APOE-ε4-related differences in left thalamic microstructure in cognitively healthy adults

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APOE-ε4 is a main genetic risk factor for developing late onset Alzheimer’s disease (LOAD) and is thought to interact adversely with other risk factors on the brain. However, evidence regarding the impact of APOE-ε4 on grey matter structure in asymptomatic individuals remains mixed. Much attention has been devoted to characterising APOE-ε4-related changes in the hippocampus, but LOAD pathology is known to spread through the whole of the Papez circuit including the limbic thalamus. Here, we tested the impact of APOE-ε4 and two other risk factors, a family history of dementia and obesity, on grey matter macro- and microstructure across the whole brain in 165 asymptomatic individuals (38–71 years). Microstructural properties of apparent neurite density and dispersion, free water, myelin and cell metabolism were assessed with Neurite Orientation Density and Dispersion (NODDI) and quantitative magnetization transfer (qMT) imaging. APOE-ε4 carriers relative to non-carriers had a lower macromolecular proton fraction (MPF) in the left thalamus. No risk effects were present for cortical thickness, subcortical volume, or NODDI indices. Reduced thalamic MPF may reflect inflammation-related tissue swelling and/or myelin loss in APOE-ε4. Future prospective studies should investigate the sensitivity and specificity of qMT-based MPF as a non-invasive biomarker for LOAD risk.

As the global population ages, an increasing number of people over 65 will develop dementia due to late onset Alzheimer’s disease (LOAD)1. LOAD is characterized by the development of amyloid-β plaques and neurofibrillary tau tangles that spread from limbic regions to neocortical areas2–4. As these pathological processes are thought to accumulate over many years5, it may be possible to identify brain changes related to heightened risk in asymptomatic individuals prior to the onset of memory impairment.

Carriage of the Apolipoprotein E (APOE)-ε4 genotype is the best-established genetic risk factor of LOAD6. APOE is the main cholesterol carrier in the brain that supports lipid transport, myelination, synaptic repair and the regulation of amyloid-β aggregation and clearance5. Individuals who carry the APOE-ε4 isoform compared to those with APOE-ε2 and -ε3 show an earlier onset of LOAD6,8, and a larger burden of amyloid-β plaques10–14. Such harmful effects of APOE-ε4 are heightened in individuals with a family history of LOAD15,16, probably due to the presence of other polygenic risk variants such as those of APOE10. In addition, APOE-ε4 is known to combine adversely with lifestyle-related risk notably central obesity19,20. Excessive abdominal visceral fat can lead to the metabolic syndrome, type 2 diabetes, and cardiovascular disease21 and obese APOE-ε4 carriers are more likely to develop hypertension, inflammation and insulin resistance22,23.

Much attention has been devoted to characterizing APOE-ε4-related changes in medial temporal lobe regions, notably in the hippocampus and parahippocampal regions24–26 due to their importance for episodic memory. Hippocampal volume loss on magnetic resonance imaging (MRI) is also one of the diagnostic biomarkers of LOAD27. However, hippocampal atrophy is lacking in specificity28 and usually occurs in more advanced disease stages29. Indeed, evidence regarding hippocampal atrophy in APOE-ε4 carriers is mixed and is often thought to result from the inclusion of older participants with underlying LOAD pathology30,31. It, therefore, stands to reason that hippocampal volume loss may not be sufficiently sensitive to detect very early disease changes and it has been proposed that focusing on specific hippocampal subregions such as CA1 and subiculum may be

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Table 1. Summary of demographic, genetic, and lifestyle risk information of CARDS participants. 

| Sample size n | Mean (SD) (range) |
|---------------|-------------------|
| Age (in years) | 55.7 (8.2) (38–71) |
| Females       | 57%               |
| NART-IQ       | 116.8 (6.7) (96–128) |
| MMSE          | 29.1 (0.9) (27–30) |
| FH+           | 35.8%             |
| APOE4 +       | 38.8%             |
| WHR           | 1.4 (0.5) (0.7–2.2) |
| Systolic BP (mm Hg) | 132 (18.8) (68.3–196) |
| Diastolic BP (mm Hg) | 83.3 (9.4) (58.7–118.7) |
| Smokers       | 5.5%              |
| Diabetes      | 1.8%              |
| Alcohol units per week | 7.4 (9.4) (0–60) |
| PHQ-9 Depression score | 2.6 (2.9) (0–13) |

APOE = Apolipoprotein-E based on DNA extraction and APOE genotyping of saliva samples using TaqMan genotyping of single nucleotide polymorphism (SNP) rs7412 and KASP genotyping of SNP rs429358. FH = Family History of a first degree relative affected by Alzheimer’s or Lewy body disease or vascular dementia. MMSE = Mini Mental State Exam (maximum score = 30)42, NART-IQ = National Adult Reading Test- Intelligence Quotient44, PHQ-9 = Patient Health Questionnaire (maximum score = 27)109. WHR = Waist-to-Hip-Ratio.

more promising22,23. However, it is also possible that limbic regions other than the hippocampus may play an important role in the development of LOAD. Notably, it has been recognised for a while that LOAD pathology may spread through the whole of the Papez circuit and may critically involve the limbic thalamus4. For instance, neurofibrillary accumulations in the anterodorsal thalamic nucleus have been found at the same time as those in the hippocampus in LOAD brains24 and reduced thalamic MRI volume has been observed in amnestic Mild Cognitive Impairment (MCI)36, LOAD35 and presymptomatic presenilin 1 mutation carriers37. Similarly, Positron Emission Tomography (PET) studies have found APOE-ε4 state to accelerate longitudinal reductions in glucose metabolism in the thalamus and frontal, parietal, and posterior cingulate regions in MCI38. Reduced glucose metabolism in anterior and posterior cingulate cortices, retrosplenial, precuneus, parietal cortex, hippocampus and thalamus was also observed in cognitively healthy middle-aged APOE-ε4 carriers39, suggesting that metabolic tissue changes in regions beyond the hippocampus can already occur at asymptomatic stages40.

While PET imaging is sensitive to metabolic changes and can identify amyloid-β and tau burden41, it is invasive and expensive and, therefore, difficult to scale up. Recent advances in non-invasive multi-parametric quantitative MRI (qMRI) methods can reveal subtle microstructural brain changes and promise to provide alternative imaging markers that may be sensitive to early risk-related changes. Up to now qMRI measurements have primarily been studied in LOAD patients and animal models, thus evidence with regards to the effects of risk factors in asymptomatic individuals is sparse.

To address this gap in the literature, we went beyond morphological analyses by employing multi-parametric qMRI to study the effects of APOE-ε4, Family History (FH) of dementia and obesity on cortical and subcortical grey matter in 165 asymptomatic individuals from the Cardiff Ageing and Risk of Dementia Study (CARDS)42–44 (Table 1). More specifically we applied indices sensitive to neurite dispersion and density, free water, myelin and cell metabolism from Neurite Orientation Density and Dispersion Imaging (NODDI)45, quantitative magnetization transfer (qMT)46–49 and T1-relaxometry50 (Table 2).

NODDI fits a three-compartment biophysical tissue model to diffusion-weighted data acquired with a two-shell (b-values of 1200 s/mm2 and 2400 s/mm2) High Angular Resolution Diffusion Imaging (HARDI)51 protocol to separate isotropic from intra- and extracellular diffusion compartments52. This allows the calculation of the isotropic signal fraction (ISOSF), an estimate of free water, and the intracellular signal fraction (ICSF), i.e. the fraction of the tissue comprised of neurites. In addition, NODDI yields the orientation dispersion index (ODI) that reflects the spatial configuration of neurite structures (Table 2). Recent studies reported ICSF and ODI reductions in grey and white matter of patients with MCI, LOAD and young onset AD42–44. For instance, Fu et al. (2019) found decreased ICSF and ODI in the corpus callosum in MCI and LOAD patients, while Colgan et al.55 reported positive correlations between ICSF and histological measurements of hyperphosphorylated tau protein in the hippocampus of rTg4510 mice.

The qMT method models the exchange rate between macromolecular protons and protons in surrounding free water when macromolecular protons are selectively saturated by a radiofrequency pulse with a frequency that is off-resonance for protons in free water46–49. This allows the quantification of a number of parameters including the macromolecular proton fraction (MPF) and the magnetization transfer exchange rate kิ49. In combined neuroimaging and histology studies of Shiverer mice and puppies56–58, MPF has been shown to be highly sensitive to the myelin content in white matter such that MPF increases with the amount of myelin. MPF in the anterior hippocampus was also found to distinguish healthy controls from MCI and LOAD patients59. Furthermore, MCI...
Table 2. Overview of the quantitative microstructural indices and their interpretation in grey matter. AD Alzheimer’s disease, ICSF intracellular signal fraction, ISOSF isotropic signal fraction, $k_f$ forward exchange rate, MCI mild cognitive impairment, MPF macromolecular proton fraction, NODDI neurite orientation dispersion and density imaging, ODI orientation dispersion index, qMT quantitative magnetization transfer.

| MRI modality   | Index               | Apparent grey matter property                        | Hypothesised changes with LOAD risk               |
|---------------|---------------------|------------------------------------------------------|--------------------------------------------------|
| Diffusion NODDI | ICSF                | Neurite density                                     | Increases with tau pathology\(^{32–34}\)/Reduction in MCI and AD patients\(^{32–34}\) |
|               | ODI                 | Neurite dispersion                                  | Increase/Reduction                                |
|               | ISOSF               | Free water                                          | Increase                                         |
| qMT           | MPF                 | Macromolecules (e.g. myelin)                        | Reduction                                        |
|               | $k_f$               | Mitochondrial metabolism                            | Increase in acute inflammation\(^{31}\); Reduction in low-level inflammation\(^{15}\) and in MCI and AD patients\(^{39–41}\) |
| Relaxometry   | $R_1$               | free water, myelin, iron                            | Increase/Reduction                               |

and LOAD patients exhibited a reduced rate of magnetization transfer $k_f$ in grey and white matter\(^{39–41}\), suggesting reduced cell metabolism\(^{60}\). Finally, indices from relaxometry imaging such as the longitudinal relaxation rate $R_1$, have been proposed as non-invasive biomarkers of LOAD\(^{82}\). $R_1$ values are influenced by microstructural characteristics such as tissue density, macromolecular, protein and lipid composition, and paramagnetic atoms. A number of patient and preclinical studies have reported increases in $R_1$ that may reflect LOAD pathology, although the precise mechanisms underpinning these changes remain unknown (see for review\(^{15}\)). Here, we characterised age and risk-related differences in mean values of ICSF, ISOSF, ODI, MPF, $k_f$ and $R_1$ across cortical and subcortical grey matter regions that were segmented from $T_1$—weighted images with the FreeSurfer image analysis suite (version 5.3)\(^{65}\). Microstructural changes were compared with differences in standard morphological metrics of cortical thickness and subcortical volumes. We expected to see risk effects in brain regions known to be early affected in LOAD including limbic regions of the hippocampus, parahippocampus, entorhinal cortex, posterior cingulate cortex as well as thalamus\(^{125,64}\). We hypothesised that APOE-$\varepsilon4$, a positive FH, and central obesity [measured with the Waist-Hip-Ratio (WHR)] would be associated with reduced ICSF, $R_1$, MPF and $k_f$ as well as with increased ISOSF and ODI but with no differences in cortical thickness and/or subcortical volume. In addition, we expected to see the largest differences in those individuals at greatest risk, i.e. in obese APOE-$\varepsilon4$ carriers with a positive FH.

