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Differential Effect of APOE Alleles on Brain Glucose Metabolism in Targeted Replacement Mice: An [^{18}F]FDG-μPET Study

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Abstract.

Background: The Apolipoprotein E (ApoE) alleles ε2, ε3, and ε4 are known to differentially modulate cerebral glucose metabolism and the risk for Alzheimer's disease (AD) via both amyloid-β (Aβ)-dependent and independent mechanisms.

Objective: We investigated the influence of ApoE on cerebral glucose metabolism in humanized APOE Targeted Replacement (TR) mice at ages that precede the comparison of Aβ parenchymal deposits in APOE4-TR mice.

Methods: Fludeoxyglucose ([^{18}F]FDG) positron emission tomography (PET) measures were performed longitudinally in homozygous APOE-TR mice (APOE2, APOE3, APOE4; $n = 10$ for each group) at 3, 5, 11, and 15 months. Results were quantified using standard uptake values and analyzed statistically using a linear mixed effects model. Levels of the $A\beta_{40}$ and $A\beta_{42}$ peptides were quantified *ex vivo* using enzyme-linked immunosorbent assay (ELISA) at 15 months in the same animals.

Results: APOE2 mice (versus APOE3) showed a significant increase in glucose metabolism starting at 6 months, peaking at 9 months. No evidence of hypometabolism was apparent in any region or time point for APOE4 mice, which instead displayed a hypermetabolism at 15 months. Whole brain soluble $A\beta_{40}$ and $A\beta_{42}$ levels were not significantly different between genotypes at 15 months.

Conclusions: Introduction of human APOE alleles ε2 and ε4 is sufficient to produce alterations in brain glucose metabolism in comparison to the control allele ε3, without a concomitant alteration in $A\beta_{40}$ and $A\beta_{42}$ levels. These results suggest novel Aβ-independent metabolic phenotypes conferred by ε2 and ε4 alleles and have important implications for preclinical studies using TR-mice.

Keywords: Alzheimer's disease, amyloid-β, APOE, [^{18}F]FDG, glucose, mice, PET, preclinical

INTRODUCTION

APOE, a glycoprotein widely expressed throughout the periphery and the central nervous system (CNS), has a pivotal role in the transport and metabolism of cholesterol and lipids [1, 2].

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In humans, the ApoE gene is polymorphic with three common isoforms (ApoE2, ApoE3, and ApoE4). The three alleles have a skewed distribution in most human populations with a frequency of 5–10% for the ε2 allele, 50–70% for ε3 allele, and 10–15% for the ε4 allele [3, 4]. Although the three isoforms only differ in one to two amino acids, these substitutions have a profound effect on APOE structure and function, affecting, for instance, cholesterol and triglyceride plasma levels in humans [5, 6].

In clinical neuroscience research a strong interest for ApoE has arisen from the finding that the ε4 allele is an established risk factor for Alzheimer's disease (AD) [7] while the ε2 allele, at least in some human samples, appears to be protective against AD in comparison to ε3 [8, 9].

The radiotracer [^{18}F]FDG has been used in a number of PET studies to demonstrate a pattern of decrease in glucose metabolism in distinct brain regions in AD patients. On the basis of this observation it has been suggested that [^{18}F]FDG imaging could be used as a biomarker for AD [10–13]. Interestingly, more recently it has been shown that, in patients populations with a history of AD and healthy adults, carriers of the ε4 allele have lower brain glucose metabolism compared to non-carriers [14–17].

Several rodent and cell culture studies have demonstrated that APOE status also has a direct impact on Aβ aggregation and clearance (reviewed in [18–20]). However, it is debated whether APOE4 is a risk factor for AD solely because of its role in amyloid deposition. An alternative hypothesis is that other physiological roles of APOE may contribute to the pathophysiology of AD independently of Aβ [21, 22]. For instance, a reduction in brain metabolism measured with [^{18}F]FDG in ε4 carriers has also been reported at an age that normally precede amyloid deposition (i.e., 20–39 years [23]).

A line of evidence for Aβ-independent effects of APOE on brain metabolism has also come from preclinical studies using APOE targeted replacement (TR) mice [24–26], which express human apoE (hApoE) isoforms under the control of the murine ApoE (mApoE) promoter. APOE4-TR have limited parenchymal amyloid deposits which are only evident at 18 months [27]. At ages preceding the formation of amyloid deposits it has been reported that APOE4-TR (in comparison to APOE3-TR mice) display deficits in long term potentiation in the hippocampus [28], dendritic length and spine density [29], insulin signaling [30], as well as in the function and axonal transport of mitochondria [31, 32]. All these factors may concur in producing the metabolic deficits associated with the ε4 allele in a way that is independent from effects on Aβ aggregation [22, 33].

