^1H -MRS metabolites and rate of β -amyloid accumulation on serial PET in clinically normal adults

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ABSTRACT

Objective: To assess whether noninvasive proton magnetic resonance spectroscopy (^1H -MRS) tissue metabolite measurements at baseline can predict an increase in the rate of β -amyloid ($\text{A}\beta$) accumulation on serial PET in clinically normal (CN) older adults.

Methods: Consecutive participants aged 60 years and older ($n = 594$) from the Mayo Clinic Study of Aging who were CN at baseline and who underwent ^1H -MRS from the posterior cingulate voxel and longitudinal ^{11}C -Pittsburgh compound B (PiB)-PET were included. The rate of $\text{A}\beta$ accumulation by serial cortical PiB standardized uptake value ratios was estimated as a function of baseline ^1H -MRS metabolite ratios and time using mixed-effect models adjusted for age, sex, and *APOE* $\epsilon 4$. Effect of *APOE* $\epsilon 4$ on the relationship between baseline MRS and an increased rate of $\text{A}\beta$ accumulation was also assessed.

Results: Among all participants, a higher myo-inositol (mI)/creatine ($p = 0.011$) and a lower *N*-acetylaspartate/ml ($p = 0.006$) at baseline were associated with an increased $\text{A}\beta$ accumulation over time after adjusting for age, sex, and *APOE* $\epsilon 4$. *APOE* $\epsilon 4$ did not modify the association of baseline ^1H -MRS metabolite ratios and rate of $\text{A}\beta$ accumulation. However, *APOE* $\epsilon 4$ carriers accumulated $\text{A}\beta$ faster than noncarriers regardless of the baseline $\text{A}\beta$ load ($p = 0.001$).

Conclusion: Among CN older adults, early metabolic alterations on ^1H -MRS and *APOE* $\epsilon 4$ status are independently associated with an increased rate of $\text{A}\beta$ accumulation. Our findings could have important implications for early diagnosis and identification of individuals for secondary prevention trials, because an increased rate of $\text{A}\beta$ accumulation in CN older adults may confer a higher risk for cognitive decline and mild cognitive impairment. *Neurology*® 2017;89:1391-1399

GLOSSARY

$\text{A}\beta$ = β -amyloid; **AD** = Alzheimer disease; **Cho** = choline; **CN** = clinically normal; **Cr** = creatine; **MCI** = mild cognitive impairment; **mI** = myo-inositol; **MPRAGE** = magnetization-prepared rapid acquisition gradient echo; **MRS** = magnetic resonance spectroscopy; **NAA** = *N*-acetylaspartate; **PC** = posterior cingulate; **PiB** = Pittsburgh compound B; **SUVr** = standardized uptake value ratio; **WM** = white matter.

Twenty to forty percent of clinically normal (CN) older adults have significant β -amyloid ($\text{A}\beta$) load on cross-sectional PET.¹⁻³ An increased rate of $\text{A}\beta$ accumulation may put them at a higher risk for cognitive decline⁴ and mild cognitive impairment (MCI).⁵ Cost-effective and noninvasive biomarkers that can predict a further increase in $\text{A}\beta$ accumulation over time are necessary for better identification of at-risk individuals who may benefit from preventive and disease-modifying strategies.

In CN older adults, elevated myo-inositol (mI), a marker of glial activation, has been associated with a higher $\text{A}\beta$ load on PET,^{6,7} lower CSF $\text{A}\beta_{1-42}$,⁷ and a higher $\text{A}\beta$ density in an autopsy-confirmed cohort.⁸ Moreover, an elevated mI has been found in *APOE* $\epsilon 4$ carriers with no evidence of $\text{A}\beta$ deposition on PET.⁷ Although the mechanistic relationship between *APOE* $\epsilon 4$ and elevated mI is unclear, it has been suggested that *APOE* $\epsilon 4$ enhances glial activation and

Supplemental data
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modulates the relationship between A β and glial activation.^{9–11} However, the *APOE* $\epsilon 4$ effect on magnetic resonance spectroscopy (MRS) metabolite levels among CN elderly has been equivocal.^{7,9,12} Further, all of the studies examining MRS, A β -related pathology, and *APOE* $\epsilon 4$ were cross-sectional.

