Increased cortical capillary transit time heterogeneity in Alzheimer's disease: a DSC-MRI perfusion study

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Abstract

Alzheimer's disease (AD) is characterized by accumulation of hyperphosphorylated tau and neurotoxic amyloid beta protein in the brain parenchyma. Hypoxia caused by microvascular changes and disturbed capillary flows could stimulate this build-up of AD-specific proteins in the brain. In this study we compared cerebral microcirculation in a cohort of AD and MCI patients with that of age-matched controls, all without a history of diabetes or of hypertension for more than two years, using dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI). Vascular flow disturbances were quantified using a parametric model and mapped to the mid-cortical surface for group-wise statistical analysis. We found widespread hypoperfusion in patients compared to controls and identified areas of increased relative capillary transit time heterogeneity (RTH), consistent with low tissue oxygen tension. Notably, RTH was positively correlated with white matter hyperintensities and positively correlated with symptom severity in the patient cohort. These correlations extended over large parts of the temporal, parietal and frontal cortices. The results support the hypothesis of disturbed capillary flow patterns in AD and suggest that DSC-MRI may provide imaging biomarkers of impaired cerebral microcirculation in AD.

Keywords: *Alzheimer, MRI, perfusion, capillary transit-time heterogeneity (CTH), oxygen extraction fraction (OEF), white matter hyperintensities*

Abbreviations:

AD: Alzheimer's disease
AIF: Arterial input function
APOE-4: apolipoprotein ϵ 4
BOLD: blood oxygen level dependent
CBF: Cerebral blood flow
CBV: Cerebral blood volume
CMRO2: Cerebral metabolic rate of oxygen
CSF: Cerebrospinal fluid
CTH: Capillary transit time heterogeneity
DSC: Dynamic susceptibility contrast
DSM-IV: Diagnostic and statistical manual of mental disorders, 4th edition
FACE: Fast accurate cortex extraction
FLAIR: Fluid attenuated inversion recovery
GM: Grey matter
GRE: Gradient echo

ICD-10: International classification of diseases, 10th edition
MMSE: Mini-mental state examination
MTT: Mean transit time
NAWM: Normal appearing white matter
OEF: Oxygen extraction fraction
PET: Positron emission tomography
RTH: Relative transit time heterogeneity
SE: Spin echo
SVD: Small vessel disease
T1w: T1-weighted
TE: Echo time
TI: Inversion time
TR: Repetition time
WM: White matter
WMH: White matter hyperintensities
WMHL: White matter hyperintensitiy load

1. Introduction

Disturbances in the neurovascular coupling mechanisms that secure adequate blood supply to brain tissue have long been suspected of playing a part in the development of Alzheimer's disease (AD) (Girouard and Iadecola, 2006). In support of this hypothesis, evidence suggests that vascular changes and hypoperfusion are intimately involved in the etiopathogenesis of AD (Iadecola, 2010; Kalaria, 2010; Montagne et al., 2016; Pantoni, 2010; Zlokovic, 2011). Indeed, AD shares many risk factors with cardiovascular disease, including hypertension, hypercholesterolemia, and diabetes. These risk factors are known to impair vessel function, and at times limit organ blood supply. In AD, however, blood vessels and blood flow is only mildly affected when cognitive symptoms develop, making it difficult to explain the development of AD in terms of limited blood flow or oxygen supply (Hirao et al., 2005; Ruitenberg et al., 2005).

