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Apolipoprotein E, Not Fibrillar β-Amyloid, Reduces Cerebral Glucose Metabolism in Normal Aging

William J. Jagust[1,2] **and Susan M. Landau,**[1,2] **For the Alzheimer's Disease Neuroimaging Initiative**
[1]Helen Wills Neuroscience Institute, University of California at Berkeley, Berkeley, California 94720, and [2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley California 94720

The $\varepsilon4$ allele of the polymorphic apolipoprotein E gene is associated with increased risk of Alzheimer's disease (AD), deposition of β-amyloid (Aβ), and reduction in cerebral glucose metabolism in asymptomatic people. Although ApoE4 may exert an effect on AD risk through amyloidogenic pathways, whether its effect on glucose metabolism is related to Aβ is unknown. To answer this question, we examined data from 175 cognitively normal older people (mean age, 77; 87 men, 88 women) in the Alzheimer's disease neuroimaging initiative studied concurrently with [¹⁸F]flurodeoxyglucose (FDG) positron emission tomography measures of glucose metabolism and the radiotracer [¹⁸F]florbetapir, an imaging agent which labels fibrillar Aβ *in vivo*. Based on a threshold value of florbetapir uptake determined in separate samples, subjects were categorized as florbetapir+ or florbetapir−. Glucose metabolism was measured as a continuous variable in a group of regions of interest (ROIs) selected *a priori* based on their involvement in AD, and also by using a whole-brain voxelwise approach. Among this sample, 29% of subjects were florbetapir+ and 23% were ApoE4 carriers. As expected, there was a significant association between ApoE4 genotype and florbetapir positivity. Florbetapir status, however, was not significantly associated with glucose metabolism, but the ApoE4 genotype was associated with lower metabolism in both voxelwise and ROI approaches. These results show that ApoE genotype, and not aggregated fibrillar forms of Aβ, contributes to reduced glucose metabolism in aging and adds to a growing list of neural consequences of ApoE that do not appear to be related to Aβ.

Introduction

The $\varepsilon4$ allele of the polymorphic apolipoprotein E gene (ApoE4) is a major risk factor for Alzheimer's disease (AD) (Corder et al., 1993; Schellenberg, 1995). This polymorphism affects clearance of the β-amyloid (Aβ) protein that is proposed to play a major role in AD pathogenesis (Castellano et al., 2011). The ApoE4 allele is also associated with increased aggregation of Aβ into toxic oligomeric forms (Ma et al., 1994; Näslund et al., 1995) and

greater deposition of Aβ in amyloid plaques seen at autopsy in human brains (Rebeck et al., 1993; Schmechel et al., 1993). These postmortem results have been extended by *in vivo* human studies that used positron emission tomography (PET) with radiolabeled ligands that bind to fibrillar Aβ to demonstrate increased Aβ deposition in cognitively normal older people carrying the ApoE4 allele (Reiman et al., 2009; Morris et al., 2010; Fleisher et al., 2011).

AD is also associated with reductions in cerebral glucose metabolism that can be measured with [¹⁸F]flurodeoxyglucose PET (FDG-PET) and appear primarily in lateral and medial parietal and lateral temporal cortex (Silverman et al., 2001). Older asymptomatic ApoE4 carriers also show reductions in glucose metabolism that spatially overlap with those seen AD patients (Small et al., 1995; Reiman et al., 1996). These reports appear consistent with the ApoE effects on Aβ, suggesting that genetic effects may be mediated by deposition of Aβ that then results in synaptic dysfunction (Lacor et al., 2007), reducing cortical metabolism. However, the ApoE4 allele is also associated with similar metabolic reductions in individuals at the young ages of 20−39 (Reiman et al., 2004), when Aβ plaque deposition is rare (Braak and Braak, 1997; Kok et al., 2009). This raises the question as to whether metabolic alterations seen in older ApoE4 carriers are due to Aβ deposition or another process.