To our knowledge, a crucial aspect of APOE-TR mice has not been investigated to date, namely, do APOE4-TR mice display the hypometabolism phenotype identified in humans? In addition, no human [^{18}F]FDG study has so far concentrated on the metabolic profile of carriers of the protective ε2 allele, probably because of its low frequency in human populations [33].

The aim of the present [^{18}F]FDG PET study was to examine longitudinally alterations in glucose metabolism in adult APOE2/3/4-TR mice up to an age (<18 months) that precedes the appearance of Aβ plaque deposits. To test for any concurrent alteration in Aβ aggregation, brain $A\beta_{40}$ and $A\beta_{42}$ levels were quantified *ex vivo* after the last PET measurement.

METHODS

Animals

All animal experiments were conducted according to Swedish regulations with the approval of the Animal Research Ethics Committee of the Swedish Animal Welfare Agency (Northern Stockholm Region) and were performed according to the Karolinska Institutet guidelines for working with experimental animals (Dnr.: N557/11).

APOE2/3/4 TR female mice (C57BL/6 background) were obtained from Taconic (Denmark). Female mice were selected because previous work has shown that behavioral and molecular deficits in APOE4-TR mice are more pronounced for female gender (reviewed in [34]), consistent with observations in humans showing higher risk for AD in female ApoE4 carriers [35]. Animals were housed under a 12 : 12 h light/dark cycle at constant temperature (∼22°C) and humidity with *ad libitum* access to food and water. Animal were fed on a regular chow diet (LABFOR r70 pellets with 4.5% fat content). Information on the time-course for animal weight is reported in Supplementary Table 1.

Study design

The study employed a balanced longitudinal design in which $n = 10$ mice for each genotype were scanned at 3, 6, 11, and 15 months of age. The 30 animals were imaged over 3 days at each time point with scan order being randomized according to genotype.

In vivo imaging with small animal PET

Mice were fasted for at least 4 h before the start of the experiments. Animals were anesthetized with isofluorane for a short period of time (induction 4–5% mixed with oxygen and maintenance 1.5–2% mixed with air/oxygen (50/50)) and [^{18}F]FDG was administered via a bolus-injection in the tail vein. After injection of [^{18}F]FDG, animals were allowed to wake and each mouse was transferred to an individual box with temperature controlled via a heating pad to allow distribution of the tracer (45 min total). The animals were then re-anesthetized with isofluorane and positioned into the nanoScan PET/MRI or nanoScan PET/CT (Mediso Ltd., Budapest, Hungary) in a transaxial position with their heads in the center of the field of view. Values for injected radioactivity were in the range of 10.1 MBq–15.6 MBq, except 2 out of 120 measurements where injected radioactivity was 21.2 and 21.8 MBq, respectively (Supplementary Table 2).

The PET data was acquired in list mode and was reconstructed to one 40-min static scan with Maximum Likelihood Estimation Method (MLEM) reconstruction (20 iterations). The acquired PET data was not corrected for attenuation or scatter.

$A\beta_{40}$ and $A\beta_{42}$ ELISA

Upon termination of the last [^{18}F]FDG measurement animals were returned to their home-cage. Animals were sacrificed 2–4 days after the last PET measurement and the whole brain was dissected and preserved at –85°C. Soluble brain $A\beta_{40}$ and $A\beta_{42}$ levels were quantified using a commercial sandwich ELISA (KMB3481 and KMB3441; Invitrogen), following a diethylamine extraction procedure described previously [36].

PET data analysis

The reconstructed static images were manually aligned to a standard mouse template [37] using PMOD (PMOD 3.7, Zurich, Switzerland). Images were then normalized voxel-wise to represent standardized uptake value (SUV) according to equation 1:

$$\mathrm{SUV} = \frac{\text{tissue activity concentration (Bq/cm3)}}{\text{injected dose (Bq)} / \text{body weight (g)}} \quad (1)$$

Four regions of interest (ROIs) and the whole brain were defined using the mouse template atlas available in PMOD. The ROIs were cortex, cingulate cortex, hippocampus and cerebellum. We selected these regions because in human studies they have been consistently associated either with hypometabolism or, in case of the cerebellum, display no metabolic change [38–40].

To examine potential alterations in glucose metabolism outside of the brain, we also defined a ROI in the Harderian glands. First, a template ROI for Harderian glands was manually drawn using PMOD on the average PET image for the study (i.e., average of 120 PET measurements). Then the Harderian gland ROI was manually realigned to the average PET image for each time point and genotype.

Custom made MATLAB (MATLAB 2015a, The MathWorks Inc., USA) scripts based on SPM12 (Wellcome Department of Cognitive Neurology, University College London, UK) were used to compute regional average SUV values.