AD and cardiovascular risk factors share a less studied feature, namely morphological changes in the walls of the smallest blood vessels, the capillaries. Such changes are likely to disturb, but not necessarily block, the flow of blood through tissue. According to the classical understanding of oxygen supply, the availability of oxygen in tissue depends on the blood flow and the capillary density in tissue, but not the way in which blood is distributed among capillaries (Crone, 1963; Krogh, 1919; Renkin, 1985). This fundamental assumption was recently shown to be in error (Jespersen and Ostergaard, 2012): changes in capillary flow patterns invariably increase the proportion of erythrocytes that pass through the capillary bed too fast to permit efficient extraction of their oxygen by the tissue. Direct microscopy studies indeed show that capillary flows shift to a more homogenous pattern during functional activation (Gutierrez-Jimenez et al., 2016; Jespersen and Ostergaard, 2012; Lee et al., 2016). This mechanism serves to maintain efficient oxygen extraction during episodes of high flow by reducing 'functional shunting'. If, however, changes in capillary morphology or function prevent this homogenization, the consequences are striking: functional shunting of oxygenated blood can reach levels such that tissue becomes severely hypoxic, even at non-ischemic cerebral blood flow (CBF) levels, and such that increased CBF no longer improves oxygen availability at the level of individual neurons (Jespersen and Ostergaard, 2012).

The classical relation between CBF and tissue oxygenation (Renkin, 1985) was recently extended to include the effects of capillary transit time heterogeneity (CTH) (Jespersen and Ostergaard, 2012). Elevated CTH, and the failure of CTH to decrease during episodes of increased metabolic needs, is referred to as *capillary dysfunction* and thought to result from the gradual changes in capillary wall morphology and function that accompany AD risk factors (Ostergaard et al., 2013). As CTH increases, the resulting fall in oxygen extraction fraction (OEF) is initially predicted to elicit compensatory increases in CBF and blood oxygen level dependent (BOLD) responses during functional activation to maintain tissue oxygenation - a puzzling phenomenon observed in college-age carriers of the apolipoprotein $\epsilon 4$ (APOE-4) AD risk gene (Filippini et al., 2009; Scarmeas et al., 2005). As CTH increases further, however, the resulting shunting is predicted to reach a critical limit at which increased CBF is no longer sufficient to maintain and increase tissue oxygen availability. Biophysically, functional shunting can now only be limited by suppressing flow velocities across the microvascular network, allowing oxygen extraction efficacy to improve while tissue oxygen tension falls. Attenuated flow-responses, a phenomenon referred to as neurovascular dysfunction, are the earliest known signs of imminent hypertension (Iadecola and Davisson, 2008), hypercholesterolemia, diabetes, and AD (Bell and Zlokovic, 2009; Chow et al., 2007; Montagne et al., 2016; Niwa et al., 2001). According to the

proposed hypothesis, this phenomenon thus reflects flow-metabolism coupling mechanisms that maintain tissue oxygenation despite changes in capillary morphology and function in these conditions.

Irrespective of its origin, attenuation of normal flow responses comes at great costs: endothelial dysfunction is mediated by the release of free radicals in the vessel walls (Girouard et al., 2007), causing vessel walls to thicken and blood clots to form more frequently. While attenuation of flow responses allows more efficient oxygen extraction, tissue oxygen tension falls in parallel. Hypoxia, in turn, stimulates neurodegeneration, inflammation (Eltzschig and Carmeliet, 2011), the formation of A β and neurofibrillary tangles, and impairs A β degradation and its clearance across the blood-brain barrier (Sun et al., 2006; Zhang and Le, 2010).

The heterogeneity of vascular flows can be inferred from the retention of intravascular contrast media in the brain circulation by means of dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI) (Mouridsen et al., 2006b; Ostergaard et al., 1999; Ostergaard et al., 2000). Recently, these methods have been extended to estimate both the mean transit time (MTT) and CTH, as well as the accompanying oxygen extraction efficacy (Mouridsen et al., 2014) based on the extended model of capillary tissue oxygenation (Jespersen and Ostergaard, 2012). In this study, we apply DSC-MRI to measure cerebral perfusion, including capillary transit time properties, in patients with AD or mild cognitive impairment(MCI) and in healthy controls to examine whether signs of capillary dysfunction can be detected in AD (Ostergaard et al., 2013).