The ApoE gene, in fact, has a multiplicity of neural effects that are not necessarily related to its role in association with Aβ (Kim et al., 2009). This includes effects on synaptic plasticity and repair (Slezak and Pfrieger, 2003) and on synaptic development (Ji et al., 2003; Levi et al., 2003; Dumanis et al., 2009). Functional MRI

(fMRI) studies of neural activity in young adults with the ApoE4 allele have largely, although not invariably, showed increases in brain activation during cognitive tasks (Trachtenberg et al., 2012) that are also suggestive of possible synaptic alterations at ages when Aβ deposition is unlikely. In cognitively normal older adults, both increases and decreases in brain activation during cognition have been reported with ApoE4 (Bookheimer et al., 2000; Lind et al., 2006); until recently, such results have been difficult to interpret due to the possibility of concomitant unmeasured Aβ. In the present report, we studied a group of cognitively normal older people who were part of the Alzheimer's disease neuroimaging initiative (ADNI) and who had been examined with both FDG-PET and PET amyloid imaging to differentiate the contributions of Aβ and ApoE genotype to resting glucose metabolism.

Materials and Methods

Subjects. ADNI is a longitudinal multisite study supported by the National Institutes of Health, private pharmaceutical companies, and nonprofit organizations with ~50 medical center and university sites across the United States and Canada (www.loni.ucla.edu/ADNI). Individuals are recruited and followed longitudinally with imaging (FDG-PET and MRI), cognitive assessments, and blood and CSF biomarkers. In the second phase of ADNI, PET amyloid imaging with the [^{18}F]-labeled amyloid imaging agent florbetapir (Clark et al., 2011) was added to the protocol. The subjects of this report are 175 cognitively normal ADNI participants who had available FDG-PET, ApoE genotyping, and [^{18}F]Florbetapir-PET as of June 2012. Full inclusion/exclusion criteria can be found at www.adni-info.org, but briefly, all normal subjects had a clinical dementia rating (CDR; Morris, 1993) of 0, did not present with cognitive complaints, and scored normally on a range of cognitive tests.

FDG-PET. Methods for FDG-PET data acquisition and analysis have been published previously (Jagust et al., 2009; Landau et al., 2011). FDG scans were acquired between 30 and 60 min after injection of ~5 mCi of tracer. Images underwent a rigorous quality control protocol and were processed to produce final images with standard orientation, voxel size, and 8 mm resolution (Jagust et al., 2010).

The initial approach to image analysis used a group of regions of interest (ROIs) that were derived from a literature review as described previously (Landau et al., 2011). In brief, we found published papers listing Talairach coordinates representing areas in which FDG uptake differed significantly between AD patients and normal subjects. Intensity values were generated for each study representing the size of group differences, mapped into Talairach coordinates, superimposed, transformed into z-scores, smoothed, intensity normalized, and thresholded. These regions correspond to areas in left and right hemispheres of temporal, lateral parietal, and medial parietal cortex in which AD–normal differences were maximal (Fig. 2) and which we use as a single composite ROI averaged together. For this study, FDG uptake from this composite AD signature ROI, normalized to a reference region of cerebellar vermis and pons, comprised the dependent measure of cerebral glucose metabolism that was extracted from PET data using the ROI template and brain images transformed to standard MNI coordinates using SPM5. While we did not use FDG-PET data in dichotomous form, in a previous study values below 1.22 differentiated AD patients from controls with the best overall accuracy (Landau et al., 2010).

Florbetapir-PET. [^{18}F]Florbetapir and FDG-PET were generally performed within a week of one another. Florbetapir scans were acquired 50–70 min after injection of ~10 mCi of tracer. Images underwent quality control and processing steps similar to those of the FDG-PET data. Scans were analyzed in native space using the subjects' MRI images (one or usually two averaged MPRAGE images acquired close to the time of the florbetapir scans) that were segmented into cortical regions using Freesurfer version 4.5.0 (surfer.nmr.mgh.harvard.edu/). These cortical ROIs were used to extract florbetapir uptake from gray matter in lateral and medial frontal, anterior, and posterior cingulate, lateral parietal, and lateral temporal cortex. Values were normalized to florbetapir uptake in

Table 1. Participant characteristics

	Total sample (N = 175)	ApoE4+ (N = 40)	ApoE4− (N = 135)	Florbetapir+ (N = 50)	Florbetapir− (N = 125)
Age	77.2 (6.0)	76.8 (5.6)	77.3 (6.1)	78.3 (4.8)	76.7 (6.4)
Sex (M/F)	87/88	20/20	67/68	22/28	65/60
Education	16.3 (2.8)	15.9 (2.9)	16.5 (2.7)	16 (3)	16.5 (2.7)
MMSE[a]	29 (1.2)	28.6 (1.3)	29.1 (1.2)	29 (1.2)	29 (1.2)
ADAS-Cog[b]	6 (3.1)	6.8 (3.6)	5.9 (2.9)	6.4 (3.3)	6 (3)
ApoE2 carriers[c]	23	1	22	4	19