Results

Microstructural and morphological dependent variables were fitted to a general linear model in SPSS version 26\(^{69}\). All data were examined for outliers defined as above or below three times of the interquartile range (75th percentile value–25th percentile value). This led to an exclusion of 0.6% of the microstructural but no exclusions of the morphological data.

Separate multivariate analyses of covariance (MANCOVA) were carried out to test for the effects of APOE genotype ($\varepsilon4$+, $\varepsilon4$), FH (FH+, FH−) and WHR (WHR+, WHR−) on brain morphology (cortical thickness and subcortical volume measures) and on each of the microstructural indices (MPF, $k_f$, ISOSF, ICSF, ODI) across 68 cortical and 14 subcortical regions of interest, whilst controlling for age, sex, and IQ estimates from the revised National Adult Reading Test (NART-R)\(^{69}\). Significant omnibus effects were further investigated with post-hoc comparisons across all outcome measures. All first and post-hoc models were corrected for multiple comparisons with a False Discovery Rate (FDR) of 5% using the Benjamini–Hochberg procedure\(^{47}\) ($P_{\text{BHadj}}$). As the aim of the study was to explore microstructural indices that could potentially provide novel biomarkers of dementia risk in future studies, a false positive rate of below 5% was regarded as an acceptable threshold to control for false positives while minimising the risk of missing any true risk-related microstructural differences. Information about effects sizes was provided with the partial eta squared index $\eta^2$ for MANCOVA analyses, Cohen’s $d_{\text{p}}$ for group comparisons and Pearson’s $r$ for correlational analyses.

MANCOVAs of microstructural qMT metrics. MPF omnibus effects. There were main effects of sex [F(78,46) = 2.2, $P_{\text{BHadj}}$ = 0.015, $\eta^2 = 0.8$] and of APOE genotype [F(78,46) = 2.6, $P_{\text{BHadj}}$ < 0.001, $\eta^2 = 0.8$] but not of FH ($P_{\text{BHadj}}$ = 0.137), WHR ($P_{\text{BHadj}}$ = 0.348), age ($P_{\text{BHadj}}$ = 0.385) or NART-IQ ($P_{\text{BHadj}}$ = 0.497). There were no interaction effects between APOE and FH ($P_{\text{BHadj}}$ = 1.000), APOE and WHR ($P_{\text{BHadj}}$ = 0.974), FH and WHR ($P_{\text{BHadj}}$ = 1.000) or APOE, FH and WHR ($P_{\text{BHadj}}$ = 0.935).
**MPF post-hoc effects.** APOE-ε4 carriers relative to non-carriers had lower MPF in the left thalamus (Table 3) (Fig. 1). Women had higher MPF than men in the left and right rostral middle frontal cortices, in the left superior temporal cortex and the right transverse temporal cortex (Table 3) (Fig. 2).

**R₁ omnibus effects.** A significant omnibus effect was only observed for APOE genotype [F(82,43) = 2.1, p(BHadj) = 0.040, η² = 0.08]. No main effects were present for FH [p(BHadj) = 0.215], WHR [p(BHadj) = 0.167], age [p(BHadj) = 0.085] sex [p(BHadj) = 0.060] or NART-IQ [p(BHadj) = 0.866] and no interaction effects between APOE and FH [p(BHadj) = 0.256], APOE and WHR [p(BHadj) = 0.582], FH and WHR [p(BHadj) = 0.782] or APOE, FH and WHR [p(BHadj) = 0.548] were observed.

**R₁ post-hoc effects.** No APOE post-hoc effects survived FDR correction (see Supplementary Table 1).

**k₁ omnibus effects.** There were no significant main effects of APOE [p(BHadj) = 0.813], FH [p(BHadj) = 0.908], WHR [p(BHadj) = 1.000], age [p(BHadj) = 0.075], sex [p(BHadj) = 0.975] or NART-IQ [p(BHadj) = 0.870] and no interaction effects between APOE and FH [p(BHadj) = 0.888], APOE and WHR [p(BHadj) = 0.840], FH and WHR [p(BHadj) = 0.990] or APOE, FH and WHR [p(BHadj) = 0.436].

**MANCOVAs of microstructural NODDI metrics.** ISOSF omnibus effects. There were main effects for age [F(78,42) = 2.0, p(BHadj) = 0.03, η² = 0.08], sex [F(78,42) = 3.4, p(BHadj) < 0.001, η² = 0.09], and NART-IQ [F(78,42) = 2.2, p(BHadj) = 0.020, η² = 0.08]. No main effects were present for the risk factors of APOE [p(BHadj) = 1.000], FH [p(BHadj) = 0.060] or WHR [p(BHadj) = 0.717] and no interaction effects between APOE and FH [p(BHadj) = 0.374], APOE and WHR [p(BHadj) = 0.551], FH and WHR [p(BHadj) = 0.986] or APOE, FH and WHR [p(BHadj) = 0.678] were observed.

**ISOSF post-hoc effects.** Ageing was associated with bilateral increases in ISOSF in medial regions including the cingulate, precuneus and cuneus cortices and in lateral regions including superior temporal, supramarginal, postcentral, pars opercularis and insula cortices. Age-related increases in ISOSF were also observed in left middle temporal and pars triangularis regions as well as in subcortical hippocampi, thalami, nuclei accumbens and right putamen (Table 4) (Fig. 1). Women relative to men had higher ISOSF in widespread frontal, temporal, parietal and cingulate cortices and in caudate nuclei, hippocampi, thalami and right nucleus accumbens (Table 4) (Fig. 2). In addition, NART-IQ correlated positively with ISOSF in the superior temporal sulci (left: r = 0.253, p(BHadj) = 0.026) but no main effects for sex [p(BHadj) = 0.241], NART-IQ [p(BHadj) = 0.006], left superior parietal (r = 0.227, p(BHadj) = 0.006), and right lingual (r = 0.182, p(BHadj) = 0.026) cortices (Table 4). After parting out of age only correlations on the left hemisphere remained significant [superior parietal cortex (r = 0.206, p(BHadj) = 0.048), superior temporal sulcus (r = 0.197, p(BHadj) = 0.032)] but those on the right did not [superior temporal sulcus (p(BHadj) = 0.053), lingual (p(BHadj) = 0.08)].

**ODI omnibus effects.** There was a significant main effect of age [F(78,51) = 2.0, p(BHadj) = 0.040, η² = 0.08] and a significant interaction effect between FH and WHR [F(78,51) = 2.3, p(BHadj) = 0.010, η² = 0.08] but no main effects for sex [p(BHadj) = 0.270], NART-IQ [p(BHadj) = 0.949], APOE [p(BHadj) = 0.153], FH [p(BHadj) = 0.520] or WHR [p(BHadj) = 0.330] and no interaction effects between APOE and FH [p(BHadj) = 0.436], APOE and WHR [p(BHadj) = 0.295] or APOE, FH and WHR [p(BHadj) = 0.228] were observed.

**ODI post-hoc effects.** Age-related increases in ODI were observed in left hippocampus, amygdala, caudate and right transverse temporal cortex (Table 3) (Fig. 3).

**Post-hoc effects for the interaction between FH and WHR did not survive 5% FDR correction (Supplementary Table 2).**

**ICSF effects.** There were no significant main or interaction effects on ICSF [age (p(BHadj) = 0.170), sex (p(BHadj) = 0.130), NART-IQ (p(BHadj) = 0.451), APOE (p(BHadj) = 0.324), FH (p(BHadj) = 0.342), WHR (p(BHadj) = 0.517), APOE × FH (p(BHadj) = 0.541), APOE × WHR (p(BHadj) = 0.236), FH × WHR (p(BHadj) = 0.883), APOE × FH × WHR (p(BHadj) = 0.912)].

**MANCOVA on cortical thickness and subcortical volume (ICV corrected).** Omnibus effects. There were main effects for age [F(82,68) = 1.8, p(BHadj) = 0.035, η² = 0.07] and sex [F(82,68) = 1.9, p(BHadj) = 0.040, η² = 0.07]. No main effects were observed for APOE (p(BHadj) = 0.597), FH (p(BHadj) = 0.144), WHR (p(BHadj) = 0.152) or NART-IQ (p(BHadj) = 0.651). No interaction effects between APOE and FH [p(BHadj) = 0.844], APOE and WHR [p(BHadj) = 0.978], FH and WHR [p(BHadj) = 0.053] or APOE, FH and WHR [p(BHadj) = 0.123] were observed.

**Post-hoc effects.** Ageing was associated with widespread thinning in bilateral frontal, temporal, and parietal cortical regions as well as with volume loss in subcortical structures, i.e. in the left hippocampus, left nucleus accumbens, bilateral thalamus and putamen (Table 6) (Fig. 3). Women relative to men had larger volumes in left hippocampus, left nucleus accumbens, left putamen, right caudate and right pallidum. They also had larger cortical thickness in the right isthmus cingulate but lower cortical thickness in the left insula (Table 6) (Fig. 2).

**Exploring interaction effects between APOE, age and sex.** Potential interaction effects between APOE, age and sex on left thalamus MPF were explored. Univariate analysis of variance revealed an effect of
| Effect   | Side | ROI                                    | F_{(1,32)}-value | p_{BHadj} |
|----------|------|----------------------------------------|------------------|-----------|
| Left     |      | Accumbens                              | 3.985            | 0.214     |
|          |      | Amygdala                               | 0.171            | 0.869     |
|          |      | Caudate                                | 6.710            | 0.090     |
|          |      | Hippocampus                            | 5.327            | 0.143     |
|          |      | Pallidum                               | 0.099            | 0.891     |
|          |      | Putamen                                | 1.416            | 0.511     |
|          |      | Thalamus                               | 10.772           | 0.026     |
| Right    |      | Accumbens                              | 0.310            | 0.790     |
|          |      | Amygdala                               | 0.125            | 0.868     |
|          |      | Caudate                                | 3.433            | 0.264     |
|          |      | Hippocampus                            | 6.700            | 0.095     |
|          |      | Pallidum                               | 0.039            | 0.919     |
|          |      | Putamen                                | 1.226            | 0.561     |
|          |      | Thalamus                               | 5.233            | 0.144     |
| APOE     | Left | Banks of superior temporal sulcus       | 3.424            | 0.261     |
|          |      | Caudal anterior cingulate              | 1.518            | 0.483     |
|          |      | Cuneus                                 | 0.631            | 0.689     |
|          |      | Entorhinal                             | 0.002            | 0.986     |
|          |      | Frontal pole                           | 2.579            | 0.320     |
|          |      | Fusiform                               | 0.771            | 0.669     |
|          |      | Inferior parietal                      | 0.886            | 0.631     |
|          |      | Inferior temporal                      | 0.942            | 0.635     |
|          |      | Insula                                 | 6.754            | 0.097     |
|          |      | Lateral occipital                      | 0.307            | 0.788     |
|          |      | Lateral orbito frontal                 | 0.355            | 0.777     |
|          |      | Lingual                                | 0.641            | 0.690     |
|          |      | Medial orbito frontal                  | 0.001            | 0.993     |
|          |      | Middle temporal                        | 2.653            | 0.318     |
|          |      | Paracentral                            | 0.035            | 0.924     |
|          |      | Parahippocampal                        | 0.150            | 0.865     |
|          |      | Pars opercularis                       | 8.341            | 0.097     |
|          |      | Pars orbitalis                         | 0.028            | 0.932     |
|          |      | Pars triangularis                      | 0.019            | 0.945     |
|          |      | Postcentral                            | 2.459            | 0.331     |
|          |      | Posterior cingulate                    | 1.065            | 0.592     |
|          |      | Precentral                             | 3.040            | 0.297     |
|          |      | Precuneus                              | 0.000            | 0.997     |
|          |      | Rostral anterior cingulate             | 0.531            | 0.714     |
|          |      | Rostral middle frontal                 | 0.112            | 0.880     |
|          |      | Superior frontal                       | 0.515            | 0.719     |
|          |      | Superior parietal                      | 0.222            | 0.836     |
|          |      | Superior temporal                      | 1.096            | 0.594     |
|          |      | Supramarginal                          | 2.657            | 0.312     |
|          |      | Temporal pole                          | 3.597            | 0.252     |
|          |      | Transverse temporal                    | 5.752            | 0.117     |
| Right    |      | Banks of superior temporal sulcus       | 0.085            | 0.892     |
|          |      | Caudal anterior cingulate              | 6.693            | 0.100     |
|          |      | Cuneus                                 | 0.077            | 0.897     |
|          |      | Entorhinal                             | 0.088            | 0.892     |
|          |      | Frontal pole                           | 0.070            | 0.882     |
|          |      | Fusiform                               | 2.047            | 0.416     |
|          |      | Inferior parietal                      | 0.736            | 0.673     |
|          |      | Inferior temporal                      | 0.162            | 0.865     |
|          |      | Insula                                 | 4.235            | 0.198     |
|          |      | Isthmus cingulate                      | 0.927            | 0.635     |
|          |      | Lateral occipital                      | 0.072            | 0.891     |