2. Methods

2.1. Participants and imaging

Patients were referrals to the Dementia Clinic, Department of Neurology, Aarhus University Hospital who fulfilled the following inclusion criteria: Mini Mental State Examination (MMSE) (Folstein et al., 1975) score ≥ 20 , age > 40 years, and clinical suspicion of AD or MCI after mental state examination by a neuro-psychologist (Waldemar et al., 1994). Exclusion criteria included diabetes type 1 or 2, a history of hypertension for more than two years (treated or untreated), clinical suspicion of major depression (also if treated), clinical suspicion of alcohol-related dementia or of other organic or psychiatric cause the patients symptoms, and any contraindications to contrast-enhanced MRI. The patients' final diagnosis was recorded.

Control subjects were recruited through an advertisement in a local newspaper. Inclusion criteria included age > 40 , MMSE ≥ 28 , normal neurological examination, normal CAMCOG test scores (Roth et al., 1986), and normal mental state examination by a neuropsychologist. Exclusion criteria were identical to those of the patients.

The study was approved by the Local Committee for Ethics in Biomedical Research (Permission no. 2001-0326). After information by one of the study physicians (LG, HB), written consent was obtained from all study participants. Data storage and handling was approved by the Danish Data Protection Agency (Permission no. 2002-41-1815)

A total of 27 patients were enrolled in the study. Of these, MRI could not be completed in four patients due to movement artifacts or technical issues, while five patients received a final diagnosis other than AD or

MCI. Twenty-two controls were included in the study, but full MRI data sets could not be obtained in three subjects due to movement artifacts or technical issues. In total, 18 patients with AD or MCI and 19 controls are hence reported here – See Table 1 for demographic and risk factor data.

Imaging was performed on a 1.5T GE Signa LX scanner. DSC-MRI was acquired using gradient echo (GRE) and spin echo (SE) EPI with i.v.-bolus injection (5 ml/s) of 0.1 mmol/kg and 0.2 mmol/kg gadobutrol (Gadovist® 1.0 M, Schering), respectively, followed by injection of 20 ml of saline at a rate of 5 ml/s. Due to the microvascular weighting of the SE sequence (See below), the contrast-to-noise of SE DSC-MRI is inherently lower than for GRE DSC-MRI. Therefore, double contrast agent dose is generally used to obtain sufficient perfusion image quality. In each sequence, a time series of 32 whole brain image volumes were acquired at TR=1.5 s. Bolus injection was administered after 10-15 repetitions. In the GRE sequence, 16 axial slices were imaged in an interleaved fashion, at a spatial resolution of 1.9x1.9x6.5mm. With the SE sequence, 12 slices were imaged at the same spatial resolution. Slice time correction was performed in a post-processing step.

DSC-MRI by SE and GRE EPI differ in terms of their sensitivity to contrast agent in the microvasculature. While GRE EPI is sensitive to intravascular contrast irrespective of vessel size in a linear, tissue-concentration dependent fashion, the SE EPI sequence is predominantly sensitive to contrast agent in capillary-size vessels (Boxerman et al., 1995; Weisskoff et al., 1994). By the dual injection scheme, we were thus able to address changes in ‘overall’ hemodynamics measured by GRE and compare our results to CBF, MTT and CBV estimates in the literature for any modality. With the SE measurements, which are rarely used due to their lesser sensitivity, we could address capillary level hemodynamics, which we hope more specifically reflect CTH and capillary density.

Fluid attenuated inversion recovery (FLAIR) images were acquired for quantification of white matter hyperintensities (WMHs) in order to identify signs of cerebral small vessel disease (TE=142.5 ms, TR=9 s, TI=2.2 s, reconstruction matrix = 256x256, in-plane resolution = 0.94x0.94 mm², slice thickness = 6.5 mm, 16 slices). For co-registration purposes and for the assessment of cerebral atrophy, whole brain 3D T1-weighted (T1w) images were acquired with a spatial resolution of 0.9x0.9x1.5 mm³ and a reconstruction matrix of 256x256x116.