Our objective was to investigate the association of MRS metabolite ratios from a posterior cingulate (PC) gyrus voxel at baseline with the change in A β accumulation over time on serial amyloid PET in CN older adults drawn from a population-based sample. A secondary objective was to assess whether *APOE* $\epsilon 4$ modifies the relationship between MRS metabolites and the rate of A β accumulation.

METHODS Participants. Consecutive participants aged ≥ 60 years were drawn from the ongoing population-based, longitudinal Mayo Clinic Study of Aging,^{13,14} between January 2006 and May 2016. To be included in this imaging study, participants were required to be CN at baseline when MRS and amyloid PET were performed and have at least one follow-up amyloid PET. The diagnostic process and criteria for being clinically normal are described in appendix e-1 at Neurology.org. A total of 594 participants meeting inclusion criteria and passing image quality control were included in the analyses. The final cohort flowchart is provided as figure e-1.

Blood was collected to determine the *APOE* $\epsilon 4$ noncarrier or carrier status.

Change in A β accumulation over time was assessed using all available follow-up amyloid PET examinations on an individual. Consecutive participants had 1 ($n = 416$; 70%), 2 ($n = 144$; 24.6%), 3 ($n = 29$; 4.8%), or 4 ($n = 5$; 0.6%) follow-up PET scans performed approximately every 15 months.

Standard protocol approvals, registrations, and participant consents. The Mayo Clinic and Olmsted Medical Center institutional review boards approved the study. Every participant provided written informed consent.

¹H-MRS and MRI studies. Baseline MRS and MRI were performed at 3T using an 8-channel phase array coil (GE Healthcare, Waukesha, WI). A 3D high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) scan was acquired for anatomic segmentation and region labeling of PET and localization of ¹H-MRS voxel.

A point-resolved spectroscopic sequence was acquired with repetition time/echo time 2,000/30 ms with a single voxel of $2 \times 2 \times 2$ cm³ placed in the midsagittal MPRAGE image including right and left PC gyri and inferior precunei. Although the transverse relaxation time of *N*-acetylaspartate (NAA) and choline (Cho) is longer than mI, a single short echo time of 30 ms allowed measurements of all 3 metabolites in participants within the Alzheimer disease (AD) continuum.¹⁵ Metabolites were quantified using the automated proton brain examination/single-voxel package, and their intensities were scaled by creatine (Cr), a standard reference. Individual voxel placement and magnetic resonance spectra were visually evaluated by a trained image analyst for quality control. A trained image analyst reviewed the location of the MRS voxel and evaluated water suppression, baseline distortions, or lipid

contamination. Voxels that did not include the PC location according to the predetermined anatomic landmarks and spectra with poor water suppression, lipid contamination, or baseline distortions failed quality control and were excluded. Although the spectral fit was not measured quantitatively, the quantification of metabolite ratios failed if the spectral fit was poor.

PET studies. ¹¹C-Pittsburgh compound B (PiB)–PET/CT images were acquired using a GE scanner (GE Healthcare). Participants were injected with the PiB tracer (average activity 625 MBq; range 385–723) and a low-dose CT scan was acquired for attenuation correction. Forty to sixty minutes postinjection, a 20-minute dynamic PET scan consisting of four 5-minute frames was acquired. These 4 frames were averaged to create a single statistical image.

Cortical A β retention for each PiB image was calculated as a global cortical standardized uptake value ratio (SUVR).¹⁶ For measuring change over time in PiB uptake, we used a previously published SUVR measurement technique, which was demonstrated to improve reliability and plausibility for serial measurements compared to traditional cross-sectional approaches.¹⁷ This technique uses a reference region of eroded supratentorial white matter (WM) segmented using MRI, combined with the whole cerebellum and pons. In brief, each PiB scan is rigidly registered to its corresponding T1-weighted MRI. Each MRI is segmented using SPM12¹⁸ with an in-house population-specific template and several population-specific measure alterations previously described.¹⁹ These segmentations are used for locating supratentorial WM for the reference region.

The target region and the cerebellar/pons regions included as part of the reference region were each localized using a corresponding in-house atlas¹⁹ that was nonlinearly registered to each corresponding MRI using the advanced normalization tools symmetric normalization algorithm,²⁰ resampled using nearest-neighbor interpolation, and refined using the tissue segmentations described above. Automated registration and segmentation steps were each visually confirmed for acceptable quality. SUVRs were then calculated from PiB scans as the mean of all voxels in the target region normalized by the mean of all voxels in the reference region.