Continued
| Effect | Side   | ROI                                | F(1,123) | p-value |
|--------|--------|------------------------------------|----------|---------|
|        |        | Lateral orbito frontal             | 0.785    | 0.668   |
|        |        | Lingual                            | 3.499    | 0.262   |
|        |        | Medial orbito frontal              | 1.979    | 0.407   |
|        |        | Middle temporal                     | 0.130    | 0.876   |
|        |        | Paracentral                        | 0.071    | 0.887   |
|        |        | Parahippocampal                     | 1.994    | 0.409   |
|        |        | Pars opercularis                   | 1.551    | 0.493   |
|        |        | Pars orbitalis                      | 0.511    | 0.714   |
|        |        | Pars triangularis                  | 0.001    | 0.986   |
|        |        | Pericalcine                         | 0.875    | 0.629   |
|        |        | Postcentral                         | 0.074    | 0.895   |
|        |        | Posterior cingulate                | 1.341    | 0.532   |
|        |        | Precentral                         | 0.303    | 0.784   |
|        |        | Precuneus                          | 0.198    | 0.854   |
|        |        | Rostral anterior cingulate         | 1.850    | 0.429   |
|        |        | Rostral middle frontal             | 0.151    | 0.858   |
|        |        | Superior frontal                   | 0.026    | 0.932   |
|        |        | Superior parietal                  | 1.548    | 0.488   |
|        |        | Superior temporal                  | 1.148    | 0.579   |
|        |        | Supramarginal                      | 0.167    | 0.866   |
|        |        | Temporal pole                      | 0.764    | 0.665   |
|        |        | Transverse temporal                | 0.155    | 0.867   |
|        | Left   | Accumbens                          | 0.353    | 0.784   |
|        | Left   | Amygdala                           | 0.014    | 0.956   |
|        | Left   | Caudate                            | 1.918    | 0.418   |
|        | Left   | Hippocampus                        | 0.684    | 0.673   |
|        | Left   | Pallidum                           | 1.079    | 0.594   |
|        | Left   | Putamen                            | 2.12     | 0.405   |
|        | Left   | Thalamus                           | 2.668    | 0.321   |
|        | Right  | Accumbens                          | 0.126    | 0.874   |
|        | Right  | Amygdala                           | 0.000    | 0.993   |
|        | Right  | Caudate                            | 0.046    | 0.912   |
|        | Right  | Hippocampus                        | 0.223    | 0.842   |
|        | Right  | Pallidum                           | 0.697    | 0.673   |
|        | Right  | Putamen                            | 2.678    | 0.324   |
|        | Right  | Thalamus                           | 0.571    | 0.710   |
|        | Left   | Banks of superior temporal sulcus  | 0.559    | 0.711   |
|        | Left   | Caudal anterior cingulate          | 0.459    | 0.742   |
|        | Left   | Cuneus                             | 7.712    | 0.093   |
|        | Left   | Entorhinal                         | 5.902    | 0.115   |
|        | Left   | Frontal pole                       | 4.243    | 0.204   |
|        | Left   | Fusiform                           | 0.007    | 0.971   |
|        | Left   | Inferior parietal                  | 6.242    | 0.104   |
|        | Left   | Inferior temporal                  | 0.191    | 0.854   |
|        | Left   | Insula                             | 1.298    | 0.541   |
|        | Left   | Lateral occipital                  | 0.063    | 0.888   |
|        | Left   | Lateral orbito frontal             | 0.002    | 0.992   |
|        | Left   | Lingual                            | 3.095    | 0.293   |
|        | Left   | Medial orbito frontal              | 2.921    | 0.298   |
|        | Left   | Middle temporal                    | 2.496    | 0.331   |
|        | Left   | Paracentral                        | 0.009    | 0.968   |
|        | Left   | Parahippocampal                    | 7.180    | 0.104   |
|        | Left   | Pars opercularis                   | 1.169    | 0.578   |
|        | Left   | Pars orbitalis                     | 1.524    | 0.488   |
|        | Left   | Pars triangularis                  | 7.929    | 0.085   |
|        | Left   | Postcentral                        | 0.903    | 0.638   |

Continued
APOE [F(1,141) = 5.7, p = 0.018] and age [F(2,141) = 3.7, p = 0.027] but no interaction effects between APOE and age (p = 0.700) or APOE and sex (p = 0.900).

Exploring moderator effects of blood pressure and markers of inflammation. We then explored with two separate analyses of covariances whether controlling for differences in (i) systolic and diastolic blood pressure (BP) and (ii) inflammation-related measures of C-Reactive Protein (CRP), Interleukin-8 (IL-8) and leptin/adiponectin ratio (LAR) would account for the effect of APOE on left thalamus MPF.

| Effect          | Side                      | ROI                        | F(1,123) | p(BHadj) |
|-----------------|---------------------------|----------------------------|----------|----------|
| Posterior cingulate |                           | 15.379                     | < 0.001  |          |
| Precuneus       |                           | 0.726                      | 0.664    |          |
| Rostral anterior cingulate |               | 0.727                      | 0.669    |          |
| Rostral middle frontal   |                           | 18.725                     | < 0.001  |          |
| Superior frontal      |                           | 4.349                      | 0.202    |          |
| Superior parietal     |                           | 1.629                      | 0.474    |          |
| Superior temporal    |                           | 13.584                     | < 0.001  |          |
| Supramarginal       |                           | 7.857                      | 0.104    |          |
| Temporal pole       |                           | 3.766                      | 0.238    |          |
| Transverse temporal |                           | 7.374                      | 0.096    |          |
| BANKS of superior temporal sulcus |           | 2.881                      | 0.292    |          |
| Caudal anterior cingulate |                   | 4.038                      | 0.215    |          |
| Cuneus             |                           | 7.177                      | 0.089    |          |
| Entorhinal         |                           | 2.004                      | 0.413    |          |
| Frontal pole       |                           | 4.610                      | 0.196    |          |
| Fusiform           |                           | 0.097                      | 0.886    |          |
| Inferior parietal  |                           | 1.757                      | 0.442    |          |
| Inferior temporal  |                           | 0.352                      | 0.771    |          |
| Insula             |                           | 2.943                      | 0.308    |          |
| Isthmus cingulate  |                           | 0.443                      | 0.746    |          |
| Lateral occipital  |                           | 0.297                      | 0.782    |          |
| Lateral orbito frontal |                     | 0.356                      | 0.790    |          |
| Lingual            |                           | 3.196                      | 0.289    |          |
| Medial orbito frontal |                     | 4.570                      | 0.195    |          |
| Middle temporal    |                           | 0.360                      | 0.793    |          |
| Paracentral        |                           | 0.425                      | 0.752    |          |
| Parahippocampal    |                           | 0.973                      | 0.625    |          |
| Pars opercularis   |                           | 0.340                      | 0.774    |          |
| Pars orbitalis     |                           | 0.892                      | 0.636    |          |
| Pars triangularis  |                           | 6.046                      | 0.106    |          |
| Pericalcine        |                           | 0.553                      | 0.708    |          |
| Postcentral        |                           | 2.934                      | 0.301    |          |
| Posterior cingulate |                       | 1.783                      | 0.441    |          |
| Precuneus          |                           | 2.025                      | 0.415    |          |
| Rostral anterior cingulate |             | 0.597                      | 0.702    |          |
| Rostral middle frontal   |                       | 11.339                     | 0.031    |          |
| Superior frontal    |                           | 8.639                      | 0.089    |          |
| Superior parietal   |                           | 4.557                      | 0.188    |          |
| Superior temporal   |                           | 7.319                      | 0.083    |          |
| Supramarginal      |                           | 2.903                      | 0.295    |          |
| Temporal pole      |                           | 6.534                      | 0.093    |          |
| Transverse temporal |                       | 14.344                     | < 0.001  |          |

Table 3. Post-hoc effects of APOE genotype and sex on the macromolecular proton fraction (MPF). $p_{\text{BHadj}}$, 5% False Discovery Rate Benjamini–Hochberg adjusted $p$ value; ROI region of interest. Significant results are highlighted in bold.
Figure 1. Violin plots with overlaid box plots of the difference in the macromolecular proton fraction (MPF) in the left thalamus between APOE-ε4 carriers (n = 57) and non-carriers (n = 97) (P_{BHadj} = 0.026). Boxplots display the median and the interquartile range and violin plots the kernel probability density, i.e. the width of the yellow area represents the proportion of the data located there.