The acquisition order was 3D T1 followed by FLAIR, GRE DSC, and SE DSC with a total scan time of approximately 20 min. All patients and controls followed the same imaging protocol and acquisition order.

2.2. Calculation of perfusion parameters

A parametric approach was used for estimating CBF, CTH, cerebral blood volume (CBV), and MTT from the DSC-MRI raw data (Mouridsen et al., 2006b; Mouridsen et al., 2014). The method is validated for a range of signal-to-noise levels typical for DSC-MRI acquisitions and has been shown to provide more robust perfusion estimates than non-parametric approaches (Mouridsen et al., 2006b; Mouridsen et al., 2014). The model provides quantitative measures of MTT and CTH, while CBF and CBV are scaled by an indeterminable factor κ . Accordingly, CBF and CBV were normalized by corresponding values for normal appearing white matter (NAWM) identified on T1w images (see below).

2.2.1 Maximum oxygen extraction fraction and metabolic rate

To estimate the maximum oxygen extraction fraction (OE^{max}) and the maximum cerebral metabolic rate of oxygen ($CMRO_2^{max}$), which can be supported by the estimated combination of MTT and CTH at normal brain oxygen tension, we applied the recent model by Jespersen and Østergaard (2012). The model is composed of three parts: i) a model of the oxygen extraction along a single capillary Q as function of transit time τ , ii) a model of the capillary transit time distribution $h(\tau)$, and iii) the resulting OE^{max} defined as the sum of the single capillary contributions weighted by the capillary transit time distribution. $Q(\tau)$ is modelled as a three-compartment model consisting of tissue, blood plasma and haemoglobin, where transfer of oxygen across the capillary membrane is determined by an unknown rate constant k . The model assumes normal tissue oxygen tension, $P_tO_2 = 25$ mmHg. See (Jespersen and Østergaard, 2012) for details. Subject specific rate constants, k , was found by assuming $OE^{max}=0.3$ in NAWM (identified on T1w images) for the corresponding MTT and CTH. From OE^{max} , the upper limit of the cerebral metabolic rate of oxygen that can be supported for $P_tO_2 = 25$ mmHg is calculated as $CMRO_2^{max} = Ca \cdot CBF \cdot OE^{max}$, where Ca is the arterial oxygen concentration. $CMRO_2^{max}$ depends on CBF, and we therefore normalized this index to NAWM.

2.2.2 Selection of arterial input function

Calculation of perfusion parameters involves the identification of an arterial input function (AIF), which accounts for the delivery of intravascular tracer to the tissue. The selection of voxels from which to estimate the AIF is therefore a crucial step in the procedure. For each subject we calculated a global AIF by averaging the signal over AIF voxels selected semi-automatically: First, an experienced operator visually identified the slice and approximate region of the vertical segments of the middle cerebral arteries (lower part of M2), then an automatic selection algorithm identified arterial voxels (Mouridsen et al., 2006a). We chose the M2 segment of the middle cerebral arteries (MCA) to (i) avoid signal truncation, which may occur at larger arteries such as carotid artery and MCA M1, to (ii) minimize the effects of phase shifts on the AIF shape by measuring from arteries running in parallel with the static magnetic field (B_0), and (iii) to measure the AIF as close to the tissue as possible to avoid the inclusion of large vessel dispersion into transit time distribution estimations (Calamante, 2013). All AIF curves were visually inspected to ensure arterial shape characteristics, i.e. early tracer arrival, high peak height, and quick wash-out.

2.3. Image processing

T1w images were preprocessed using the framework described in (Aubert-Broche et al., 2013). In this framework images are denoised (Coupe et al., 2008) using an estimated standard deviation of noise (Coupe et al., 2010), bias field corrected (Sled et al., 1998), rigidly (Collins et al., 1994) and non-rigidly (Collins and Evans, 1997) registered to MNI space and skull stripped (Eskildsen et al., 2012). Brain tissue is classified into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using an artificial neural network classifier (Zijdenbos et al., 1998).