Statistics

Statistical analysis was performed in R (R Development Core Team, 2009). For the PET data, a linear mixed-effects (LME) model was employed for the statistical analysis (*lme4* package [41]). The LME applied included genotype, region, weight and injected radioactivity as fixed effects (descriptive statistics in Supplementary Tables 1-2). Time was included as a categorical variable due to its nonlinear effect on SUV (see Fig. 3). Second order interactions between time and genotype were also included in the model. Likelihood ratio test was performed to assess the significant inclusion of each fixed effect and of the interactions in the model. Animal-specific intercepts were included as random effects in the model. Predicted values were estimated from the previously specified LME model for each region of interest described above.

Given that $A\beta_{40}$ and $A\beta_{42}$ concentrations were available only for the last PET measure in each animal, they were not included in the LME model. Differences in $A\beta_{40}$ and $A\beta_{42}$ levels due to genotype were tested with Kruskal–Wallis one-way analysis of variance. Correlation of $A\beta_{40}$ and $A\beta_{42}$ levels with whole brain SUV values at the last PET measure was computed with the Kendall rank correlation coefficient and tau test.

RESULTS

All PET measurements ($n = 120$) were performed according to the protocol and included in the

analysis. The average horizontal SUV images for the APOE-TR mice at the four time points investigated are presented in Fig. 1A (sagittal slice images in Supplementary Figure 1).

The uptake of [^{18}F]FDG in the whole brain was rather homogenous. In the Harderian glands mean SUV values were about twice as high as in whole brain. For each of the three genotypes, the variability for the whole brain SUV (Fig. 1B) was low in the baseline scan at 3 months (first quartile 1.58 SUV – third quartile 1.90 SUV) and there was no obvious difference between genotypes. At later time points the variability increased within groups and between group differences also became apparent.

Similarly to the whole brain data, regional differences in posterior cingulate cortex, cortex, hippocampus and cerebellum (Fig. 2A-D) were not apparent across groups at baseline. The highest [^{18}F]FDG uptake in APOE2-TR animals was at the 11 month time point in the four brain ROIs. In APOE4-TR mice a small increase in SUV was only consistently observed at the last time point (i.e., 15 months).

In the case of the extracerebral Harderian glands (Fig. 2E), differences between genotypes were already apparent at the time of the baseline scan. In addition, the variability within groups was greater than for the whole brain. APOE2-TR had higher mean