Statistical analysis. We used mixed-effects models to model repeated PiB SUVR values as a function of baseline MRS metabolite ratios for all participants. We incorporated random slopes and intercepts for each participant (estimating the correlation between the slopes and intercepts). The primary predictor of interest involved an interaction of baseline MRS ratio with time, because we were interested in longitudinal change in A β accumulation. Models included the nested time and MRS ratio at baseline as is proper for an evaluation of interaction with time. A significant interaction of MRS ratio at baseline with time would indicate that the increase in the rate of A β accumulation on serial PET depends upon the value of the MRS ratio at baseline. A significant association between baseline MRS ratio and A β accumulation in the model would indicate that the MRS ratio was associated with consistently higher A β accumulation across all serial PET measurements in a given participant but not with an increased rate of accumulation. The models also accounted for effects of baseline age, sex, and *APOE* $\epsilon 4$ status. We computed the 3-way interactions of baseline MRS ratios and *APOE* $\epsilon 4$ status with time to assess whether *APOE* $\epsilon 4$ modifies the relationship between baseline metabolite MRS ratios and change over time in A β accumulation on serial PET.

Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) and R statistical software version 3.1.1 (R-

Table 1 Participants' (n = 594) characteristics at baseline

Characteristics	Values
Age, y	74.4 (7.3)
Male	343 (58)
APOE ε4 carrier	169 (29)
Education, y	14.73 (2.70)
Short test of mental status	35.06 (2.24) ^a
NAA/Cr	1.68 (0.11)
Cho/Cr	0.63 (0.06)
mI/Cr	0.51 (0.06)
NAA/mI	3.35 (0.57)
PiB SUVR, mean (SD)	1.50 (0.33)
PiB SUVR, median (range)	1.37 (1.18–1.93)
Follow-up time, y, mean (SD)	3.26 (1.50)
Follow-up time, y, median (range)	2.67 (1–9.17)

Abbreviations: Cho = choline; Cr = creatine; mI = myo-inositol; NAA = N-acetylaspartate; PiB = ¹¹C-Pittsburgh compound B; SUVR = cortical standardized uptake value ratio. APOE ε4 status was missing in 1 and short test of mental status in 8 participants. Mean (SD) is listed for continuous variables unless otherwise noted and counts and proportions (%) are listed for categorical variables.

^aMaximum score is 38 points.⁴⁰

project.org) with 2-sided significance set at α 0.05 type I error rate. Because we were interested in the association of individual metabolite ratios with serial PiB measurements, and at this stage did not want to inflate type II error by declaring true associations null, we did not adjust for multiple comparisons.^{21,22}

RESULTS Participants' characteristics at baseline are listed in table 1. Although a higher mI/Cr at baseline was only borderline associated with a higher Aβ load across all serial PET of a given participant ($p = 0.06$), it is more important that it was significantly associated with a greater increase in rate of Aβ accumulation ($p = 0.011$), adjusting for baseline age, sex, APOE ε4, and the interaction between APOE ε4 and age (table 2). Similarly, a lower baseline NAA/mI was associated with consistently higher Aβ load across all serial PETs of a given participant ($p = 0.007$), but moreover, it was associated with an increased rate of Aβ accumulation ($p = 0.006$), adjusting for the same covariates (table 2). Figure 1 illustrates the estimated increase in rate of Aβ accumulation by baseline MRS ratios and by APOE ε4 with specific estimated values shift according to age, sex, and APOE ε4. Figure 2 shows the 3 individuals from the current cohort, their magnetic resonance spectra at baseline, and associated Aβ load at baseline and at follow-up.

Mixed-effect models for the remaining metabolite ratios showed that a lower baseline NAA/Cr was associated with consistently higher Aβ load across all

serial PET examinations in a given participant ($p = 0.02$; table e-1). However, NAA/Cr was not associated with an increased rate of Aβ accumulation ($p = 0.18$; figure e-2). Cho/Cr was associated neither with Aβ load across serial PET ($p = 0.28$) nor with rate of Aβ accumulation ($p = 0.47$; table e-1).