[a]ApoE4− has significantly higher MMSE than ApoE4+ ($t = 2.35$, $p = 0.02$).
[b]Alzheimer's Disease Assessment Scale– cognitive subscale.
[c]ApoE2 is more common with ApoE4− than ApoE4+ ($\chi^2 = 5.1$, $p = 0.02$).

the whole cerebellum. The averaged cortical uptake in this composite ROI was used as the index of florbetapir uptake in each subject.

Subjects were characterized as florbetapir positive or negative as described previously (Landau et al., 2012). A threshold value of 1.10 for the cortical/cerebellar ratio in a separate study represented the upper limit of the 95% confidence interval for a group of normal young subjects (Joshi et al., 2012) and was also the value below which no pathological evidence of plaque Aβ deposition greater than "low likelihood" was found in an imaging–autopsy correlation study (Clark et al., 2011). To account for processing differences between those studies and the methods used here, a separate data set was processed with both methods, yielding a regression equation ($y = 0.80x + 0.23$) that permitted conversion of the 1.10 threshold to a value of 1.11, which we applied to the current dataset to define Aβ positivity.

Statistics. Both florbetapir and ApoE genotype were independent binary categorical variables. Glucose metabolism, measured in the composite ROI, was the dependent measure as a continuous variable. The initial linear model evaluating these relationships included age as a covariate; a subsequent model included additional potentially confounding variables and variables of interest. The relationship between florbetapir and glucose metabolism was also analyzed as a continuous function using nonparametric statistics because of the non-normality of the florbetapir distribution. ApoE homozygosity was ignored in all analyses. All statistical tests were considered significant at $p < 0.05$, and two-tailed test results are reported. Statistical analyses used SPSS 20.

Because the selected ROIs were small (see Fig. 2), and to investigate potentially larger regions of hypometabolism that might have been missed, we followed the ROI analysis with a whole-brain voxelwise analysis using SPM5 to examine the effects of both genotype and Aβ on glucose metabolism. Voxelwise FDG images in MNI coordinate space were used to carry out an ANOVA with florbetapir and ApoE genotype as binary categorical independent variables, and age as a variable of no interest (as in the FDG ROI model described above). Voxelwise results were initially displayed at $p < 0.05$ (uncorrected) to illustrate global patterns, but subsequently were corrected for multiple dependent comparisons at cluster corrected $p < 0.001$ (cluster peak threshold, $p < 0.001$; extent threshold, $k = 210$). This was derived from the image volume, degrees of freedom, and image smoothness using Gaussian random field theory (Cao and Worsley, 2001).

Results

Subject characteristics are reported in Table 1. Forty subjects were carriers of the ApoE4 allele; all but three were heterozygotes for an overall allele frequency of 0.12. ApoE2 alleles were significantly more common in ApoE4 noncarriers. Fifty subjects (28.6%) were categorized as florbetapir+. The group as a whole was evenly distributed between men and women. Besides the ApoE2 distribution, the only other significant group differences when subjects were compared by either genotype or florbetapir status was for the Mini-Mental State Examination (MMSE); ApoE4 carriers had slightly but significantly lower mean scores than noncarriers. Of the ApoE4 carriers, 20 (50%) were florbetapir+, and of the noncarriers 30 (22%)
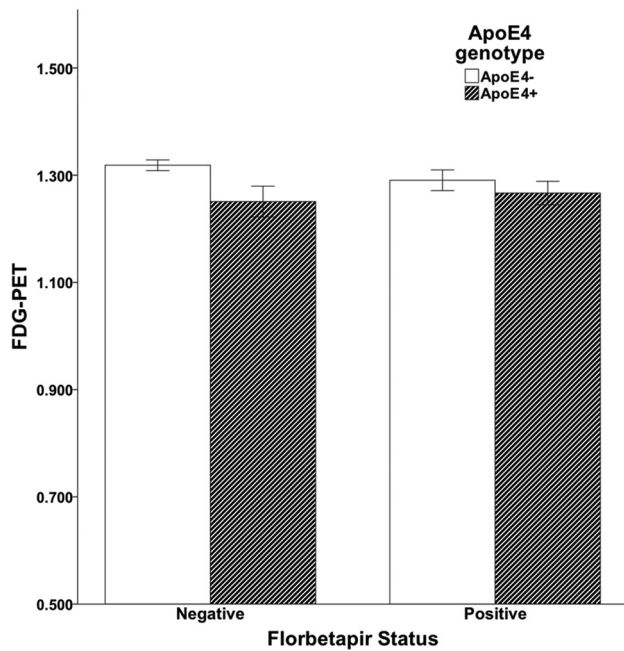
Figure 1. Relationships between florbetapir positivity, ApoE carrier status, and glucose metabolism (FDG-PET). Analysis indicates a significant effect of ApoE, but no effect of florbetapir.