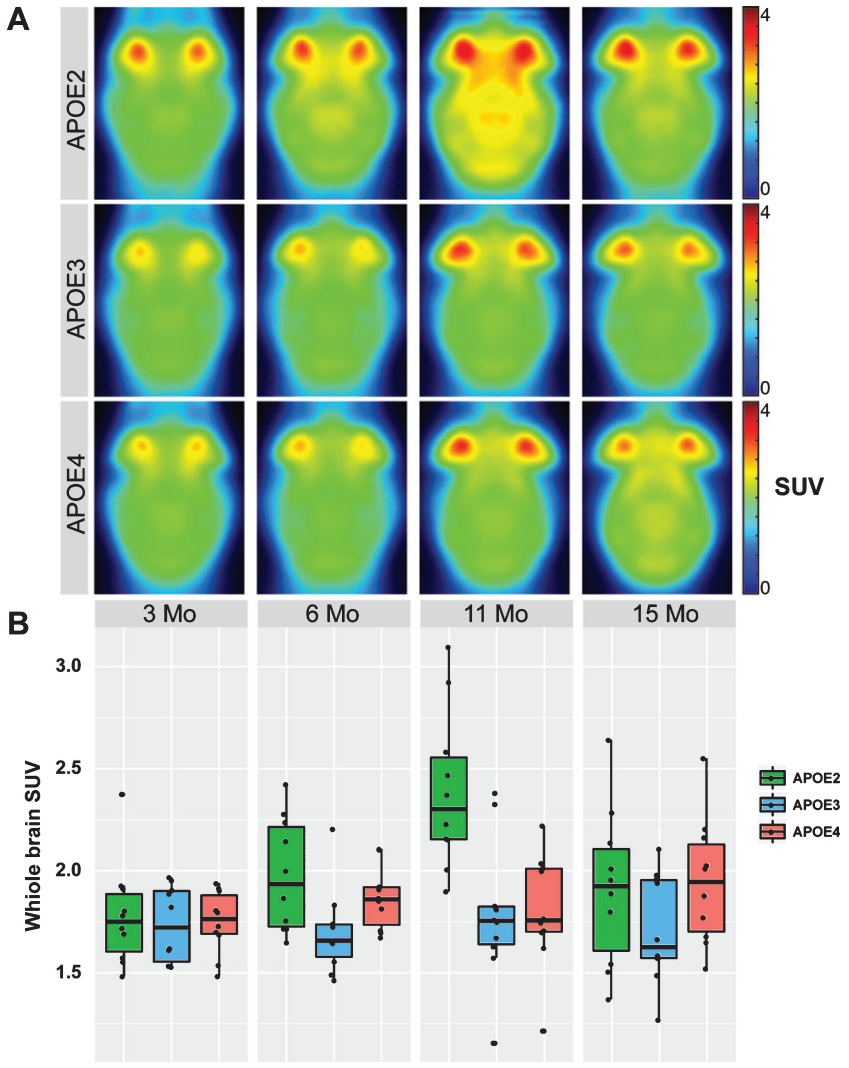

Fig. 1. Average horizontal SUV PET images of [^{18}F]FDG uptake in 3, 6, 11, and 15 months old APOE2/3/4 TR mice (A). Bright red spots in each PET image correspond to the Harderian glands. Quantification of the whole brain SUV (B). Boxplots show median and the 25th and 75th percentiles; upper and lower whisker extends ± 1.5 times the inter-quartile range. Raw data points are plotted superimposed to the boxplot

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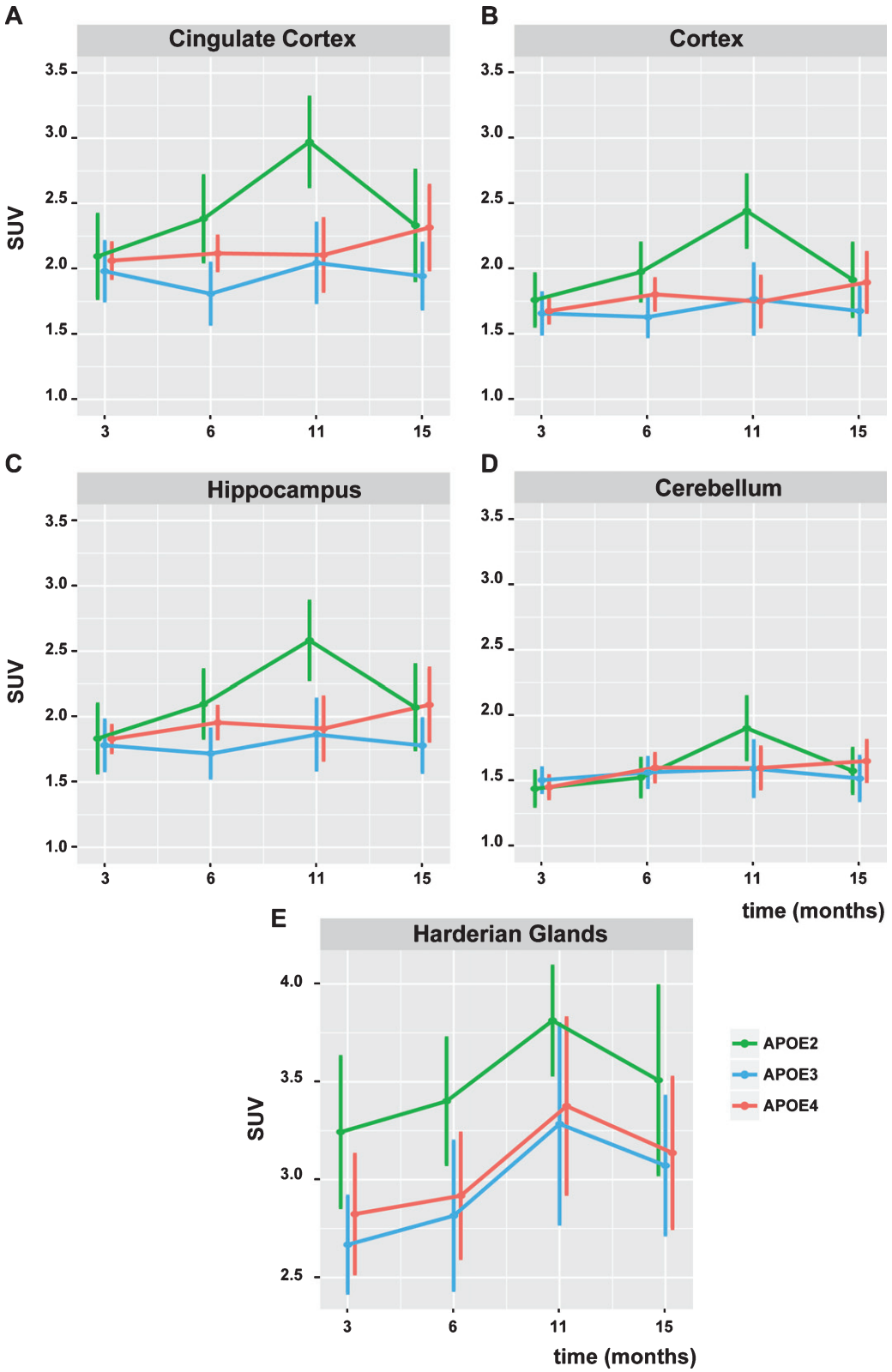

Fig. 2. SUV uptake of [^{18}F]FDG in cingulate cortex (A), cortex (B), hippocampus (C), cerebellum (D), and Harderian glands (D) at 3, 6, 11, and 15 months. Means and 95% confidence intervals are shown.