Cortical surfaces were generated with FACE (fast accurate cortex extraction) (Eskildsen and Østergaard, 2006; Eskildsen et al., 2005). In FACE, topologically correct surface meshes are iteratively fitted to the WM-GM interface and the GM-CSF with subvoxel precision. FACE has been shown to be more accurate and much faster than the FreeSurfer method (Eskildsen and Østergaard, 2007).

Cortical surfaces were transformed to perfusion native space using the transformation matrix from a rigid body co-registration between perfusion and T1w images (Collins et al., 1994). Similar to previous work (Chen et al., 2011), perfusion parameters were interpolated and mapped to the surface approximating the middle cortical layer in order to minimize the influence of partial volume effects and larger vessels at the cortical surface (Figure 1). Individual surfaces were registered to the cortical surface of an average non-linear anatomical template in MNI space (Fonov et al., 2011) using a feature driven surface registration algorithm (Eskildsen and Ostergaard, 2008). Perfusion values were then mapped to the average surface and smoothed using a 20 mm FWHM geodesic Gaussian kernel. Smoothing along the cortex eliminates the unwanted blurring across gyri caused by smoothing in voxel space.

2.3.1 Normalization and estimation of rate constants

Normal appearing white matter was used for normalizing CBF, CBV and $\text{CMRO}_2^{\text{max}}$ and for estimating subject specific rate constants. In order to obtain robust and consistent values, a whole-brain WM mask without lesions was used for the normalization and estimation of rate constants. The WM mask was generated by a single voxel erosion of the T1w WM tissue classification to avoid any overlap with GM or CSF. WMHs identified on FLAIR images (see below) were removed from the WM classification through FLAIR-T1 co-registration using a rigid body transformation. Using this mask we found subject specific rate constants in the range of 32 s^{-1} to 111 s^{-1} (mean $\pm$ sd: $57\pm17 \text{ s}^{-1}$) for SE and 37 s^{-1} to 154 s^{-1} (mean $\pm$ sd: $74\pm24 \text{ s}^{-1}$) for GRE. Finally, voxels with extreme CBV values (lower than the 5th percentile and higher than the 95th percentile) were excluded from further analysis in order to minimize partial volume- and edge-effects from CSF and large vessels.

2.4. Measurements of atrophy and WM hyperintensities

Cerebral atrophy was quantified in terms of hippocampal volume and cortical thickness. Hippocampus was segmented using a patch based label fusion method (Coupé et al., 2011), which has been shown to be sensitive to AD related atrophy (Coupe et al., 2012). Cortical thickness was calculated using the cortical surfaces determined by FACE, estimated as the distance between the GM-CSF interface and the WM-GM interface, perpendicular to the cortical surface. Thickness values were obtained for each vertex in the GM-CSF surface adding to approximately 200,000 measurements per subject. Similar to perfusion values, thickness values were mapped to the average surface and blurred using a 20 mm FWHM geodesic Gaussian kernel.

WMHs on FLAIR images were semi-automatically (Smart et al., 2011) outlined and total WMH volume was calculated by interpolating the 2D images on a 3D matrix. WMH load was estimated as the WMH volume fraction of total brain volume.

2.5. Statistical analysis

Simulation studies show that CTH tends to vary in proportion to MTT in healthy, passive, compliant microvascular networks (Rasmussen et al., 2015). This intrinsic hemodynamic property seems crucial for the maintenance of optimal oxygen extraction in tissue: functional shunting seemingly becomes critical if CTH exceeds MTT (Angleys et al., 2015; Jespersen and Ostergaard, 2012), and the attenuation of microvascular flows mentioned above would therefore be predicted to prolong MTT in proportion to CTH. To interrogate

this critical relation between transit time heterogeneity and MTT, we used the transit times coefficient of variation, also referred to as the relative transit time heterogeneity (RTH), $RTH = CTH/MTT$, below.