were florbetapir+, a significant association of genotype with Aβ ($\chi^2 = 11.7, p = 0.001$).

Glucose metabolism in the composite ROI was inversely associated with age ($r = -0.19, p = 0.01$) and was also lower in ApoE4 carriers (mean = 1.26, SD = 0.11) than noncarriers (mean = 1.31, SD = 0.10), a statistically significant difference ($t = 2.83, p = 0.005$). There was no significant difference ($t = 1.5, p = 0.14$) in glucose metabolism between those who were florbetapir+ (mean = 1.28, SD = 0.10) and florbetapir− (mean = 1.31, SD = 0.11). There was also no relationship between glucose metabolism and florbetapir as a continuous variable (Spearman $\rho = -0.09, p = 0.24$).

The initial linear model revealed no effect of florbetapir ($p = 0.64$), but a significant effect of ApoE genotype ($p = 0.007$) and age ($p = 0.008$) on glucose metabolism, with the ApoE4 genotype and older age associated with lower glucose metabolism in the composite ROI. A second model added sex, MMSE score, ApoE2 genotype, and an ApoE4 × florbetapir interaction term. None of these additional model parameters were significantly associated with glucose metabolism, and there was no effect on the original results other than changes in p values (for ApoE, $p = 0.03$; for age, $p = 0.01$; and for florbetapir, $p = 0.98$). Glucose metabolism in the composite ROI, plotted as a function of ApoE genotype and florbetapir status, is shown in Figure 1.

In the voxelwise analyses, ApoE4 carriers compared to noncarriers showed reductions in metabolism throughout the entire brain. Figure 2 shows voxels that met a threshold of at least $p < 0.05$ (uncorrected), with cluster peak voxels that met the $p < 0.001$ cluster threshold shown in Table 2. Some of these findings were in brain regions associated with metabolic reductions in AD, such as the temporal and parietal cortex, but reductions were much more widespread, including both lateral and medial prefrontal cortex. While reductions were extensive, clusters that met criteria for significance were seen particularly in temporal, parietal, and frontal cortex. There were no regions in which ApoE4 carriers showed higher metabolism than noncarriers at $p < .05$ or lower. Contrasts of subject groups by florbetapir status revealed

several small regions of increased metabolism in superior parietal cortex in florbetapir+ subjects compared to florbetapir−, and several regions in temporal lobes in which the florbetapir− subjects showed greater metabolism. These regions were not significant using the cluster threshold.

Discussion

These data show a significant effect of ApoE4 genotype on cerebral glucose metabolism in aging that is not explained by the presence of fibrillar cerebral Aβ as detected by florbetapir binding. Florbetapir uptake was not associated with glucose hypometabolism, while both age and ApoE genotype were. Even after accounting for age effects, the presence of the ApoE4 allele remained associated with lower glucose metabolism. These results were seen in a pre-specified ROI analysis that sampled regions typically affected by AD, and a whole-brain voxelwise analysis found widespread reductions in metabolism associated with the ApoE4 genotype. This adds to a growing list of functional brain abnormalities that are associated with the ApoE4 allele and do not appear mediated by fibrillar, aggregated forms of Aβ.