APOE ε4 was associated with consistently higher Aβ load across all serial PiB SUVR measurements ($p < 0.001$). Furthermore, the interaction of APOE ε4 with time ($p < 0.001$) was associated with an increased rate of Aβ accumulation, taking into account baseline age, sex, and time with baseline age interaction (table 3, model 1; figure 1). However, an accelerated rate of Aβ accumulation in APOE ε4 carriers compared to noncarriers may be because APOE ε4 carriers have higher baseline Aβ load.¹⁶ Nevertheless, when we compared rates of Aβ accumulation between a subset of our APOE ε4 carriers (n = 149) and noncarriers matched on age, sex, and baseline Aβ load to a subset of our noncarriers (n = 149), the interaction between APOE ε4 and time remained significant ($p = 0.001$; table 3, model 2) using mixed-effect model with a random block design to account for matching. Therefore, APOE ε4 carriers accumulated Aβ at an accelerated rate compared to APOE ε4 noncarriers even when they had a similar baseline Aβ load (figure 1).

Finally, we assessed whether APOE ε4 status modified the relationship between the baseline metabolites and increased rate of Aβ accumulation using a 3-way interaction of baseline metabolites, APOE ε4, and time among all. None of these interactions was significant, including for mI/Cr $\times$ APOE ε4 $\times$ time ($p = 0.35$) and for NAA/mI $\times$ APOE ε4 $\times$ time ($p = 0.90$). Therefore, longitudinally, APOE ε4 status did not modify the relationship between MRS metabolites and rate of Aβ accumulation on serial PET.

DISCUSSION In this large cohort of CN older adults, mean age of 74, drawn from a population-based sample, we demonstrated that noninvasive and inexpensive baseline MRS metabolite levels are associated with an increased rate of Aβ accumulation on serial PET. Lower NAA/mI and higher mI/Cr at baseline were associated with an increased rate of Aβ accumulation taking into account age at baseline, sex, and APOE ε4 status. APOE ε4 carriers accumulated Aβ faster than noncarriers. APOE ε4 status did not alter the relationship between baseline metabolite levels and rate of Aβ accumulation and both MRS metabolites and APOE ε4 likely are independently associated with an increased Aβ accumulation over time.

An elevated mI/Cr has been consistently associated with biomarkers of elevated Aβ in CN adults cross-sectionally.^{7,8,23,6} Moreover, in the transgenic

Table 2 Estimated rate of β -amyloid ($A\beta$) accumulation by baseline myo-inositol (mI)/creatinine (Cr) and N-acetylaspartate (NAA)/mI ratios

	Estimate (SE)	p Value
mI/Cr with time interaction as primary predictor		
Intercept	−0.863 (0.098)	<0.001
Time, y	−0.052 (0.009)	<0.001
Baseline age	0.008 (0.001)	<0.001
Male	0.033 (0.014)	0.017
<i>APOE</i> ϵ 4	0.119 (0.016)	<0.001
Baseline mI/Cr ratio	0.223 (0.016)	0.06
<i>APOE</i> ϵ 4 with time interaction	0.011 (0.002)	<0.001
Baseline age with time interaction	0.001 (0.000)	<0.001
Baseline mI/Cr ratio with time interaction	0.029 (0.011)	0.011
NAA/mI with time interaction as primary predictor		
Intercept	−0.628 (0.087)	<0.001
Time, y	−0.024 (0.009)	0.003
Baseline age	0.008 (0.001)	<0.001
Male	0.034 (0.014)	0.013
<i>APOE</i> ϵ 4	0.118 (0.016)	<0.001
Baseline NAA/mI ratio	−0.035 (0.013)	0.007
<i>APOE</i> ϵ 4 with time interaction	0.011 (0.002)	<0.001
Baseline age with time interaction	0.001 (0.000)	<0.001
Baseline NAA/mI ratio with time interaction	−0.003 (0.001)	0.006

Coefficient estimates with their SE and p values are reported for the rate of $A\beta$ accumulation as the response. The predictor of primary interest is an interaction of baseline mI/Cr ratio with time and then baseline NAA/mI ratio with time, which are 2 metabolite ratios significantly associated with the change (increase and decrease) in rate of $A\beta$ accumulation. The interaction with time is illustrated in figure 1. The individual effects of other variables that contribute to the model fit are also shown.

murine AD model, a passive immunization with anti- $A\beta$ antibodies lessened the mI/Cr in the treatment arm compared to placebo.²⁴ In the current longitudinal cohort, we demonstrated findings that suggest an elevated mI/Cr as a predictor of an accelerated $A\beta$ accumulation over time on serial PET.