Figure 2. displays the effects of sex on cortical thickness (CT), subcortical volume (corrected for intracranial volume), isotropic signal fraction (ISOSF) and macromolecular proton fraction (MPF) across 34 cortical regions per hemisphere parcellated with the Desikan–Killiany atlas and seven subcortical regions per hemisphere (hippocampus, amygdala, thalamus, caudate, putamen, globus pallidus, nucleus accumbens). Region of interest segmentations were performed with FreeSurfer (version 5.3). Regions are colour-coded according to effect sizes indicated by Cohen's d. Warm colours indicate positive and blue colours negative correlations. L = Left, R = Right.
| Effect | Side | ROI                          | \( F_{(LHS)} value \) | \( p_{BHadj} \) |
|--------|------|-----------------------------|----------------------|----------------|
| Left   |      | Accumbens                   | 16.946               | < 0.001       |
|        |      | Amygdala                    | 0.002                | 0.977         |
|        |      | Caudate                      | 2.906                | 0.174         |
|        |      | **Hippocampus**              | 32.296               | < 0.001       |
|        |      | Pallidum                     | 0.741                | 0.544         |
|        |      | Putamen                      | 3.705                | 0.121         |
|        |      | Thalamus                     | 17.881               | < 0.001       |
| Right  |      | Accumbens                   | 8.272                | 0.016         |
|        |      | Amygdala                    | 0.090                | 0.847         |
|        |      | Caudate                      | 4.359                | 0.090         |
|        |      | **Hippocampus**              | 20.305               | < 0.001       |
|        |      | Pallidum                     | 0.168                | 0.787         |
|        |      | Putamen                      | 6.089                | 0.039         |
|        |      | Thalamus                     | 21.716               | < 0.001       |
|        |      | **Banks of superior temporal sulcus** | 12.121             | 0.003         |
|        |      | Caudal anterior cingulate    | 12.152               | 0.004         |
|        |      | Cuneus                       | 17.203               | < 0.001       |
|        |      | Entorhinal                   | 0.170                | 0.788         |
|        |      | Frontal pole                 | 0.667                | 0.559         |
|        |      | Fusiform                     | 0.884                | 0.494         |
|        |      | **Inferior parietal**        | 6.381                | 0.035         |
|        |      | Inferior temporal            | 0.765                | 0.538         |
|        |      | **Insula**                   | 17.457               | < 0.001       |
|        |      | Lateral occipital            | 6.671                | 0.031         |
|        |      | Lateral orbito frontal       | 3.029                | 0.163         |
|        |      | Lingual                      | 2.481                | 0.212         |
|        |      | Medial orbito frontal        | 6.335                | 0.035         |
|        |      | **Middle temporal**          | 11.334               | 0.004         |
|        |      | Paracentral                  | 4.216                | 0.095         |
|        |      | Parahippocampal              | 0.125                | 0.819         |
|        |      | Pars opercularis             | 19.568               | < 0.001       |
|        |      | Pars orbitalis               | 0.005                | 0.961         |
|        |      | Pars triangularis            | 15.445               | < 0.001       |
|        |      | Postcentral                  | 14.471               | < 0.001       |
|        |      | Posterior cingulate          | 15.798               | < 0.001       |
|        |      | Precentral                   | 5.314                | 0.057         |
|        |      | **Precuneus**                | 19.354               | < 0.001       |
|        |      | Rostral anterior cingulate   | 16.241               | < 0.001       |
|        |      | Rostral middle frontal       | 5.017                | 0.067         |
|        |      | Superior frontal             | 1.173                | 0.410         |
|        |      | Superior parietal            | 0.963                | 0.470         |
|        |      | **Superior temporal**        | 25.891               | < 0.001       |
|        |      | Supramarginal                | 16.621               | < 0.001       |
|        |      | Temporal pole                | 1.219                | 0.410         |
|        |      | **Transverse temporal**      | 51.576               | < 0.001       |
| Right  |      | **Banks of superior temporal sulcus** | 12.346             | 0.003         |
|        |      | Caudal anterior cingulate    | 7.267                | 0.025         |
|        |      | Cuneus                       | 13.388               | < 0.001       |
|        |      | Entorhinal                   | 0.131                | 0.819         |
|        |      | Frontal pole                 | 1.185                | 0.414         |
|        |      | Fusiform                     | 0.108                | 0.835         |
|        |      | Inferior parietal            | 1.881                | 0.297         |
|        |      | Inferior temporal            | 1.475                | 0.366         |
|        |      | Insula                       | 14.803               | < 0.001       |
|        |      | **Isthmus cingulate**        | 6.659                | 0.031         |
|        |      | Lateral occipital            | 1.818                | 0.307         |

*Continued*
| Effect               | Side  | ROI                  | F_{(FWE)} \text{value} | P_{BHadj} |
|---------------------|-------|----------------------|------------------------|----------|
| Lateral orbito frontal | Left  | 1.286                | 0.406                  |
| Lingual             | Left  | 7.195                | 0.024                  |
| Medial orbito frontal | Left  | 3.288                | 0.147                  |
| Middle temporal     | Left  | 3.039                | 0.165                  |
| Paracentral         | Left  | 0.702                | 0.556                  |
| Parahippocampal     | Left  | 1.158                | 0.412                  |
| Pars opercularis    | Left  | 15.413               | < 0.001                |
| Pars orbitalis      | Left  | 2.665                | 0.195                  |
| Pars triangularis   | Left  | 0.523                | 0.605                  |
| Pericalcine         | Left  | 16.505               | < 0.001                |
| Postcentral         | Left  | 6.318                | 0.034                  |
| Posterior cingulate | Left  | 18.89                | < 0.001                |
| Precentral          | Left  | 4.015                | 0.104                  |
| Precuneus           | Left  | 15.968               | < 0.001                |
| Rostral anterior cingulate | Left  | 12.476               | 0.003                  |
| Rostral middle frontal | Left  | 2.466                | 0.212                  |
| Superior frontal    | Left  | 0.676                | 0.550                  |
| Superior parietal   | Left  | 3.634                | 0.124                  |
| Superior temporal   | Left  | 12.296               | 0.003                  |
| Supramarginal       | Left  | 8.563                | 0.013                  |
| Temporal pole       | Left  | 2.727                | 0.189                  |
| Transverse temporal | Left  | 44.346               | < 0.001                |

| Effect               | Side  | ROI                  | F_{(FWE)} \text{value} | P_{BHadj} |
|---------------------|-------|----------------------|------------------------|----------|
| Lateral orbito frontal | Right | 1.286                | 0.406                  |
| Lingual             | Right | 7.195                | 0.024                  |
| Medial orbito frontal | Right | 3.288                | 0.147                  |
| Middle temporal     | Right | 3.039                | 0.165                  |
| Paracentral         | Right | 0.702                | 0.556                  |
| Parahippocampal     | Right | 1.158                | 0.412                  |
| Pars opercularis    | Right | 15.413               | < 0.001                |
| Pars orbitalis      | Right | 2.665                | 0.195                  |
| Pars triangularis   | Right | 0.523                | 0.605                  |
| Pericalcine         | Right | 16.505               | < 0.001                |
| Postcentral         | Right | 6.318                | 0.034                  |
| Posterior cingulate | Right | 18.89                | < 0.001                |
| Precentral          | Right | 4.015                | 0.104                  |
| Precuneus           | Right | 15.968               | < 0.001                |
| Rostral anterior cingulate | Right | 12.476               | 0.003                  |
| Rostral middle frontal | Right | 2.466                | 0.212                  |
| Superior frontal    | Right | 0.676                | 0.550                  |
| Superior parietal   | Right | 3.634                | 0.124                  |
| Superior temporal   | Right | 12.296               | 0.003                  |
| Supramarginal       | Right | 8.563                | 0.013                  |
| Temporal pole       | Right | 2.727                | 0.189                  |
| Transverse temporal | Right | 44.346               | < 0.001                |

| Sex                  |       |                      |                        |          |
|---------------------|-------|----------------------|------------------------|----------|
| Left                |       |                      |                        |          |
| Banks of superior temporal sulcus | Left | 9.745                | 0.007                  |
| Caudal anterior cingulate | Left | 10.321               | 0.007                  |
| Cuneus              | Left  | 14.189               | < 0.001                |
| Entorhinal          | Left  | 2.097                | 0.263                  |
| Frontal pole        | Left  | 1.317                | 0.400                  |
| Fusiform            | Left  | 0.471                | 0.621                  |
| Inferior parietal   | Left  | 19.193               | < 0.001                |
| Inferior temporal   | Left  | 3.546                | 0.129                  |
| Insula              | Left  | 14.093               | < 0.001                |
| Lateral occipital   | Left  | 15.940               | < 0.001                |
| Lateral orbito frontal | Left  | 0.039                | 0.902                  |
| Lingual             | Left  | 1.178                | 0.414                  |
| Medial orbito frontal | Left  | 3.411                | 0.138                  |
| Middle temporal     | Left  | 17.995               | < 0.001                |
| Paracentral         | Left  | 1.542                | 0.355                  |
| Parahippocampal     | Left  | 14.537               | < 0.001                |
| Pars opercularis    | Left  | 11.519               | 0.003                  |
| Pars orbitalis      | Left  | 0.167                | 0.784                  |
| Pars triangularis   | Left  | 16.204               | < 0.001                |
| Postcentral         | Left  | 28.162               | < 0.001                |

Continued
| Effect                  | Side          | ROI                                | $F_{(1,119)}$ value | $P_{BHadj}$ |
|------------------------|---------------|------------------------------------|---------------------|------------|
| Posterior cingulate    | Right         | 16.237                             | < 0.001             |            |
| Precentral             | Right         | 22.987                             | < 0.001             |            |
| Precuneus              | Right         | 13.571                             | < 0.001             |            |
| Rostral anterior cingulate | Right             | 4.385                             | 0.088               |            |
| Rostral middle frontal | Right         | 35.530                             | < 0.001             |            |
| Superior frontal       | Right         | 13.064                             | < 0.001             |            |
| Superior parietal      | Right         | 18.143                             | < 0.001             |            |
| Superior temporal      | Right         | 26.621                             | < 0.001             |            |
| Supramarginal          | Right         | 42.479                             | < 0.001             |            |
| Temporal pole          | Right         | 4.436                              | 0.088               |            |
| Transverse temporal    | Right         | 30.601                             | < 0.001             |            |
| Banks of superior temporal sulcus | Right         | 14.697                             | < 0.001             |            |
| Caudal anterior cingulate | Right             | 10.623                             | 0.004               |            |
| Cuneus                 | Right         | 24.330                             | < 0.001             |            |
| Entorhinal             | Right         | 0.491                              | 0.616               |            |
| Frontal pole           | Right         | 0.684                              | 0.557               |            |
| Fusiform               | Right         | 3.168                              | 0.158               |            |
| Inferior parietal      | Right         | 6.885                              | 0.030               |            |
| Inferior temporal      | Right         | 3.105                              | 0.162               |            |
| Insula                 | Right         | 4.265                              | 0.094               |            |
| Isthmus cingulate      | Right         | 0.601                              | 0.578               |            |
| Lateral occipital      | Right         | 10.275                             | 0.006               |            |
| Lateral orbito frontal | Right         | 0.102                              | 0.839               |            |
| Lingual                | Right         | 7.981                              | 0.019               |            |
| Medial orbito frontal  | Right         | 3.038                              | 0.166               |            |
| Middle temporal        | Right         | 5.352                              | 0.055               |            |
| Paracentral            | Right         | 9.075                              | 0.010               |            |
| Parahippocampal        | Right         | 3.733                              | 0.121               |            |
| Pars opercularis       | Right         | 7.161                              | 0.027               |            |
| Pars orbitalis         | Right         | 3.870                              | 0.112               |            |
| Pars triangularis      | Right         | 5.958                              | 0.042               |            |
| Pericalcine            | Right         | 14.080                             | < 0.001             |            |
| Postcentral            | Right         | 19.109                             | < 0.001             |            |
| Posterior cingulate    | Right         | 14.954                             | < 0.001             |            |
| Precentral             | Right         | 17.777                             | < 0.001             |            |
| Precuneus              | Right         | 13.291                             | < 0.001             |            |
| Rostral anterior cingulate | Right             | 5.785                              | 0.046               |            |
| Rostral middle frontal | Right         | 24.380                             | < 0.001             |            |
| Superior frontal       | Right         | 16.120                             | < 0.001             |            |
| Superior parietal      | Right         | 8.266                              | 0.016               |            |
| Superior temporal      | Right         | 16.902                             | < 0.001             |            |
| Supramarginal          | Right         | 16.983                             | < 0.001             |            |
| Temporal pole          | Right         | 0.330                              | 0.691               |            |
| Transverse temporal    | Right         | 37.792                             | < 0.001             |            |