The results from the ROI approach and the voxelwise approach require integration with one another. The ROIs sampled voxels that mainly showed metabolic reductions at the $p < 0.05$ level. Because these ROIs targeted specific brain regions and were not corrected for multiple comparisons, they were highly sensitive in defining abnormalities. This ROI approach has been applied and found to be sensitive in different laboratories and different samples for the detection of AD (Landau et al., 2010; Caroli et al., 2012; Jack et al., 2012), but it is likely nonspecific for AD. Thus, the idea that these metabolic reductions represent presymptomatic AD is not necessarily supported by the widespread reductions seen in the whole-brain analysis. For the voxelwise analysis, the actual pattern of results depends on the significance threshold; widespread reductions are seen at the liberal $p < 0.05$ level, but more restricted reductions are noted when using a cluster threshold. While neither set of whole-brain results defines voxels limited to regions typically affected by AD, they are consistent with earlier studies showing widespread ApoE4 effects on metabolism (Reiman et al., 1996, 2004). Regardless of thresholding, there are numerous brain regions in which ApoE4 carriers show reductions compared to noncarriers, and no regions in which the reverse is seen. These findings are not restricted to the pattern of hypometabolism seen in patients with AD.

The metabolic reductions in some of the association cortex, especially those seen on the medial surface in Figure 2, includes regions that in PET and fMRI studies are part of the default mode network (DMN), a network that is more active in the absence of externally presented stimuli and deactivates during externally driven cognitive engagement (Raichle et al., 2001). This network becomes disconnected in AD (Greicius et al., 2004) and in normal older people with evidence of Aβ deposition detected with another amyloid imaging agent, [^{11}C]PIB (Pittsburgh Compound B; Hedden et al., 2009; Mormino et al., 2011). It is also a region in which Aβ tends to be deposited (Buckner et al., 2005). However, neither the ApoE4 effects seen here, nor the pattern of Aβ deposition conforms specifically to the topography of the DMN (Buckner et al., 2009). Based on both the pattern of hypometabolism and its lack of association with aggregated Aβ, our results do not support an explanation whereby ApoE4 increases Aβ deposition in the DMN, leading to AD. Another possibility is that the widespread metabolic reductions seen throughout the brain of ApoE4 carriers confers vulnerability to other processes,
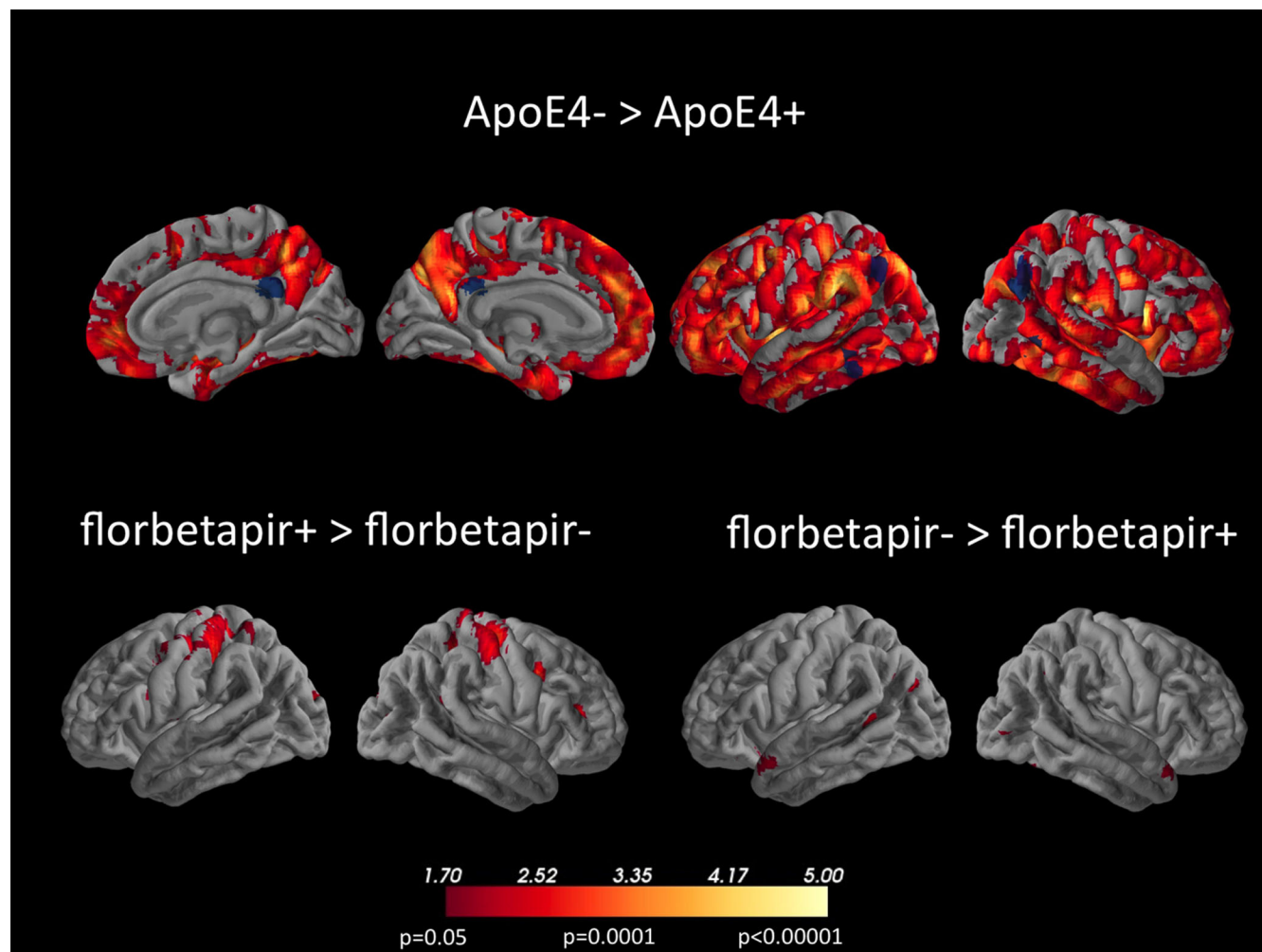
ApoE4- > ApoE4+