NAA/Cr ratio, a marker of neuronal viability and synaptic integrity, is reduced in participants with MCI²⁵ and AD,^{15,26,27} but not in CN older adults, suggesting that a decline in NAA/Cr is preceded by an increased mI/Cr during the course of AD. In line with this, an autopsy-confirmed study⁸ demonstrated an association between a lower NAA/Cr and a higher burden of tau-related pathology and loss of synaptic integrity that is believed to follow the changes in $A\beta$ biomarkers during the course of AD.²⁸ Accordingly, we observed that a lower NAA/Cr was associated with consistently higher $A\beta$ load across all serial PET examinations of a given participant but not with an increased rate of $A\beta$ accumulation. In addition to mI/Cr, significant association was observed between a lower baseline NAA/mI and an increased rate of $A\beta$

accumulation. Lower NAA/mI has predicted progression from CN to MCI in a population-based cohort²⁹ and only a lower NAA/mI among routinely examined MRS ratios correlated with both greater tau and $A\beta$ burden at autopsy.⁸ The current study supports the composite NAA/mI ratio as a marker of increased longitudinal $A\beta$ accumulation in CN older adults.

Although Cho/Cr has been associated with a higher $A\beta$ load on PET^{7,29} and a worse cognitive performance in CN older adults,^{6,9} we did not observe an association of Cho/Cr and rate of $A\beta$ accumulation. No association was found between Cho/Cr and AD-related pathology in an autopsy-confirmed cohort,⁸ and the significance of Cho/Cr during the progression of AD remains unclear.

APOE ϵ 4 status did not modify the relationship between baseline MRS metabolites and rate of $A\beta$ accumulation on serial PET. Instead, *APOE* ϵ 4 was independently associated with an accelerated $A\beta$ accumulation. Whereas the relationship between a higher $A\beta$ load in *APOE* ϵ 4 carriers has been well-established cross-sectionally,^{3,30,31} the effect of *APOE* ϵ 4 status on longitudinal $A\beta$ accumulation in CN older adults has not been clarified, likely due to small sample sizes, various PiB uptake measurement approaches, and different interpretation of contributing effects of baseline $A\beta$ load, age, sex, and number of available follow-up PET scans.^{5,32–34} Moreover, *APOE* ϵ 4 effect on longitudinal $A\beta$ accumulation may be mediated by baseline $A\beta$ load,¹⁶ which is higher in *APOE* ϵ 4 carriers than noncarriers. Higher baseline $A\beta$ load is a risk factor for increased $A\beta$ accumulation over time.¹⁶ However, our finding of longitudinally accelerated $A\beta$ accumulation in *APOE* ϵ 4 carriers with similar baseline $A\beta$ load to *APOE* ϵ 4 noncarriers indicates that *APOE* ϵ 4 carriers accumulate $A\beta$ faster than noncarriers, regardless of baseline $A\beta$ levels.

A cross-sectional study by Voevodskaya et al.⁷ demonstrated that already cognitively normal *APOE* ϵ 4 carriers with still normal $A\beta$ biomarker levels had elevated mI/Cr. It was suggested that mI/Cr may be an early biomarker of $A\beta$ accumulation.³⁵ Our findings support this hypothesis by showing that an elevated mI/Cr ratio in older adults is associated with an increased rate of $A\beta$ accumulation. In addition, we showed that the relationship between MRS metabolite alterations and rates of $A\beta$ accumulation is independent of *APOE* ϵ 4 status. Taken together, these findings suggest that cross-sectional MRS metabolite alterations may occur in *APOE* ϵ 4 carriers because of their risk of increased $A\beta$ accumulation over time.