Group differences were examined using Student t tests for continuous variables and Pearson χ^2 tests for categorical variables. CBF, CBV, and $CMRO_2^{max}$ were normalized by dividing with the subject's corresponding NAWM value and multiplying with the grand average of NAWM values across subjects. Statistical maps of differences in perfusion parameters between patients and controls were calculated at each surface vertex using a vertex specific general linear model with cortical thickness, white matter hyperintensity load (WMHL, given as percentage WMH volume of whole brain volume), age, and gender as covariates. We added cortical thickness as covariate to account for any systematic effects of cortical thickness on the perfusion measurements, such as partial volume effects. Similarly, regression with perfusion parameter as dependent variable and total MMSE score, cortical thickness, WMHL, age, and gender as independent variables were calculated. Statistical maps were family-wise error corrected using random field theory (Worsley et al., 1996) with $\alpha=0.001$ as cluster defining threshold. All statistical maps were thresholded at $p=0.05$ (uncorrected and corrected). We chose to visualize uncorrected statistical maps in this explorative study in order to compare maps across parameters. All statistical tests were carried out using R version 3.2.2 (The R Foundation for Statistical Computing) and SPM12 (Wellcome Trust Centre for Neuroimaging) running on Matlab R2016a (MathWorks Inc).

3. Results

As expected, patients had significantly higher WMH volume ($p<0.04$), smaller brains ($p=0.02$) and hippocampi ($p<0.01$) compared to controls (Table 1). Widespread bilateral cortical atrophy was observed in patients (Figure 2). In addition to the well-known AD atrophy pattern (Eskildsen et al., 2013) of thinner cortex in temporal lobes, parietal lobes, posterior cingulate cortex and precuneus, the patients exhibited cortical thinning in parts of the prefrontal cortex.

3.1. Perfusion group differences

Cortical perfusion was reduced in patients compared to controls as measured by both SE and GRE CBF (Figure 3, first column). This reduction was especially prominent on GRE maps, which showed reductions across all major lobes, but with parietal, temporal and cingulate cortices being the areas most severely affected by hypoperfusion. GRE CBV was also reduced in patients, following a similar pattern as CBF (Supplementary Material). Accordingly, CBV was lower in patients (GRE: $M=-9.4\%$, 95% CI $[-18.1\%, -0.6\%]$; SE: $M=-5.8\%$, 95% CI $[-12.7\%, 1.2\%]$) within regions with reduced CBF. CTH and MTT were generally elevated in patients compared to controls. Areas with elevated CTH and MTT generally overlapped with, but were smaller than, areas with significant CBF reductions (Figure 3, second column and Supplementary Material), and we keep in mind that CBF is related to MTT and CBV through $CBF=CBV/MTT$ (Stewart, 1893). RTH was also found to be elevated in patients, but mostly for SE measurements (Figure 3, last column). Contrary to CTH, areas of elevated RTH did not overlap with areas of reduced CBF.

Maps of estimated oxygen extraction capacity (OEF^{max}) revealed significant increases bilaterally in the posterior cingulate, precuneus and parietal lobes for both SE and GRE based measurements (figure 4, left column). The SE based OEF^{max} map showed additional increases in the frontal lobes. GRE based $CMRO_2^{max}$ es-

timates showed extensive reductions in oxygen availability following a pattern similar to the GRE based CBF measurements (Figure 4, right column). Similarly, the lower $\text{CMRO}_2^{\text{max}}$ in patients found using SE measurements overlapped with SE-based CBF reductions.