florbetapir+ > florbetapir-　　　　**florbetapir- > florbetapir+**

1.70　　2.52　　3.35　　4.17　　5.00

p=0.05　　　　p=0.0001　　　　p<0.00001

Figure 2. Results of whole brain voxelwise analysis showing three contrasts as indicated in the label. Lower threshold for all images is $t = 1.70$, $p = 0.05$ uncorrected, with brighter shades indicating higher t and p values as indicated. No significant voxels were found for the contrast ApoE4+ > ApoE4−. The blue set of ROIs represents the composite, pre-specified ROI as defined in the text. The peak voxels of clusters meeting the $p < 0.001$ clusterwise criterion (see Materials and Methods) for the ApoE4− > ApoE4+ contrast (top row) are listed in Table 2.

Table 2. Hypometabolic regions for ApoE4 carriers relative to non-carriers

	k	t	x	y	z
Left inferior frontal gyrus	383	4.8	−30	44	−2
Right rolandic operculum	653	4.6	42	−28	20
Left supramarginal gyrus	1124	4.4	−60	−32	28
Left middle occipital gyrus	292	4.3	−32	−72	38
Left fusiform gyrus	314	4.1	−34	−30	−16
Left precentral gyrus	232	4.0	−24	−24	58
Right insula	244	4.0	32	14	−14
Right fusiform gyrus	222	3.8	40	−16	−20

such as metabolic stress (Vaishnavi et al., 2010; Vlassenko et al., 2010), that are involved in the pathogenesis of AD.

Other reports confirm an effect of the ApoE polymorphism on brain function that is independent of Aβ. An fMRI study examined the DMN in normal older subjects who were characterized as Aβ negative using PIB-PET (Sheline et al., 2010). Contrasts between ApoE4 carriers and noncarriers revealed multiple nodes in which connectivity was increased in the carriers relative to noncarriers, as well as areas where connectivity was decreased. Another recent report examined relationships between Aβ, ApoE genotype, and cognition and found an interaction such that Aβ had more deleterious effects

on cognitive function in ApoE4 carriers (Kantarci et al., 2012), another indication of a genotypic effect on brain function in aging that is independent of the amount of Aβ. The numerous studies indicating hypometabolism (Reiman et al., 2004), brain atrophy (Shaw et al., 2007), and alterations in resting state connectivity and brain activation (Filippini et al., 2009) in young adults at ages well before likely fibrillar Aβ pathology support the idea of amyloid-independent effects on neural processes. Finally, ApoE has also been shown to have an effect on mitochondrial function, with young ApoE4 carriers without Aβ showing reduced cytochrome oxidase activity compared to noncarriers (Valla et al., 2010).