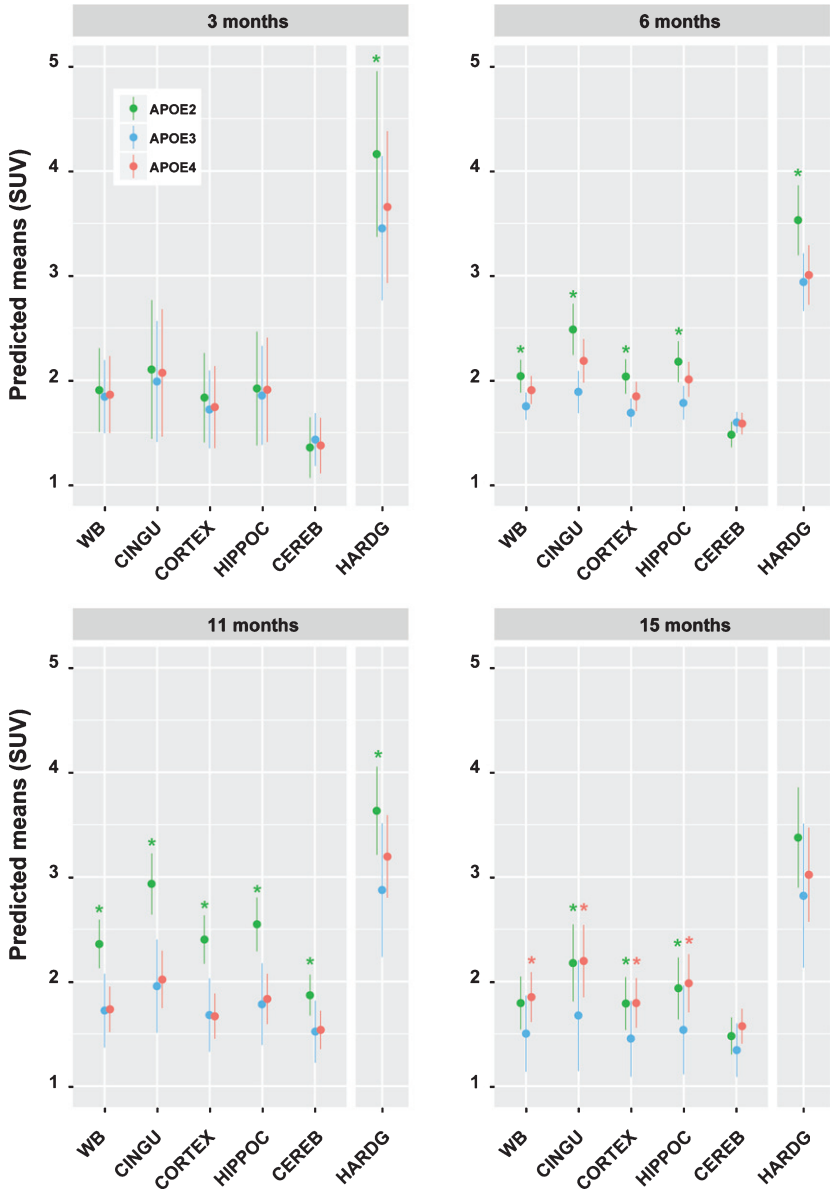

Fig. 3. Predicted means for the linear mixed model showing effect of age by genotype and by region at the four time points. Asterisks represent $p < 0.05$ for the comparison APOE2 or APOE4 versus APOE3 for each ROI; lines represent confidence intervals. Whole brain (WB), cingulate cortex (CINGU), hippocampus (HIPPOC), cerebellum (CEREB), Harderian glands (HARDG).

[^{18}F]FDG uptake in the Harderian glands compared to the other genotypes at all time points, while in APOE4-TR mice no difference in Harderian glands SUVs was apparent in comparison to the control group APOE3-TR mice.

To analyze the differences in our longitudinal dataset we employed a linear mixed effects (LME) model (the coefficients of the LME are listed in Supplementary Table 3). Predicted means from the fitted LME model, computed at weight = 25 g, and injected radioactivity = 13 MBq, for the three genotypes and four time points are presented in Fig. 3. In the following paragraph, we report statistical significance (p-value < 0.05) for the comparison of APOE2 and APOE4 ROIs vs the respective ROI of the control group APOE3 at each time point. Absolute p-values are listed in Supplementary Table 4.

At baseline, no statistically significant differences were found for the whole brain and brain ROIs. APOE2-TR mice displayed an increase in [^{18}F]FDG uptake at later time-points, in particular in whole brain, cingulate cortex, cortex, and hippocampus at 6 months and a global increase at 9 months (p-value <0.05 in all brain ROIs), with the strongest effect in cingulate cortex. At 15 months, the significant increase persisted in all brain ROIs except the cerebellum.