**NART-IQ**

| Effect                  | Side          | ROI                                | $F_{(1,119)}$ value | $P_{BHadj}$ |
|------------------------|---------------|------------------------------------|---------------------|------------|
| Accumbens              | Left          | 0.789                              | 0.556               |            |
| Amygdala               | Left          | 3.741                              | 0.120               |            |
| Caudate                | Left          | 0.016                              | 0.932               |            |
| Hippocampus            | Left          | 0.065                              | 0.864               |            |
| Pallidum               | Left          | 0.022                              | 0.922               |            |
| Putamen                | Left          | 1.221                              | 0.411               |            |
| Thalamus               | Left          | 0.000                              | 0.995               |            |
| Accumbens              | Right         | 0.022                              | 0.924               |            |
| Amygdala               | Right         | 1.266                              | 0.410               |            |
| Caudate                | Right         | 1.809                              | 0.306               |            |
| Hippocampus            | Right         | 0.067                              | 0.866               |            |
| Pallidum               | Right         | 0.206                              | 0.764               |            |
| Effect | Side | ROI | \(F_{\text{FSL,Y}}\)/value | \(p_{\text{BHadj}}\) |
|--------|------|-----|-----------------|----------------|
| Putamen |      | 0.606 | 0.579 |
| Thalamus |  | 0.481 | 0.618 |
| **Banks of superior temporal sulcus** | Left |  |  |
| Caudal anterior cingulate |  | 0.035 | 0.901 |
| Cuneus |  | 0.200 | 0.767 |
| Entorhinal |  | 0.343 | 0.684 |
| Frontal pole |  | 1.745 | 0.315 |
| Fusiform |  | 0.039 | 0.904 |
| Inferior parietal |  | 2.029 | 0.274 |
| Inferior temporal |  | 0.019 | 0.925 |
| Insula |  | 4.834 | 0.073 |
| Lateral occipital |  | 0.306 | 0.697 |
| Lateral orbito frontal |  | 0.037 | 0.901 |
| Lingual |  | 0.621 | 0.574 |
| Medial orbito frontal |  | 0.000 | 0.993 |
| Middle temporal |  | 0.402 | 0.655 |
| Paracentral |  | 0.199 | 0.764 |
| Parahippocampal |  | 0.010 | 0.943 |
| Pars opercularis |  | 0.207 | 0.768 |
| Pars orbitalis |  | 1.006 | 0.459 |
| Pars triangularis |  | 0.636 | 0.570 |
| Postcentral |  | 1.370 | 0.388 |
| Posterior cingulate |  | 1.243 | 0.411 |
| Precuneus |  | 0.401 | 0.653 |
| Rostral anterior cingulate |  | 0.582 | 0.581 |
| Rostral middle frontal |  | 1.208 | 0.411 |
| Superior frontal |  | 1.224 | 0.414 |
| **Superior parietal** | Left |  |  |
| Superior temporal |  | 0.266 | 0.724 |
| Supramarginal |  | 0.879 | 0.493 |
| Temporal pole |  | 0.084 | 0.849 |
| Transverse temporal |  | 2.832 | 0.180 |
| **Banks of superior temporal sulcus** | Right |  |  |
| Caudal anterior cingulate |  | 0.530 | 0.605 |
| Cuneus |  | 2.829 | 0.179 |
| Entorhinal |  | 4.702 | 0.077 |
| Frontal pole |  | 1.644 | 0.332 |
| Fusiform |  | 2.222 | 0.246 |
| Inferior parietal |  | 2.952 | 0.170 |
| Inferior temporal |  | 0.001 | 0.987 |
| Insula |  | 0.090 | 0.843 |
| Isthmus cingulate |  | 1.257 | 0.409 |
| Lateral occipital |  | 0.126 | 0.821 |
| Lateral orbito frontal |  | 0.014 | 0.933 |
| Lingual |  | 5.866 | 0.044 |
| Medial orbito frontal |  | 0.318 | 0.692 |
| Middle temporal |  | 0.097 | 0.842 |
| Paracentral |  | 2.527 | 0.208 |
| Parahippocampal |  | 1.980 | 0.280 |
| Pars opercularis |  | 0.242 | 0.741 |
| Pars orbitalis |  | 0.050 | 0.888 |
| Pars triangularis |  | 0.502 | 0.613 |
| Pericalcine |  | 2.623 | 0.198 |
| Postcentral |  | 1.806 | 0.306 |
| Posterior cingulate |  | 1.662 | 0.331 |

Continued
While no covariate showed a main effect (systolic BP ($p = 0.680$), diastolic BP ($p = 0.750$), CRP ($p = 0.150$), IL-8 ($p = 0.400$), LAR ($p = 0.500$)), the APOE effect on the left thalamus MPF remained significant ($F(1,149) = 6.7$, $p_{\text{BHadj}} = 0.030$) after accounting for BP measures, but was not significant anymore after controlling for CRP, IL-8 and LAR ($p = 0.060$).

### Table 4. Post-hoc effects of age, sex and NART-IQ on the isotropic signal fraction (ISOSF). $p_{\text{BHadj}}$, 5% False Discovery Rate Benjamini–Hochberg adjusted $p$ value; ROI, Region of Interest. Significant results are highlighted in bold.

| Effect     | Side | ROI                      | $F_{\text{LPPH}}$-value | $p_{\text{BHadj}}$ |
|------------|------|--------------------------|--------------------------|--------------------|
| Precentral |      | Precuneus                | 2.629                    | 0.197              |
|            |      | Rostral anterior cingulate | 0.453                   | 0.628              |
|            |      | Rostral middle frontal   | 0.394                    | 0.653              |
|            |      | Superior frontal         | 1.525                    | 0.355              |
|            |      | Superior parietal        | 4.186                    | 0.096              |
|            |      | Superior temporal        | 0.002                    | 0.978              |
|            |      | Supramarginal            | 1.407                    | 0.381              |
|            |      | Temporal pole            | 4.445                    | 0.087              |
|            |      | Transverse temporal      | 0.024                    | 0.923              |

**Figure 3.** displays the effects of age on cortical thickness (CT), subcortical volume (corrected for intracranial volume), isotropic signal fraction (ISOSF) and orientation dispersion index (ODI) across 34 cortical regions per hemisphere parcellated with the Desikan–Killiany atlas\(^1\) and seven subcortical regions per hemisphere (hippocampus, amygdala, thalamus, caudate, putamen, globus pallidus, nucleus accumbens). Region of interest segmentations were performed with FreeSurfer (version 5.3). Regions are colour-coded according to the size of the age effect indicated by Pearson correlation coefficient $r$. Warm colours indicate positive and blue colours negative correlations.
| Effect       | Side | ROI                                      | F_{(118)} Value | P_{BHadj} |
|--------------|------|-----------------------------------------|----------------|----------|
|              | Left | Accumbens                               | 3.529          | 0.307    |
|              |      | Amygdala                                | 16.646         | < 0.001  |
|              |      | Caudate                                 | 13.995         | < 0.001  |
|              |      | Hippocampus                             | 15.638         | < 0.001  |
|              |      | Pallidum                                | 0.017          | 0.958    |
|              |      | Putamen                                 | 3.880          | 0.306    |
|              |      | Thalamus                                | 2.111          | 0.505    |
|              | Right| Accumbens                               | 1.265          | 0.594    |
|              |      | Amygdala                                | 7.018          | 0.156    |
|              |      | Caudate                                 | 0.040          | 0.925    |
|              |      | Hippocampus                             | 8.834          | 0.124    |
|              |      | Pallidum                                | 0.365          | 0.755    |
|              |      | Putamen                                 | 2.142          | 0.506    |
|              |      | Thalamus                                | 0.148          | 0.828    |
|              |      | Banks of superior temporal sulcus       | 2.793          | 0.398    |
|              |      | Caudal anterior cingulate               | 7.199          | 0.156    |
|              |      | Cuneus                                  | 0.001          | 0.992    |
|              |      | Entorhinal                              | 5.518          | 0.222    |
|              |      | Frontal pole                            | 2.182          | 0.515    |
|              |      | Fusiform                                | 2.889          | 0.387    |
|              |      | Inferior parietal                       | 0.029          | 0.943    |
|              |      | Inferior temporal                       | 1.654          | 0.559    |
|              |      | Insula                                  | 0.579          | 0.698    |
|              |      | Lateral occipital                       | 1.619          | 0.563    |
|              |      | Lateral orbito frontal                  | 1.572          | 0.560    |
|              |      | Lingual                                 | 0.919          | 0.616    |
|              |      | Medial orbito frontal                   | 5.107          | 0.253    |
|              |      | Middle temporal                         | 1.088          | 0.598    |
|              |      | Paracentral                             | 0.634          | 0.693    |
|              |      | Parahippocampal                         | 0.173          | 0.826    |
|              |      | Pars opercularis                        | 0.076          | 0.892    |
|              |      | Pars orbitalis                          | 2.068          | 0.507    |
|              |      | Pars triangularis                       | 0.055          | 0.914    |
|              |      | Postcentral                             | 0.526          | 0.705    |
|              |      | Posterior cingulate                     | 1.419          | 0.575    |
|              |      | Precentral                              | 0.305          | 0.776    |
|              |      | Precuneus                               | 0.063          | 0.907    |
|              |      | Rostral anterior cingulate              | 1.459          | 0.576    |
|              |      | Rostral middle frontal                  | 2.006          | 0.496    |
|              |      | Superior frontal                        | 1.109          | 0.595    |
|              |      | Superior parietal                       | 4.078          | 0.326    |
|              |      | Superior temporal                       | 2.666          | 0.409    |
|              |      | Supramarginal                           | 0.291          | 0.760    |
|              |      | Temporal pole                           | 8.362          | 0.130    |
|              |      | Transverse temporal                     | 0.200          | 0.817    |
|              | Right| Banks of superior temporal sulcus       | 0.534          | 0.712    |
|              |      | Caudal anterior cingulate               | 2.715          | 0.408    |
|              |      | Cuneus                                  | 0.628          | 0.691    |
|              |      | Entorhinal                              | 1.911          | 0.516    |
|              |      | Frontal pole                            | 3.977          | 0.312    |
|              |      | Fusiform                                | 2.329          | 0.479    |
|              |      | Inferior parietal                       | 0.004          | 0.984    |
|              |      | Inferior temporal                       | 4.430          | 0.288    |
|              |      | Insula                                  | 4.760          | 0.268    |
|              |      | Isthmus cingulate                       | 5.750          | 0.216    |
|              |      | Lateral occipital                       | 1.311          | 0.591    |

Continued
Here, we investigated whether qMRI indices of apparent neurite density and dispersion, free water, myelin, and cell metabolism were sensitive to grey matter differences related to LOAD risk in cognitively healthy individuals. Such microstructural measurements hold the potential for novel imaging biomarkers to identify asymptomatic individuals at heightened risk of developing LOAD. As such they may provide non-invasive and cheaper alternatives to PET and cerebrospinal fluid (CSF)-based biomarkers, that are currently employed in clinical trials, in the future.

