05	-.18	<b>.41**</b>
Plasma HCY						1	-.14	-.22	.02	-.06	-.04	.06
Plasma cholesterol							1	.27	-.11	-.12	<b>-.27*</b>	-.13
Hip-to-waist								1	<b>-.69***</b>	.04	-.10	.23
Body Mass Index									1	<b>-.38**</b>	.02	-.23
QUICKI scores											-.03	.14
Hypertension											1	.04
Global cognition												1

Partial *r* correlation coefficients adjusting for age, sex, and APOE4 status; \**p*<.05; \*\**p*<.01; \*\*\**p*<.001

Abbreviations: frontal cortex (FC), posterior cingulate cortex (PCC), homocysteine (HCY)

## eAppendix

### Sensitivity analysis

#### Power calculations

Since we generally did not find significant associations of physical activity and intellectual activity with biomarker values, we wanted to ensure that our null findings were not primarily attributable to our sample size. To do so, we utilized a series of simulations to estimate minimum sample sizes required to detect our observed effect sizes at an alpha level of 0.05 (two-tailed).

Holding the observed variability constant as well as the influence of all other variables in our full model, we found that physical activity only predicted .12% of unique variance in FDG changes overtime. This corresponded to an  $f^2$  effect size of 0.0012. Based on the observed effect size, we estimated that 6,535 participants would be needed to obtain 80% power to detect significant associations between physical activity and FDG changes.

Additionally, with the same model specifications described above, we found that intellectual activity only accounted for .09% of unique variance in FDG changes overtime. This corresponded to an  $f^2$  effect size of 0.0009. Based on the observed effect size, we estimated that 8,716 participants would be needed to obtain 80% power to detect significant associations between intellectual activity and FDG changes over 3 years.

In contrast to these results, MeDi scores uniquely predicted 8.83% of the variance in FDG changes over time. This corresponded to an  $f^2$  effect size of 0.097. Based on the observed effect size, we estimated that as few as 84 participants are needed to obtain 80% power to detect significant associations between MeDi adherence and FDG changes over 3 years.

Given the small magnitude of the observed effect sizes of physical and intellectual activity, as well as the unrealistically large sample sizes required to obtain satisfactory power of detecting significant differences, we conclude that our null results are not attributable to sample size concerns. Lack of associations between physical activity, intellectual activity, and AD biomarker changes are also consistent with previous studies in the elderly.

Breaking down global cognition

Global cognition was the main cognitive outcome measure in this study. We then conducted an exploratory analysis to independently assess domain-specific associations (i.e. association with memory, executive function and language scores) using the same procedures as with the global cognition scores and  $p<0.05$ .

Longitudinal results are summarized in the tables below.

None of the predictors were directly associated with changes in memory (Table 1) and executive function (Table 2).

As shown in table 3, after accounting for baseline language, higher baseline plasma homocysteine was negatively, though marginally associated with faster rates of decline in language scores in the full model (model 1  $p=0.076$ ), in the reduced model (model 2,  $p=0.069$ ), and reached significance in the model with vascular variables only (model 4,  $p=0.036$ ). Additionally, MeDi scores were positively, though marginally associated with language in the model with lifestyle variables only (model 3,  $p=0.063$ ).

**Table 1.** Prediction of changes in memory.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
Mediterranean diet	.025 (.065)	.027 (.066)	.038 (.060)	
Physical activity	-.000 (.011)	-.002 (.012)	-.002 (.012)	
Intellectual activity	-.044 (.172)	-.058 (.175)	-.063 (.168)	
Plasma homocysteine	.022 (.046)	.009 (.045)		.008 (.044)
Plasma cholesterol	.000 (.003)	.000 (.003)		.000 (.003)
Body mass index	-.007 (.020)	-.005 (.021)		-.005 (.021)
QUICKI scores	-6.940 (5.867)	-4.758 (5.800)		-5.306 (5.637)
Hypertension	-.077 (.154)	-.083 (.148)		-.085 (.146)
Memory at baseline	.666 (.137) <sup>***</sup>	.677 (.149) <sup>***</sup>	.652 (.144) <sup>***</sup>	.672 (.148) <sup>***</sup>
Sex	-.108 (.114)			
APOE status	.051 (.105)			
Age	-.010 (.016)			
Time to follow-up	-.001 (.000)			
Constant	1.062 (.983)	.725 (.969)	-.002 (.094)	.816 (.944)

\* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; † $0.05< p<0.10$

Values are presented as unstandardized beta coefficients (standard error).