APOE4-TR mice showed no significant decrease in [^{18}F]FDG uptake in any brain region in comparison with APOE3-TR, but at 15 months a significant increase in [^{18}F]FDG uptake was present (p-value < 0.05) in whole brain and all brain ROIs except the cerebellum.

As for the predicted means for the Harderian glands, APOE2-TR had significantly higher [^{18}F]FDG uptake at baseline, an effect that persisted at 6 and 11 months. In APOE4-TR mice no statistically significant difference was observed for the Harderian glands at any time point.

Ex vivo quantification of $A\beta_{40}$ and $A\beta_{42}$ levels immediately after the last PET measurement revealed no effect of genotype on the brain soluble $A\beta_{40}$ or $A\beta_{42}$ fraction ($A\beta_{40}$: Kruskal-Wallis chi-squared = 1.0661, p-value = 0.59; $A\beta_{42}$: Kruskal-Wallis chi-squared = 2.7948, p-value = 0.25) (Fig. 4A-B, left panel). In one animal in the APOE4-TR group the $A\beta_{40}$ quantification was not possible due to a technical error.

The levels of $A\beta_{40}$ were not significantly correlated with the whole brain [^{18}F]FDG uptake at 15 months in any of the three genotypes (APOE2, tau = 0.02, p-value = 0.19; APOE3, tau = 0.11, p-value = 0.36; APOE4, tau = 0.11, p-value = 0.38). As for $A\beta_{42}$, we note that in the case of APOE4 the Kendall's tau coefficient was 0.38 (p-value = 0.08), while in the case of APOE2 and APOE4 no such trend was apparent (APOE2, tau = 0.02, p-value = 0.50; APOE3, tau = –0.06, p-value = 0.64) (Fig. 4A-B, right panel).

DISCUSSION

In this longitudinal study, we used [^{18}F]FDG PET to investigate age-related changes in glucose metabolism in APOE-TR mice. We demonstrated that hAPOE alleles ε2 and ε4 produce alterations in brain glucose metabolism in comparison to the control allele ε3 without a concomitant change in $A\beta_{40}$ and $A\beta_{42}$ levels.

Effect of the ε2 allele on brain glucose metabolism

There was no evident difference in glucose metabolism between the alleles at 3 months. However, beyond 3 months the APOE2-TR mice exhibited a global increase in [^{18}F]FDG brain uptake compared to both APOE3 and APOE4-TR mice. The size of the change was highest in the cingulate cortex, cortex and hippocampus, and lowest in the cerebellum.

The ε2 allele is known to be protective for AD but, to our knowledge, no human [^{18}F]FDG study has been carried out to investigate differences in glucose metabolism in homozygous ε2 carriers. Indeed, ε2/ε2 are only present in 1% of the human population which imposes obvious practical limitations in the design of powered studies [33]. Thus, our study is the first to demonstrate a modulation of glucose metabolism driven by the human ε2 allele.

Although human [^{18}F]FDG data is lacking, there is some structural MRI evidence that young ε2 carriers have higher cortical thickness compared to the other ApoE genotypes [42, 43]. Moreover APOE2-TR mice have been shown to have more complex dendritic arborization and spine length in cortical neurons compared to both APOE3 and APOE4-TR mice [44]. We can speculate that these morphological changes may contribute to the protective role of the ε2 allele [43] and result in a relative increase in cerebral glucose metabolism as demonstrated by the current study.

Effect of the ε4 allele on brain glucose metabolism

In the case of the ε4 allele, differences in brain metabolism were only observed in the LME model analysis at the last time-point 15 months. APOE4-TR mice exhibited a general increase in [^{18}F]FDG brain uptake in comparison with APOE3. The increase in glucose metabolism was significant in cingulate cortex, cortex and hippocampus, whereas the cerebellum was unaffected, showing some consistency with the altered regional pattern seen in human ε4 carriers [33, 45].

However, the direction of the metabolic change was unexpected given that the vast majority of human [^{18}F]FDG studies in ε4 carriers have instead identified patterns of hypometabolism in the brain [45]. Instead, we observed hypermetabolism in APOE4-TR mice at 15 months. The transgenic mice employed in our study were generated with a back-translation