3.2. Correlations with white matter hyperintensities

WMHL was significantly positively correlated with age in both patients ($p=0.03$) and controls ($p=0.04$), while no correlations with gender, MMSE or hippocampal volume were found when adjusted for age. Within patients, WMHL correlated positively with RTH as determined by SE in large parts of the temporal, parietal and frontal lobes (Figure 5). A single cluster located where the surface cuts through the lower part of the thalamus and caudate nucleus survived FWE correction (Supplemental Material). We did not observe this relation using GRE. Both SE and GRE perfusion maps showed positive correlations between WMHL and CTH in the anterior cingulate cortex. This was accompanied by a negative correlation with CBF using GRE maps, while SE maps demonstrated a positive correlation mainly in the temporal and parietal lobes (Figure 5). Similar to the correlations found for CBF maps, WMHL was negatively correlated with SE MTT in large parts of the cortex, except for the medial frontal and cingulate cortex, which exhibited a positive correlation similarly to the one found for CTH (Supplementary Material). Finally, a strong positive correlation with SE OEF^{max} , which survived FWE correction, was found in the cingulate cortex (predominantly anterior) possibly driven by the MTT correlation (Figure 5 and Supplementary Material).

3.3. Correlation with cognitive impairment

RTH measured by SE was the only parameter found to widely correlate with total MMSE score within the patient group (Supplementary Material). Only areas with negative correlations were found, and these were located symmetrically in all lobes except the occipital lobe (Figure 6, right). GRE based CBF exhibited positive correlations confined to the posterior cingulate, precuneus, and parahippocampal region (predominantly right hemisphere) (Supplementary Material).

4. Discussion

Earlier studies have reported regional reductions in CBF (Alsop et al., 2010; Alsop et al., 2000; Binnewijzend et al., 2015; Dai et al., 2009; Hu et al., 2010; Johnson et al., 2005; Lacalle-Auriales et al., 2014; Yoshiura et al., 2009) and elevated OEF (Nagata et al., 2000; Nagata et al., 2002; Tohgi et al., 1998) in patients with suspected or definite AD compared to controls. Our study extends these studies by demonstrating reductions in both CBF and CBV, as well as an inverse relation between cognitive scores and intra-voxel flow patterns, as measured by SE based DSC-MRI. Due to the microvascular sensitivity of SE based DSC-MRI, we interpret these findings as evidence of capillary loss and capillary flow disturbances, consistent with capillary pathology in AD (Ostergaard et al., 2013).

According to the classical understanding of CBF and its relation to oxygen availability, AD-related hypoperfusion never reaches ischemic levels. Our work points to two mechanisms which may cause us to overestimate tissue oxygenation based on CBF alone: first, oxygen availability falls in proportion to capillary density, and the observed fall in CBV as measured by SE DSC thus suggests a more severe reduction in oxygen availability in the affected brain region. Second, elevated CTH reduces oxygen extraction efficacy for a

given CBF, and the observed increase in CTH as measured by the capillary-weighted SE DSC sequence thus again suggests a more severe reduction in oxygen availability. We noted a net *increase* in OEF^{max} , rather than a reduction as one might have expected. OEF^{max} increases with MTT, and this finding is thus attributed to a parallel increase in MTT (reduction of CBF). This finding is consistent with the prediction that CBF is attenuated to compensate for functional shunting in capillary dysfunction. The observed correlation between symptom severity and CTH/MTT further supports a link between capillary dysfunction and AD.

The gradual reduction in CBF observed in AD patients has been attributed to a lower demand of oxygen caused by neurodegeneration. However, studies show that hypoperfusion precedes the development of AD symptoms (Hirao et al., 2005; Knopman and Roberts, 2010; Ruitenberg et al., 2005). Our findings suggest that these CBF reductions in reality serve to increase OEF, albeit at the expense of tissue hypoxia. Recent observations confirm that capillary function is disturbed prior to neurodegenerative changes in animal models of dementia (Bell et al., 2010; Bell et al., 2012), and we have pointed out that capillary morphology and function is likely to be disturbed in most AD risk factors, suggesting that capillary dysfunction may be an important contributing factor to tissue hypoxia and the development of AD pathology (Ostergaard et al., 2013).