<sup>a</sup>Model 1: full model with all variables examined

<sup>b</sup>Model 2: full model without non-significant adjustment variables

<sup>c</sup>Model 3: model with lifestyle variables only, and without non-significant adjustment variables

<sup>d</sup>Model 4: model with vascular variables only, and without non-significant adjustment variables

Abbreviations: QUICKI, Quantitative Insulin Sensitivity Check Index.

**Table 2.** Prediction of changes in executive function (EF).

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
Mediterranean diet	.019 (.044)	.022 (.044)	.015 (.042)	
Physical activity	-.000 (.009)	-.002 (.009)	-.002 (.009)	
Intellectual activity	.218 (.133)	.173 (.132)	.193 (.130)	
Plasma homocysteine	-.032 (.038)	-.027 (.034)		-.030 (.035)
Plasma cholesterol	.000 (.003)	.000 (.002)		.000 (.002)
Body mass index	.012 (.014)	.012 (.014)		.010 (.014)
QUICKI scores	2.367 (4.078)	2.623 (4.006)		2.346 (3.921)
Hypertension	-.024 (.121)	-.036 (.108)		-.062 (.110)
EF at baseline	.861 (.082) <sup>***</sup>	.849 (.084) <sup>***</sup>	.842 (.080) <sup>***</sup>	.885 (.085) <sup>***</sup>
Sex	-.044 (.087)			
APOE status	.086 (.078)			
Age	.005 (.012)			
Time to follow-up	.000 (.000)			
Constant	-.337 (.674)	-.389 (.662)	.071 (.071)	-.360 (.649)

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001; †0.05<p<0.10

See legend to Table 1.

**Table 3.** Prediction of changes in language.

	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>	Model 4 <sup>d</sup>
Mediterranean diet	.115 (.073)	.116 (.072)	.134 (.070) <sup>†</sup>	
Physical activity	-.002 (.014)	-.002 (.009)	-.007 (.013)	
Intellectual activity	.189 (.203)	.173 (.132)	.180 (.189)	
Plasma homocysteine	-.087 (.048) <sup>†</sup>	-.027 (.034) <sup>†</sup>		-.098 (.046) <sup>*</sup>
Plasma cholesterol	-.000 (.004)	.000 (.002)		.001 (.004)
Body mass index	.013 (.021)	.012 (.014)		.014 (.021)
QUICKI scores	1.254 (7.056)	2.623 (4.006)		3.486 (6.308)
Hypertension	.006 (.177)	-.036 (.108)		.061 (.157)

Language at baseline	.737 (.143) <sup>***</sup>	.849 (.084) <sup>***</sup>	.717 (.139) <sup>***</sup>	.731 (.134) <sup>***</sup>
Sex	-.032 (.136)			
APOE status	.078 (.120)			
Age	.003 (.015)			
Time to follow-up	-.000 (.001)			
Constant	-.249 (1.154)	-.389 (.662)	-.050 (.106)	-.582 (1.019)

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001; †0.05<p<0.10

See legend to Table 1.

For peer review only



**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies***

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
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	5-7
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7-8
		(b) Describe any methods used to examine subgroups and interactions	7-8
		(c) Explain how missing data were addressed	7-8
		(d) If applicable, explain how loss to follow-up was addressed	7
		(e) Describe any sensitivity analyses	e-appendix
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	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8-9
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	9-14
		(b) Report category boundaries when continuous variables were categorized	9-14
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9-14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15, eappendix, etables
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
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	18

\*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](http://www.strobe-statement.org).