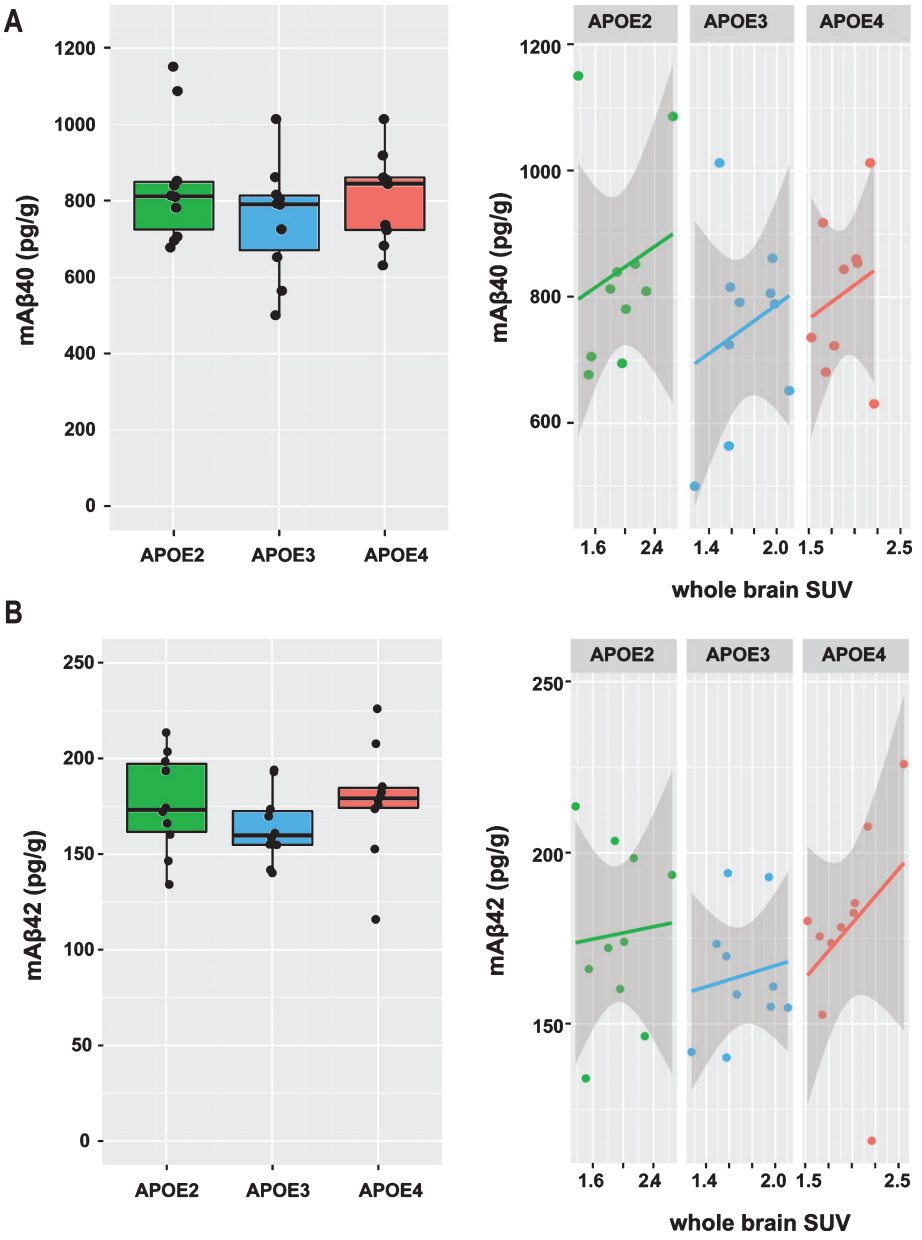

Fig. 4. *Ex vivo* quantification of soluble mouse $A\beta_{40}$ (A) and $A\beta_{42}$ (B) in whole brain homogenate at the 15-months in the same APOE-TR animals for which [^{18}F]FDG PET measures were obtained (left panel). Correlation plot between whole brain SUV and $A\beta_{40}$ (A) or $A\beta_{42}$ (B) concentrations according to genotype; estimated linear regression lines and shaded 95% confidence regions are superimposed (right panel).

approach in which hApoE alleles replace the mApoE gene and APOE isoforms are expressed at physiological levels [46]. We did not confirm the human finding of glucose hypometabolism associated with the ε4 allele and we cannot exclude that this is due to species difference between human and murine hosts. Indeed, APOE4-TR mice replicate some, but not all, phenotypes related to cholesterol clearance and lipid energetics found in human ε4 carriers [26, 47].

Extracerebral modulation of glucose metabolism

While at 3 months, the time of the first PET measurement, there was no effect of genotype on brain glucose metabolism, our analysis demonstrated an already increased [^{18}F]FDG uptake in the extracerebral Harderian glands of APOE2-TR mice. This ε2-driven relative hypermetabolism persisted at 6 and 11 months.

This observation suggests that the ε2 allele also has a role in modulating glucose metabolism in extracerebral areas; the direction of the change is consistent with the increase in [^{18}F]FDG uptake in brain, but temporally precedes cerebral hypermetabolism. Moreover, the fact that APOE2-TR mice display increased plasma cholesterol and lipid levels starting from an age of 2 months [25, 48], taken together with our data, suggests that the ε2 allele may modulate peripheral energy metabolism before having an effect on cerebral metabolism.