The only significant difference between asymptomatic APOE-ε4 carriers relative to non-carriers was in the qMT measure MPF in the left thalamus with APOE-ε4 related reductions in MPF (Fig. 1). This effect was observed independently of age, sex, and verbal intelligence. Reduced MPF may arise from processes that lead to an increase in free water and/or a reduction in the macromolecular content of grey matter including changes in myelin, proteins, and and/or iron concentrations. Such changes may be consistent with the presence of inflammatory processes leading to tissue swelling associated with glia activation and/or with a deficit in cholesterol transport in APOE-ε4 carriers. Consistent with this interpretation we observed that the effect of APOE genotype on left thalamus MPF was moderated by plasma markers of inflammation (CRP, IL-8, LAR). Furthermore, evidence suggests that APOE-ε4 carriage may increase susceptibility to inflammation and that inflammatory processes contribute significantly to the pathogenesis of LOAD.

Notably these APOE-ε4-related differences in MPF were only observed in the left thalamus but not in any other cortical or subcortical region. The limbic thalamic nuclei maintain dense reciprocal connections with the hippocampal formation and the retrosplenial cortex, which, together with the fornix, mammillary bodies and posterior cingulate cortex, comprise the Papez circuit important for episodic memory function. As outlined above it is increasingly recognised that the Papez circuit, including the anterior thalamus, can be affected early in LOAD. Neurofibrillary accumulations are found in the anterodorsal thalamic nucleus at the same time as those in the hippocampus in LOAD brains and neuroimaging studies have revealed reduced thalamic volume in both amnestic MCI and LOAD. Furthermore, studies into the effects of APOE in middle-aged asymptomatic adults found reduced glucose metabolism in the thalamus, hippocampus and cingulate cortex as well as increased metabolism in bilateral thalami and superior temporal gyrus in amyloid-β positive APOE-ε4 carriers with a maternal history of LOAD. Cacciaglia et al. studied the effects of APOE on grey matter volume in over 500 middle-aged asymptomatic individuals and identified reduced hippocampus, caudate, precentral gyrus, and cerebellum volumes but increased volumes in the thalamus, superior frontal and middle occipital gyri in APOE-ε4 carriers. While it remains unknown why APOE-ε4 may be related to increased thalamic volume it was suggested that this could reflect brain swelling associated with glial activation in response to larger amyloid-β.

| Effect         | Side            | ROI                              | p_{F(1,128)} | Value | p_{BHadj} |
|----------------|-----------------|----------------------------------|-------------|-------|----------|
| Lateral orbito frontal | 1.274           | 0.598                            |             |       |          |
| Lingual        | 0.173           | 0.819                            |             |       |          |
| Medial orbito frontal | 0.734           | 0.666                            |             |       |          |
| Middle temporal | 4.509           | 0.295                            |             |       |          |
| Paracentral    | 0.899           | 0.611                            |             |       |          |
| Parahippocampal | 0.373           | 0.754                            |             |       |          |
| Pars opercularis | 2.490           | 0.445                            |             |       |          |
| Pars orbitalis | 1.778           | 0.544                            |             |       |          |
| Pars triangularis | 0.023           | 0.952                            |             |       |          |
| Pericalcerine  | 0.293           | 0.765                            |             |       |          |
| Postcentral    | 1.564           | 0.553                            |             |       |          |
| Posterior cingulate | 0.042           | 0.926                            |             |       |          |
| Precentral     | 0.100           | 0.870                            |             |       |          |
| Precuneus      | 0.000           | 0.985                            |             |       |          |
| Rostral anterior cingulate | 0.284 | 0.760                          |             |       |          |
| Rostral middle frontal | 0.268 | 0.768                          |             |       |          |
| Superior frontal | 0.485           | 0.716                            |             |       |          |
| Superior parietal | 3.130           | 0.352                            |             |       |          |
| Superior temporal | 5.045           | 0.238                            |             |       |          |
| Supramarginal  | 1.426           | 0.581                            |             |       |          |
| Temporal pole  | 6.156           | 0.198                            |             |       |          |
| Transverse temporal | 10.589         | 0.039                            |             |       |          |

Table 5. Post-hoc effects of age on the orientation dispersion index (ODI). p_{BHadj}, 5% False Discovery Rate Benjamini–Hochberg adjusted p value; ROI region of interest. Significant results are highlighted in bold.
| Effect          | Side   | ROI                          | Index      | $F_{(1,40)}$-value | $p$-value |
|-----------------|--------|-----------------------------|------------|--------------------|-----------|
|                  | Left   | Accumbens                   | $\text{Vol}_{\text{ICVadj}}$ | 7.037              | 0.027     |
|                  | Left   | Amygdala                    | $\text{Vol}_{\text{ICVadj}}$ | 3.360              | 0.146     |
|                  | Left   | Caudate                     | $\text{Vol}_{\text{ICVadj}}$ | 0.073              | 0.873     |
|                  | Left   | Hippocampus                 | $\text{Vol}_{\text{ICVadj}}$ | 12.023             | 0.004     |
|                  | Left   | Pallidum                    | $\text{Vol}_{\text{ICVadj}}$ | 1.141              | 0.448     |
|                  | Left   | Putamen                     | $\text{Vol}_{\text{ICVadj}}$ | 8.886              | 0.012     |
|                  | Left   | Thalamus                    | $\text{Vol}_{\text{ICVadj}}$ | 26.144             | < 0.001   |
|                  | Right  | Accumbens                   | $\text{Vol}_{\text{ICVadj}}$ | 4.944              | 0.071     |
|                  | Right  | Amygdala                    | $\text{Vol}_{\text{ICVadj}}$ | 3.723              | 0.120     |
|                  | Right  | Caudate                     | $\text{Vol}_{\text{ICVadj}}$ | 0.225              | 0.778     |
|                  | Right  | Hippocampus                 | $\text{Vol}_{\text{ICVadj}}$ | 2.828              | 0.190     |
|                  | Right  | Pallidum                    | $\text{Vol}_{\text{ICVadj}}$ | 2.444              | 0.221     |
|                  | Right  | Putamen                     | $\text{Vol}_{\text{ICVadj}}$ | 7.732              | 0.021     |
|                  | Right  | Thalamus                    | $\text{Vol}_{\text{ICVadj}}$ | 45.557             | < 0.001   |
|                  | Agr    | Banks of superior temporal sulcus | CT        | 5.798              | 0.047     |
|                  | Agr    | Caudal anterior cingulate   | CT         | 0.583              | 0.589     |
|                  | Agr    | Caudal middle frontal       | CT         | 8.485              | 0.016     |
|                  | Agr    | Cuneus                      | CT         | 3.911              | 0.110     |
|                  | Agr    | Entorhinal                  | CT         | 0.120              | 0.836     |
|                  | Agr    | Frontal pole                | CT         | 0.076              | 0.885     |
|                  | Agr    | Fusiform                    | CT         | 5.474              | 0.057     |
|                  | Agr    | Inferior parietal           | CT         | 11.874             | 0.004     |
|                  | Agr    | Inferior temporal           | CT         | 7.261              | 0.027     |
|                  | Agr    | Insula                      | CT         | 20.522             | < 0.001   |
|                  | Agr    | Inthmus cingulate           | CT         | 0.130              | 0.836     |
|                  | Agr    | Lateral occipital           | CT         | 4.536              | 0.086     |
|                  | Agr    | Lateral orbito frontal      | CT         | 12.478             | 0.006     |
|                  | Agr    | Lingual                     | CT         | 6.891              | 0.030     |
|                  | Agr    | Medial orbito frontal       | CT         | 7.171              | 0.026     |
|                  | Agr    | Middle temporal             | CT         | 12.759             | < 0.001   |
|                  | Agr    | Paracentral                 | CT         | 20.354             | < 0.001   |
|                  | Agr    | Parahippocampal             | CT         | 7.647              | 0.022     |
|                  | Agr    | Pars opercularis            | CT         | 14.469             | < 0.001   |
|                  | Agr    | Pars orbitalis              | CT         | 18.893             | < 0.001   |
|                  | Agr    | Pars triangularis           | CT         | 19.089             | < 0.001   |
|                  | Agr    | Pericalcetine               | CT         | 2.678              | 0.203     |
|                  | Agr    | Postcentral                 | CT         | 12.426             | 0.006     |
|                  | Agr    | Posterior cingulate         | CT         | 1.032              | 0.467     |
|                  | Agr    | Precentral                  | CT         | 28.246             | < 0.001   |
|                  | Agr    | Precuneus                   | CT         | 12.353             | 0.006     |
|                  | Agr    | Rostral anterior cingulate  | CT         | 7.759              | 0.022     |
|                  | Agr    | Rostral middle frontal      | CT         | 13.280             | < 0.001   |
|                  | Agr    | Superior frontal            | CT         | 24.962             | < 0.001   |
|                  | Agr    | Superior parietal           | CT         | 9.821              | 0.009     |
|                  | Agr    | Superior temporal           | CT         | 27.155             | < 0.001   |
|                  | Agr    | Supramarginal               | CT         | 22.159             | < 0.001   |
|                  | Agr    | Temporal pole               | CT         | 0.682              | 0.555     |
|                  | Agr    | Transverse temporal         | CT         | 2.574              | 0.211     |
|                | Right  | Banks of superior temporal sulcus | CT         | 11.955             | 0.006     |
|                | Right  | Caudal anterior cingulate   | CT         | 3.192              | 0.150     |
|                | Right  | Caudal middle frontal       | CT         | 2.576              | 0.209     |
|                | Right  | Cuneus                      | CT         | 1.553              | 0.363     |
|                | Right  | Entorhinal                  | CT         | 0.121              | 0.840     |
|                | Right  | Frontal pole                | CT         | 0.015              | 0.938     |
|                | Right  | Fusiform                    | CT         | 18.048             | < 0.001   |
|                | Right  | Inferior parietal           | CT         | 22.640             | < 0.001   |