Higher baseline $A\beta$ load increases the risk of cognitive decline over time in CN older adults.^{34,36} However, a recent cut point for $A\beta$ positivity on PET in CN older adults³⁷ was based on repeated

Figure 1 Estimated rate of β -amyloid ($A\beta$) accumulation on serial PET by baseline magnetic resonance spectroscopy (MRS) metabolite ratios and $APOE \epsilon 4$ status

The estimates for the rate of $A\beta$ accumulation for a 75-year-old clinically normal man from the Mayo Clinic Study of Aging are shown. His rate of $A\beta$ accumulation is estimated using an interaction between his baseline MRS metabolite ratios and time as primary predictor. The 1st and 3rd quartiles of MRS ratios are based on the models in table 2. (A) With a higher baseline myo-inositol (ml)/creatine (Cr) ratio, rate of $A\beta$ accumulation increases more than it does with lower baseline ml/Cr ratio. Interaction of baseline MRS metabolites with time is visualized by gradually diverging slopes between quartiles. (B) A lower baseline *N*-acetylaspartate (NAA)/ml ratio is associated with an increase in rate of $A\beta$ accumulation, a negative association represented by inverse order of quartiles. (C) The rate of $A\beta$ accumulation is compared between a 75-year-old male $APOE \epsilon 4$ noncarrier and an $APOE \epsilon 4$ carrier of the same age and sex (table 3). The carrier slope is increasing and diverging from the noncarrier slope with time. However, note an obvious difference in baseline $A\beta$ load between the $APOE \epsilon 4$ carrier and noncarrier, and because of this $A\beta$ accumulation might be faster in the $APOE \epsilon 4$ carrier. (D) The difference in rate of $A\beta$ accumulation is shown between the $APOE \epsilon 4$ carrier matched on age, sex, and baseline $A\beta$ load to the noncarrier. The $APOE \epsilon 4$ carrier accumulates $A\beta$ faster, regardless of baseline $A\beta$ load. Note that the baseline Pittsburgh compound B (PiB) standardized uptake value ratio (SUVR) is derived using the serial PiB SUVR measurement approach.

measurements of $A\beta$ accumulation. Using repeated measurements, the reliable worsening in $A\beta$ accumulation was identified and served as cut point. Moreover, a few studies demonstrated that an increased rate of $A\beta$ accumulation on PET is associated with cognitive decline over time in CN⁴ and progression to MCI.⁵ Therefore identifying those who accumulate $A\beta$ faster over time provides additional and valuable information on at-risk individuals beyond cross-sectional measurements, which do not provide any information on disease progression. However, so far, the biomarkers that would identify such individuals have been scarce. Identifying those who accumulate $A\beta$ faster can have important implications for early diagnosis and selecting at-risk individuals for secondary preventive interventions targeted to reduce

$A\beta$ accumulation rate. It is possible that interventions might be more effective in those who are still clinically normal but on the way to higher rates of $A\beta$ accumulation. Our current findings suggest that both MRS metabolite alterations and $APOE \epsilon 4$ status independently are associated with accelerated rates of $A\beta$ accumulation.

We did not dichotomize the participants as amyloid-positive or -negative by a cut point, although this popular approach may have practical advantages. Instead, we treated serial PiB SUVR as continuous measures that allowed us to include the CN participants with the full range of PiB SUVR values. Cut point may create a gray zone where sub-threshold but important relationships might be obscured. For example, the biological difference

These are 3 individuals from the Mayo Clinic Study of Aging. (A) A 76-year-old woman with high N-acetylaspartate (NAA)/myo-inositol (ml) ratio 3.25 and low ml/creatine (Cr) ratio 0.35 and minimal A β load (Pittsburgh compound B [PiB] standardized uptake value ratio [SUVR] 1.29) at baseline and minimal progression on follow-up 32 months later (PiB SUVR 1.30). (B) A 78-year-old man with a high ml/Cr ratio 0.63 and higher amyloid load at baseline (PiB SUVR 1.40) further shows a considerable progression on follow-up PET (PiB SUVR 1.82) 45 months later. (C) A 76-year-old woman with low NAA/ml ratio 2.74 with baseline PiB SUVR 1.35 shows progression on PET (PiB SUVR 1.58) 30 months later.

between those participants who are close to the cut point, but arbitrarily fall into opposite groups, or a minor longitudinal change that moves a participant from one group to another by a given cut point might be negligible. On the contrary, a large difference in PiB SUVR among 2 participants who are in the same PiB group and a large change in PiB SUVR in a participant over time without a change in PiB group designation by cut point may be very meaningful. Finally, the cut point for amyloid positivity for longitudinal amyloid measurements remains to be established.