Interplay between cerebral glucose metabolism and levels of Aβ$_{40}$ and Aβ$_{42}$

Brain soluble mouse Aβ$_{40}$ and Aβ$_{42}$ concentrations were not significantly different between the three APOE-TR strains at 15 months, indicating that APOE alleles can drive a modulation of cerebral [^{18}F]FDG uptake before any dysregulation of Aβ levels can be detected.

These results were surprising given that APOE4-TR mice have been reported to have parenchymal Aβ deposits (albeit to a very limited extent) that are detectable with immunohistochemistry at 18 months age [27]. However, in the only study in adult animals where parenchymal deposits of Aβ have been reported, no absolute quantification was performed and Aβ deposits were only described qualitatively for the cortex. Moreover, the authors reported that the Aβ pathology effect did not appear with complete penetrance in the animals. Thus it is possible that that our quantitative analysis, performed at the "whole-brain homogenate level" *ex vivo*, did not pick up potential subtle, region specific changes in Aβ levels.

In addition, one study described an increase in Aβ$_{42}$ levels in hippocampal homogenates of young APOE4-TR compared to APOE3-TR mice, but the effect was mainly driven by the hippocampal CA3 area [49]. The same authors also reported that the strain difference in Aβ$_{42}$ immunoreactivity in the hippocampus was abolished by behavioral testing [50], indicating that the differences in Aβ$_{42}$ may be sensitive to subtle manipulations of the environment of the animals.

Therefore the most parsimonious interpretation of our study is that, in our experimental conditions, the introduction of hAPOE alleles is sufficient to elicit a modulation of cerebral glucose metabolism and that this effect is independent from global changes in soluble Aβ$_{40}$ or Aβ$_{42}$ levels.

Notably, in the case of APOE4 we detected a trend for a positive correlation between Aβ$_{42}$ levels and the brain [^{18}F]FDG uptake. Although this result should be replicated in a larger cohort, it cannot be excluded that the hypermetabolism phenotype observed in APOE4-TR mice at 15 months is related to an increase in endogenous Aβ$_{42}$ levels.

Limitations

In our experimental design, animals fasted for a different amount of time according to when during the day the PET measure was performed. Fasting duration is known to have an effect on [^{18}F]FDG uptake in μPET studies [51]; nonetheless, in our design the PET scan order was randomized across genotypes, so this factor is unlikely to have affected our results.

Moreover, in our experiments, [^{18}F]FDG was injected during isofluorane anesthesia, which is expected to decrease [^{18}F]FDG uptake in brain and Harderian glands [51]. However, to our knowledge there is no evidence suggesting that this baseline reduction in [^{18}F]FDG uptake would be different across genotypes.

It has been previously shown that APOE2 and APOE4 TR mice fed on a high-fat diet exhibit significant changes in peripheral cholesterol and triglycerides levels compared to APOE-3 TR mice ([24, 26], but see [52]). In view of the interplay between brain glucose metabolism and peripheral lipid homeostasis [53], this could suggest that some of the brain glucose metabolism changes observed were driven by peripheral changes in lipid metabolism. However, all animal strains in the study were fed on the same, standard, low-fat diet. Therefore, we think that this is unlikely to explain the differences in [^{18}F]FDG uptake observed in the current study, but this hypothesis could be explicitly tested in future studies comparing the effect of low vs high fat diet on brain glucose metabolism in APOE-TR mice.

Implications for future preclinical studies using APOE-TR mice

The increase in [^{18}F]FDG uptake in APOE2-TR mice is highly interesting because it indicates a novel example of cerebral and extra cerebral modulation of glucose metabolism conferred by the ε2 allele. It was recently shown that in the same transgenic animals, the ε2 allele is protective against cognitive decline independently from Aβ accumulation [54]. In future studies it would be of interest to correlate the

performance of APOE-TR mice in memory tasks with their [^{18}F]FDG uptake to investigate any causal role of glucose hypermetabolism on neuroprotection.

Conclusion

In the current study, we have shown novel metabolic phenotypes conferred by human ApoE alleles, in particular we demonstrated that the ε2 and ε4 alleles can modulate brain glucose metabolism (in comparison to the control allele ε3) in the absence of a concomitant change in Aβ_{40} and Aβ_{42} levels. The increase in glucose metabolism in APOE2-TR mice may indicate a novel mechanism for the neuroprotective role of the ε2 allele in AD.

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DISCLOSURE STATEMENT

M.V., A.R., A.B, A.M., and L.F. were full-time employees of AstraZeneca R&D at the time of the study conduct and/or manuscript preparation.

M.T., J.H. and P.F. declare no conflict of interest. The study was carried out under research agreement between AstraZeneca and Department of Clinical Neuroscience, PET Centre, Karolinska Institutet, Stockholm, Sweden.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/ADR-170006.

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