Continued
| Effect            | Side | ROI                           | Index | £(1,149)-value | P(BHadj) |
|-------------------|------|-------------------------------|-------|---------------|---------|
| Superior frontal  |      | CT                            | 18.426| < 0.001       |         |
| Superior parietal |      | CT                            | 7.745 | 0.021         |         |
| Superior temporal |      | CT                            | 19.439| < 0.001       |         |
| Supramarginal     |      | CT                            | 10.607| 0.005         |         |
| Temporal pole     |      | CT                            | 0.020 | 0.950         |         |
| Transverse temporal|    | CT                            | 1.548 | 0.359         |         |
| Sex               | Left | Accumbens                      | Vol$_{\text{ICVadj}}$ 8.927 | 0.012 |         |
|                   |      | Amygdala                       | Vol$_{\text{ICVadj}}$ 0.074 | 0.878 |         |
|                   |      | Caudate                        | Vol$_{\text{ICVadj}}$ 4.492 | 0.086 |         |
|                   |      | Hippocampus                    | Vol$_{\text{ICVadj}}$ 10.913 | 0.007 |         |
|                   |      | Pallidum                       | Vol$_{\text{ICVadj}}$ 1.649 | 0.343 |         |
|                   |      | Putamen                        | Vol$_{\text{ICVadj}}$ 6.103 | 0.042 |         |
|                   |      | Thalamus                       | Vol$_{\text{ICVadj}}$ 1.934 | 0.289 |         |
|                   | Right| Accumbens                      | Vol$_{\text{ICVadj}}$ 3.833 | 0.113 |         |
|                   |      | Amygdala                       | Vol$_{\text{ICVadj}}$ 0.513 | 0.623 |         |
|                   |      | Caudate                        | Vol$_{\text{ICVadj}}$ 7.183 | 0.025 |         |
|                   |      | Hippocampus                    | Vol$_{\text{ICVadj}}$ 4.695 | 0.080 |         |
|                   |      | Pallidum                       | Vol$_{\text{ICVadj}}$ 7.633 | 0.020 |         |
|                   |      | Putamen                        | Vol$_{\text{ICVadj}}$ 4.265 | 0.096 |         |
|                   |      | Thalamus                       | Vol$_{\text{ICVadj}}$ 4.360 | 0.090 |         |
|                   | Left | Banks of superior temporal sulcus| CT 3.183| 0.157 |         |
|                   |      | Caudal anterior cingulate      | CT 0.019| 0.935 |         |
|                   |      | Caudal middle frontal          | CT 0.018| 0.934 |         |
|                   |      | Cuneus                         | CT 1.857| 0.302 |         |
|                   |      | Entorhinal                     | CT 0.075| 0.881 |         |
|                   |      | Frontal pole                   | CT 0.794| 0.519 |         |
|                   |      | Fusiform                       | CT 0.285| 0.761 |         |
|                   |      | Inferior parietal              | CT 2.104| 0.268 |         |
|                   |      | Inferior temporal              | CT 0.229| 0.780 |         |
|                   |      | Insula                         | CT 9.485| 0.008 |         |
|                   |      | Isthmus cingulate              | CT 0.031| 0.928 |         |
|                   |      | Lateral occipital              | CT 0.244| 0.772 |         |
|                   |      | Lateral orbito frontal         | CT 0.058| 0.886 |         |
|                   |      | Lingual                        | CT 0.891| 0.503 |         |
|                   |      | Medial orbito frontal          | CT 1.146| 0.455 |         |
|                   |      | Middle temporal                | CT 0.206| 0.783 |         |

Continued
| Effect                      | Side                        | ROI                          | Index | $F_{(1,149)}$-value | $p_{BHadj}$ |
|-----------------------------|-----------------------------|------------------------------|-------|---------------------|------------|
| Paracentral                 | CT                          | 2.266                        | 0.244 |                     |            |
| Parahippocampal             | CT                          | 0.936                        | 0.490 |                     |            |
| Pars opercularis            | CT                          | 1.245                        | 0.436 |                     |            |
| Pars orbitalis              | CT                          | 0.134                        | 0.837 |                     |            |
| Pars triangularis           | CT                          | 2.647                        | 0.204 |                     |            |
| Pericalcine                 | CT                          | 0.202                        | 0.782 |                     |            |
| Postcentral                 | CT                          | 4.122                        | 0.100 |                     |            |
| Posterior cingulate         | CT                          | 0.295                        | 0.759 |                     |            |
| Precentral                  | CT                          | 0.008                        | 0.948 |                     |            |
| Precuneus                   | CT                          | 0.098                        | 0.859 |                     |            |
| Rostral anterior cingulate  | CT                          | 0.038                        | 0.917 |                     |            |
| Rostral middle frontal      | CT                          | 0.019                        | 0.941 |                     |            |
| Superior frontal            | CT                          | 1.171                        | 0.451 |                     |            |
| Superior parietal           | CT                          | 0.459                        | 0.649 |                     |            |
| Superior temporal           | CT                          | 0.141                        | 0.835 |                     |            |
| Supramarginal               | CT                          | 4.028                        | 0.105 |                     |            |
| Temporal pole               | CT                          | 1.133                        | 0.447 |                     |            |
| Transverse temporal         | CT                          | 1.466                        | 0.377 |                     |            |
| Banks of superior temporal sulcus | CT                  | 3.084                        | 0.166 |                     |            |
| Caudal anterior cingulate   | CT                          | 0.069                        | 0.872 |                     |            |
| Caudal middle frontal       | CT                          | 0.809                        | 0.527 |                     |            |
| Cuneus                      | CT                          | 0.855                        | 0.513 |                     |            |
| Entorhinal                  | CT                          | 0.746                        | 0.536 |                     |            |
| Frontal pole                | CT                          | 1.243                        | 0.433 |                     |            |
| Fusiform                    | CT                          | 0.799                        | 0.522 |                     |            |
| Inferior parietal           | CT                          | 5.173                        | 0.063 |                     |            |
| Inferior temporal           | CT                          | 0.019                        | 0.946 |                     |            |
| Insula                      | CT                          | 5.346                        | 0.059 |                     |            |
| Isthmus cingulate           | CT                          | 6.254                        | 0.037 |                     |            |
| Lateral occipital           | CT                          | 0.625                        | 0.574 |                     |            |
| Lateral orbito frontal      | CT                          | 2.769                        | 0.193 |                     |            |
| Lingual                     | CT                          | 0.267                        | 0.770 |                     |            |
| Medial orbito frontal       | CT                          | 0.941                        | 0.493 |                     |            |
| Middle temporal             | CT                          | 0.167                        | 0.811 |                     |            |
| Paracentral                 | CT                          | 2.089                        | 0.267 |                     |            |
| Parahippocampal             | CT                          | 1.127                        | 0.444 |                     |            |
| Pars opercularis            | CT                          | 0.993                        | 0.478 |                     |            |
| Pars orbitalis              | CT                          | 0.670                        | 0.556 |                     |            |
| Pars triangularis           | CT                          | 0.007                        | 0.944 |                     |            |
| Pericalcine                 | CT                          | 0.008                        | 0.959 |                     |            |
| Postcentral                 | CT                          | 2.954                        | 0.178 |                     |            |
| Posterior cingulate         | CT                          | 0.704                        | 0.550 |                     |            |
| Precentral                  | CT                          | 0.252                        | 0.771 |                     |            |
| Precuneus                   | CT                          | 0.806                        | 0.524 |                     |            |
| Rostral anterior cingulate  | CT                          | 1.113                        | 0.444 |                     |            |
| Rostral middle frontal      | CT                          | 0.008                        | 0.953 |                     |            |
| Superior frontal            | CT                          | 0.003                        | 0.959 |                     |            |
| Superior parietal           | CT                          | 4.903                        | 0.072 |                     |            |
| Superior temporal           | CT                          | 0.220                        | 0.777 |                     |            |
| Supramarginal               | CT                          | 1.145                        | 0.451 |                     |            |
| Temporal pole               | CT                          | 0.005                        | 0.951 |                     |            |
| Transverse temporal         | CT                          | 0.262                        | 0.768 |                     |            |

Table 6. Post-hoc effects of age and sex on cortical thickness and subcortical volume measures. CT cortical thickness; Vol$_{ICVadj}$ volume adjusted for intracranial volume. $p_{BHadj}$ 5% False Discovery Rate Benjamini–Hochberg adjusted p value; ROI region of interest.
qMRI indices revealed the following pattern: Women compared to men, had lower ISOSF in widespread cortical reductions of neocortical dendritic spine density in fronto-parietal regions. These opposing patterns in cortical and subcortical regions may reflect a gender-related and lower ISOSF for white matter in women than men and subcortical regions and larger MPF in frontal and temporal regions. Previously we also reported higher MPF to this effect. For instance, childhood cognitive abilities have been found to account for relationships between cognitive performance and brain cortical thickness decades later in older adults from the Lothian birth cohort.

Adverse effects of reported in the basolateral nucleus of the amygdala of rats volumetric or cortical thickness measurements. We propose that these differences may reflect subtle changes risk-related microstructural differences in limbic grey and white matter that were not apparent in conventional volumetric or cortical thickness measurements. We propose that these differences may reflect subtle changes related to neuroglia activation and that limbic structures including the thalamus are particularly susceptible to adverse effects of APOE-e4 on glia cells. Inconsistencies in previous studies may have arisen from standard morphological and DTI measurements not being sensitive and/or specific enough to detect such glia-related changes.

It is important to note that while we did not find any risk-related effects on brain morphology we did replicate the well-established pattern of widespread age-related thinning in frontal, temporal and parietal regions as well as volume loss in subcortical structures including the hippocampi and thalami (Fig. 3). The subcortical volume loss was accompanied by age-related increases in ISOSF in bilateral hippocampi and thalami but effects on cortical regions were more localised: increased ISOSF was apparent along medial regions of the cingulate and parietal cortices including the precuneus as well as in superior temporal and lateral and orbito prefrontal cortices. Age-related increases in ISOSF have been previously observed and most likely reflect lost tissue being replaced by CSF. Consistent with a previous study we also observed a positive correlation between age and ODI, an estimate of neurite dispersion, in the hippocampus and the left caudate and amygdala. In contrast to Nazari et al. however, we did not find any effects in cortical regions, while they reported reduced ODI with age in fronto-parietal regions. These opposing patterns in cortical and subcortical regions may reflect age-related reductions of neocortical dendritic spine density with accompanying compensatory increases in the dendritic extent of dentate gyrus granular cells. Similar age-related increases in the dendritic tree have also been reported in the basolateral nucleus of the amygdala of rats.

Furthermore, we observed positive correlations between ISOSF and NART-IQ in superior temporal, parietal and lingual cortices that were partly driven by age. NART requires the reading of irregularly pronounced words and older relative to younger adults tended to perform better in the NART. However, positive albeit weak correlations between NART-IQ and ISOSF remained for the left superior temporal sulcus and left superior parietal cortex. Developmental imaging studies have revealed cortical thinning during adolescence that may be due to increased myelination or synaptic pruning and dendritic arborization. It may therefore be possible that childhood developmental differences in cortical maturation as well as in education may have contributed to this effect. For instance, childhood cognitive abilities have been found to account for relationships between cognitive performance and brain cortical thickness decades later in older adults from the Lothian birth cohort.

Consistent with previous reports we did not observe widespread sex-differences in brain morphology measurements with the exception of larger volumes in the left hippocampus in women than men. However, qMRI indices revealed the following pattern: Women compared to men, had lower ISOSF in widespread cortical and subcortical regions and larger MPF in frontal and temporal regions. Previously we also reported higher MPF and lower ISOSF for white matter in women than men. Overall this pattern of sex differences suggests higher cortical myelination and lower free water signal in women as they tended to be overall in better health i.e. were less obese, had lower systolic BP, and reported drinking less alcohol than men. All of these factors may have contributed to women showing “healthier” grey and white matter in the CARDS cohort.

Finally, some study limitations need to be considered. First of all, CARDS is a cross-sectional study that cannot answer whether the observed APOE effects on left thalamus MPF are predictive of accelerated development of LOAD pathology, cognitive, or neuronal decline. Future prospective longitudinal studies are required to address this question. We also propose that our findings require replication in larger samples that can control for possible interactions between APOE and other LOAD risk genes such as variants of TREM2 and polygenic risk hazards as the number of participants in the CARDS study was too small to do so. It is also worth mentioning that other qMRI measurements, that were not included in the current study, may prove helpful in characterising risk effects on the brain. Notably quantitative $T_1$ and $T_2$ measurements have been proposed to be sensitive to neurodegenerative processes. For instance, prolonged $T_1$ relaxometry has been reported in the hippocampus of LOAD patients and has been proposed to increase the sensitivity and specificity of MCI and LOAD detection. Finally, it should be noted that we only studied the thalamus as a whole structure while neuropathological evidence suggests a specific vulnerability of the anterodorsal thalamic nucleus to LOAD pathology. Future studies may investigate
risk-related effects on specific subthalamic nuclei, which was beyond the scope of the current study as we were focusing on risk effects across the whole brain.