While the results of this study indicate that fibrillar, aggregated forms of Aβ are an unlikely cause of hypometabolism in ApoE4 carriers, Aβ also occurs in soluble, oligomeric forms that may affect neural function and are not detected by PET imaging agents (Shankar et al., 2007; Walsh and Selkoe, 2007). Therefore, it is possible that there is undetected soluble Aβ in ApoE4 carriers that would account for the hypometabolism in this group. However, aggregated fibrillar forms of Aβ in amyloid plaques are accompanied by soluble Aβ in human brains (Klunk et al., 2005), and the concentration of soluble Aβ is correlated with Aβ plaque burden (Kuo et al., 1996). Thus, failure to find an association between fibrillar Aβ and hypometabolism cannot be explained by

the absence of soluble Aβ. Second, a recent study of biomarkers in presymptomatic, dominantly inherited AD suggests that soluble Aβ may become elevated and then begin to decline 25 years before symptoms, before fibrillar aggregation can be detected in the brain (Bateman et al., 2012). These forms of Aβ could contribute to hypometabolism and yet go undetected with amyloid imaging agents. However, glucose hypometabolism was not seen at early time points, but only years later, well after Aβ was detectable in fibrillar form with PET. ApoE4 also plays a role in enhancing the synaptotoxic effects of oligomeric forms of Aβ (Koffie et al., 2012). While it seems unlikely that soluble Aβ explains the hypometabolism in our study, we cannot definitively exclude that possibility. These data do not suggest that the risk for AD seen with the ApoE4 genotype is unrelated to Aβ, as we confirmed the well-described association between ApoE4 and Aβ deposition (Morris et al., 2010; Rodrigue et al., 2012) that, along with other extensive data, certainly makes it likely that this is one pathway through which ApoE may exert its effects. However, these results indicate that more than one such ApoE-mediated route to AD is likely to exist.

A conceptual model that unifies these results with theories of AD pathogenesis has recently been proposed (Jagust and Mormino, 2011). This model builds upon the observation, made through extensive animal studies, that neural activity modulates the secretion of Aβ through synaptic exocytosis (Nitsch et al., 1993; Kamenetz et al., 2003; Cirrito et al., 2005). In young transgenic mice, neural activity is related to both Aβ release and later regional deposition of plaques (Bero et al., 2011). This has led to the conjecture that neural activity itself may play a role in Aβ deposition in humans, an idea supported by findings that Aβ release parallels fluctuations in synaptic activity in human sleep/wake cycles (Gilestro et al., 2009) and is associated with level of consciousness in coma (Brody et al., 2008). The ApoE4 genotype could play a role in this process by reducing neural efficiency. Mice expressing human ApoE4 display abnormalities of synaptic transmission and dendritic arborization (Wang et al., 2005), reduction in dendritic spines (Ji et al., 2003; Dumanis et al., 2009), and alterations in LTP (Trommer et al., 2004). Taken in conjunction with the human imaging data showing lower resting metabolism and greater activation in the brains of young ApoE4 carriers performing cognitive tasks (Reiman et al., 2004; Filippini et al., 2009; Dennis et al., 2010; Filippini et al., 2011), these findings raise the possibility that resting metabolic dysfunction due to either ApoE4-related synaptic alterations or mitochondrial energetics results in the necessity for increased synaptic activity that might lead to more Aβ release.

Finally, these data have implications for existing models of the AD pathological cascade, which suggest that Aβ deposition precedes synaptic dysfunction (Jack et al., 2010). Our results argue for the reverse, at least in ApoE4 carriers. Changes in glucose metabolism may reflect synaptic activity, because the majority of the energy budget of the neuron is devoted to signaling (Attwell and Laughlin, 2001), and because correlations between glucose metabolism and synaptic markers have been reported (Rocher et al., 2003). In studies of AD and presymptomatic stages of AD to date, it appears that Aβ is detectable before metabolic decline (Lo et al., 2011). However, our ability to measure Aβ *in vivo* is relatively new, so examination of changes in metabolism and brain function in individuals who might be at risk for AD but have no detectable Aβ is limited. Future studies of unaffected individuals with different risks for AD should expand our understanding of the sequence of events in the development of this disorder.

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