Strengths of this study are the large sample of individuals drawn from a single population with serial amyloid PET including a large subset of *APOE* ϵ 4 carriers matched to noncarriers on demographics and baseline A β load. In this cohort, A β accumulation over time was measured using a modified reference region that has demonstrated an improved reliability

and plausibility for serial measurements compared to traditional cross-sectional approaches.¹⁷

The limitations of our study are similar to those of other studies using participants drawn from a population-based sample, such as the presence of various subthreshold pathologies in CN older adults, which may increase the variability of MRS metabolite measurements and weaken some of the studied relationships. However, the levels of MRS ratios in the current cohort of CN older adults were consistent with previous studies by others and by our group,^{9,29,38} including an autopsy-confirmed study on MRS correlates of A β accumulation.⁸ Our proportion of CN *APOE* ϵ 4 carriers (29%) was similar to previous reports.^{5,32,34} However, we cannot exclude the potential for participation or survival bias because more educated and generally healthier participants may be more willing to participate longitudinally in imaging studies,²⁹ and our findings may not be

Table 3 Estimated rate of β -amyloid (A β) accumulation by APOE ϵ 4 status

	Estimate (SE)	p Value
Model 1: APOE ϵ4 as primary predictor in the whole cohort		
Intercept	−0.747 (0.076)	<0.001
Time, y	−0.037 (0.008)	<0.001
Baseline age	0.008 (0.001)	<0.001
Male	0.034 (0.014)	0.014
APOE ϵ 4	0.117 (0.016)	<0.001
Baseline age with time interaction	0.001 (0.000)	<0.001
APOE ϵ 4 with time interaction	0.011 (0.002)	<0.001
Model 2: APOE ϵ4 as primary predictor in subset of APOE ϵ4 carriers matched to noncarriers		
Intercept	−0.036 (0.018)	0.043
Time, y	0.017 (0.002)	<0.001
APOE ϵ 4	0.004 (0.004)	0.34 ^a
APOE ϵ 4 with time interaction	0.006 (0.002)	0.001

Coefficient estimates with their SE and p values are reported for the rate of A β accumulation on serial PET as response in the whole cohort (n = 594). The effects of individual variables contributing to the model fit are shown. Model 1 shows that APOE ϵ 4 status is associated with a consistently higher A β load across all serial PET in a given participant. However, it is an interaction of APOE ϵ 4 status with time, which is associated with an increased rate of A β accumulation (visualized in figure 1). Model 2 compares estimated rate of A β accumulation between a subset of our APOE ϵ 4 carriers (n = 149) matched on baseline age, sex, and baseline A β load to noncarriers (n = 149), showing that APOE ϵ 4 carriers accumulate A β faster than noncarriers.

^aNote this is a matched analysis.

entirely generalizable to other populations of CN adults. Finally, an inclusion of those ≥ 60 years old does not allow studying the relationship between rate of A β accumulation and MRS metabolites at an even earlier stage of AD pathophysiology. However, younger adults do not show sufficient increase in rate of A β accumulation to model longitudinal process. Increase in the rate of A β accumulation on serial PET in those younger than 60 is minimal^{33,39} and limited in capturing potential associations with baseline MRS metabolites.

MRS is a noninvasive and inexpensive technique that can be part of a standard clinical magnetic resonance examination, including in clinical trials. However, for these purposes, the standardization and optimization of multicenter MRS studies are necessary. Moreover, a longitudinal investigation of serial MRS metabolites to estimate progression of A β in participants within the AD continuum would provide additional information on the temporal ordering between the alterations in MRS metabolites and A β pathophysiology.

AUTHOR CONTRIBUTIONS

Dr. Nedelska: study concept and design, analysis and interpretation of the data, drafting the manuscript. S.A. Przybelski: design and analysis or interpretation of the data, critical revision of the manuscript for important intellectual content. T. Lesnick: design and analysis or interpretation

of the data, drafting manuscript, critical revision of the manuscript for important intellectual content. Dr. Lowe: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Schwarz: data analysis, critical revision of the manuscript for important intellectual content. Dr. Machulda: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Mielke: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Roberts: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Kremers: analysis or interpretation of the data, critical revision of the manuscript for important intellectual content. Dr. Boeve: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Knopman: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Petersen: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Jack: acquisition of data, critical revision of the manuscript for important intellectual content. Dr. Kantarci: study concept and design, acquisition of data, interpretation of the data, drafting the manuscript, critical revision of the manuscript for important intellectual content.

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