In summary, we have shown APOE-ε4 related reductions in the qMT measure MPF in the left thalamus that were moderated by peripheral markers of inflammation. This effect occurred independently of age, sex and NART-IQ and was not observed in morphological or microstructural indices from diffusion-weighted imaging. In addition, the effect was specific to the left thalamus and was not present in other cortical and subcortical grey matter regions. We propose that MPF reductions may reflect the effects of glia-mediated inflammatory and demyelination processes in APOE-ε4 carriers. As such qMT measurements hold the potential for non-invasive and cheaper biomarker alternatives to PET, that may aid our understanding of the pathological processes leading to LOAD. In addition, qMT may help with the identification of asymptomatic individuals at heightened risk of LOAD for stratification into clinical trials for future preventative therapeutics.

Materials and methods

The Cardiff Ageing and Risk of Dementia Study (CARDS) has been described previously including a detailed description of the participant sample, assessment of genetic and metabolic risk factors and the acquisition and processing of the MRI data. Here we provide a brief summary of the most important points. CARDS received ethical approval from the School of Psychology Research Ethics Committee at Cardiff University (EC.14.09.09.3843R2) and all participants provided written informed consent in accordance with the Declaration of Helsinki. All research methods were performed in line with Cardiff University’s Research Integrity and Governance Code of Practice and relevant data protection regulations.

Participants. The CARDS cohort comprised 166 community-dwelling individuals between the age of 38 and 71 years who underwent cognitive and health assessment as well as MRI scanning (Table 1). Exclusion criteria were a history of neurological and/or psychiatric disease, head injury, drug/alcohol dependency, high risk cardio-embolic source, large-vessel disease or MRI incompatibility due to pacemaker, stents or other surgical implants. As a group, participants intellectual functioning was above average as assessed with the National Adult Reading Test (NART)44. All but one participant scored > 26 on the Mini Mental State Exam (MMSE)42 thus the remaining 165 participants were classified as cognitively healthy. Eight participants scored ≥ 10 in the Patient Health Questionnaire (PHQ)-946, suggesting moderate levels of depression but no participant was severely depressed.

Assessment of risk factors. Saliva samples were collected with the Genotek Oragene-DNA kit (OG-500) and APOE genotypes ε2, ε3, and ε4 were determined with TaqMan genotyping of single nucleotide polymorphism (SNP) rs7412 and KASP genotyping of SNP rs429358. Participants self-reported their family history of dementia, i.e., whether a first-grade relative was affected by Alzheimer’s disease, vascular dementia or any other type of dementia.

Central obesity was assessed from the waist-hip ratio (WHR)44 with abdominal obesity defined as a WHR ≥ 0.9 for males and ≥ 0.85 for females. Resting systolic and diastolic blood pressure (BP) readings were taken with a digital blood pressure monitor (Model UA-631; A&D Medical, Tokyo, Japan) and the means of three readings were calculated. Participants self-reported other metabolic risk factors, including diabetes mellitus, high levels of blood cholesterol controlled with statin medication, history of smoking, and weekly alcohol intake. There were only few diabetics, smokers, and individuals on statins and, hence, these variables were not included in the analyses.

Blood plasma analysis. As previously reported44,46, venous blood samples were drawn into 9 ml heparin coated plasma tubes after 12 h overnight fasting and were centrifuged for 10 min at 2000 × g within 1 h from blood collection. Plasma samples were then transferred into 0.5 ml polypropylene microtubes and stored in a freezer at −80 °C. Circulating levels of high-sensitivity C-Reactive Protein (CRP) in mg/dL were assayed using a human CRP Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, USA). Six individuals had a CRP value > 10 mg/ml indicative of acute infection and were, therefore, excluded from the statistical analyses testing for moderating effects of inflammation. Leptin concentrations in pg/ml were determined with the DRP300 Quantikine ELISA kit (R & D Systems) and adiponectin in ng/ml with the human total adiponectin/Acrp30 Quantikine ELISA kit (R & D Systems). Leptin/adiponectin ratios for each participant were calculated. Interleukin IL-8 levels in pg/mL were determined using a high sensitivity CXCL8/INTERLEUKIN-8 Quantikine ELISA kit (R & D Systems). Determination of interleukin-1β, interleukin-6 and Tumor Necrosis Factor α (TNFα) were trialled with high-sensitivity Quantikine ELISA kits but did not result in reliable measurements consistently above the level of detection for each assay.

MRI data acquisition. MRI data were acquired on a 3 T MAGNETOM Prisma clinical scanner (Siemens Healthcare, Erlangen, Germany) as described in43,44,49,108. T1-weighted images (1 × 1 × 1 mm voxel) were collected with a three-dimension (3D) magnetization-prepared rapid gradient-echo (MP-RAGE) sequence (256 × 256 acquisition matrix, TR = 2300 ms, TE = 3.06 ms, TI = 850 ms, flip angle θ = 9°, 176 slices, 1 mm slice thickness, FOV = 256 mm and acquisition time of ~6 min).

High Angular Resolution Diffusion Imaging (HARDI)101 data (2 × 2 × 2 mm voxel) were collected with a spin-echo echo-planar dual shell HARDI sequence with diffusion encoded along 90 isotropically distributed orientations101 (30 directions at b-value = 1200 s/mm² and 60 directions at b-value = 2400 s/mm²) and six non-diffusion weighted scans with dynamic field correction and the following parameters: TR = 9400 ms, TE = 67 ms,
80 slices, 2 mm slice thickness, FOV = 256 × 256 × 160 mm, GRAPPA acceleration factor = 2 and acquisition time of ~15 min.

Quantitative magnetization transfer weighted imaging (qMT) data were acquired with a prototype sequence, i.e. an optimized 3D MT-weighted gradient-recalled echo sequence to obtain magnetization transfer-weighted data with the following parameters: TR = 32 ms, TE = 2.46 ms, Gaussian MT pulses, duration t = 12.8 ms; FA = 5°; FOV = 24 cm, 2.5 × 2.5 × 2.5 mm³ resolution. The following off-resonance irradiation frequencies (Θ) and their corresponding saturation pulse nominal flip angles (ΔSAT) for the 11 MT-weighted images were optimized using Cramer-Rao lower bound optimization; Θ = [1000 Hz, 1000 Hz, 2750 Hz, 2768 Hz, 2790 Hz, 2890 Hz, 1000 Hz, 1000 Hz, 12,060 Hz, 47,180 Hz, 56,360 Hz] and their corresponding ASAT values = [332°, 333°, 628°, 628°, 628°, 628°, 628°, 628°, 332°]. The longitudinal relaxation time, T₁, of the system was estimated by acquiring three 3D gradient recalled echo sequence (GRE) volumes with three different flip angles (Θ = 3°, 7°, 15°) using the same acquisition parameters as used in the MT-weighted sequence (TR = 32 ms, TE = 2.46 ms, FOV = 24 cm, 2.5 × 2.5 × 2.5 mm³ resolution). Data for computing the static magnetic field (B₀) were collected using two 3D GRE volumes with different echo-times (TE = 4.92 ms and 7.38 ms respectively; TR = 330 ms; FOV = 240 mm; slice thickness 2.5 mm). The acquisition time for the complete qMT sequence including all fieldmaps was ~30 min.

**HARDI and qMT data processing.** As described in [23], the dual-shell HARDI data were split and b = 1200 and 2400 s/mm² data were corrected separately for distortions induced by the diffusion-weighted gradients and motion artifacts with appropriate reorientation of the encoding vectors in ExploreDTI. The acquisition time for the complete qMT sequence including all fieldmaps was ~30 min.

EPI-induced geometrical distortions were corrected by warping the diffusion-weighted image volumes to the T₁—weighted anatomical images. After pre-processing, the NODDI model was fitted to the HARDI data with the fast, linear model fitting algorithms of the Accelerated Microstructure Imaging via Convex Optimization (AMICO) framework to gain ISOSF, ICSF, and ODI maps.

Using Elastix, MT-weighted GRE volumes were coregistered to the MT-volume with the most contrast using a rigid body (6 degrees of freedom) registration to correct for inter-scan motion. Data from the 11 MT-weighted GRE images and T₁-maps were fitted by a two-pool model using the Ramani pulsed-MT approximation. This approximation provided MPF and kₜ maps. To remove voxels with noise-only data, MPF maps were thresholded to an upper intensity limit of 0.3 and kₜ maps to an upper limit of 5.0 using the fslmaths imaging calculator from the Functional Magnetic Resonance Imaging of the Brain (FMRIB) library (version 6).

All image modality maps were spatially aligned to the T₁-weighted anatomical volume as reference image with linear affine registration (12 degrees of freedom) in within-subject space using FMRIB’s Linear Image Registration Tool (FLIRT).

**Cortical and subcortical grey matter region segmentation.** Grey matter cortical and subcortical regions were automatically segmented from T₁—weighted images with the Freesurfer image analysis suite (version 5.3), which is documented online (https://surfer.nmr.mgh.harvard.edu/). The images were processed by running the “recon-all” script using the default analysis settings. In brief, the images were registered to the Montreal Neurological Institute standard space and intensity normalization was performed. This was followed by automatic skull stripping to remove extracerebral structures, the cerebral and the brain stem, followed by segmentation into grey matter, white matter and CSF and separation of the hemispheres. Pial surfaces were obtained by tessellating the grey and white matter boundary and by surface deformation following intensity gradients for optimal placement of grey and white matter and grey matter and CSF boundaries. Surface inflation and registration to a spherical atlas were then performed and the cerebral cortex was parcellated into 34 regions per hemisphere based on gyral and sulcal structures following the Desikan-Killiany atlas. Cortical thickness measurements were estimated as the average shortest distance between the pial surface and the white matter boundary. For each hemisphere, seven deep grey matter structures (hippocampus, amygdala, thalamus, caudate, putamen, pallidum, and nucleus accumbens) were automatically parcellated using a probabilistic atlas so that average volumetric measurements could be determined. Mean intracranial volume fractions (ICV) were estimated for each brain as estimates of individual differences in head sizes and all volumetric measurements were adjusted for ICV by dividing each participant’s subcortical volume by their ICV.

Finally, the mean values of all microstructural indices were extracted from each participants’ cortical and subcortical region of interests. Mean measurements were taken in each participants’ native space. This was done by first converting each participants’ cortical and subcortical masks from the FreeSurfer Massachusetts General Hospital volume file format (MGZ) into the Neuroimaging Informations Technology Initiative (NIFTI) analyze-style data format and then uploading the microstructural maps onto each region of interest mask using the fslmaths command from the FMRIB library. Mean values of each index for each mask were then extracted using the FMRIB fslstats command. NODDI and qMT indices of ISOSF, ICSF, OD, MPF and kₜ could not be extracted from bilateral caudal middle frontal, left isthmus cingulate and left pericalcarine regions and Rₙ could not be extracted from the right postcentral region.

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**Author contributions**
C.M.B.: conceptualization, methodology, formal analysis, writing—original draft preparation, writing—review and editing, visualization, funding acquisition; J.P.M.: investigation, formal analysis, data curation, project administration; R.S., E.K.: Resources; F.F., J.E.: Software; J.A.: reviewing and editing.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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