Our measurements of capillary transit times did indeed indicate capillary flow disturbances as shown by the elevated RTH in patients compared to controls. The capillary dysfunction hypothesis of AD (Ostergaard et al., 2013) predicts that microvascular transit times are attenuated to limit functional shunting and thereby optimize oxygen extraction under such conditions. Applying our extended model of tissue oxygenation to take capillary flow patterns into account (Jespersen and Ostergaard, 2012) we indeed observed elevated OEF^{max} in patients as predicted. The elevated OEF^{max} was primarily found in the parietal and cingulate cortices. These observations are in line with previous studies using positron emission tomography (PET). Nagata and colleagues found hypoperfusion accompanied by significant increase in OEF in parietotemporal areas of AD patients using PET H_2O^{15} (Nagata et al., 2000; Nagata et al., 2002). Also, Tohgi et al. found increased OEF in the parietal cortex in AD patients using PET ^{15}O (Tohgi et al., 1998). Contrary to these results and the capillary dysfunction hypothesis, a study found decreased OEF in the medial temporal lobes of AD patients using PET ^{15}O , while other cortical regions were similar to controls (Ishii et al., 1996). This finding may be explained by differences in CBV between patients and controls, as the OEF estimate method applied by Ishii and colleagues is highly sensitive to CBV.

Regions of reduced CBF reported here confirm previous studies demonstrating hypoperfusion in the precuneus, posterior cingulate and temporoparietal regions in AD and MCI patients (Alsop et al., 2000; Chao et al., 2009; Dai et al., 2009; Hauser et al., 2013; Hu et al., 2010; Johnson et al., 2005; Xu et al., 2007; Yoshiura et al., 2009). Our index of capillary dysfunction, the SE based RTH, was found to be elevated within these regions. CBF changes cannot account for the changes in capillary flow distribution found here, as RTH is flow-normalized (CTH/MTT). In addition, RTH involvement extended to frontal and medial temporal lobes – especially when considering the correlation with WMHL. Our finding of capillary loss, as indicated by reduced CBV, is consistent with previous reports (Bozzao et al., 2001; Lacalle-Auriales et al., 2014; Uh et al., 2010). It remains unclear whether capillary loss can account for the micro-vascular hemodynamic changes: the topology and function of the microvasculature is carefully orchestrated to provide oxygen delivery to cells (Pries and Secomb, 2014). On one hand, the elimination of poorly functioning capillaries might lead to

a more homogenous flow across the remaining capillaries, but on the other hand, any accompanying disruption of metabolic signalling across the capillary bed might also lead to excessive functional shunting.

4.1. Perfusion in aging

Cerebral perfusion changes in aging have been studied for more than six decades with divergent results and conclusions. In recent years, with improved measuring techniques, converging evidence suggests regional decreases of CBF and to some extent also reduced CMRO₂ with aging (Biagi et al., 2007; Borghammer et al., 2008; Ibaraki et al., 2010; Parkes et al., 2004; Aanerud et al., 2012), while age related changes in OEF seem more controversial (Aanerud et al., 2012). These studies indicate widespread hypoperfusion involving frontal, temporal and parietal cortical and subcortical regions (Hays et al., 2016). In agreement with these studies we found decreased regional cortical CBF and CMRO₂^{max} with age measured by SE, while OEF^{max} increased in some regions (particularly the cingulate cortex) and decreased in others with advancing age (data not shown). Accordingly, age was used as covariate in all statistical maps.

4.2. Capillary dysfunction and white matter hyperintensities

White matter hyperintensities on T2-weighted FLAIR images are markers of small vessel disease (SVD) and associated with cognitive decline (Poggesi et al., 2011; Prins and Scheltens, 2015). WMHs are commonly observed in aging, though more prevalent in dementia (Barber et al., 1999). We observed WMHs in both groups studied here with significantly more WMHs in patients. Most WMHs have a hypoxic-ischaemic origin (Prins and Scheltens, 2015), and studies have demonstrated clear relations between WMHs and subsequent cognitive decline (Carmichael et al., 2010; De Groot et al., 2002; Garde et al., 2005). One study has shown that only periventricular and not subcortical WMH progression is associated with cognitive decline (van Dijk et al., 2008), which may explain why we did not observe a correlation between WMHL and MMSE, since our estimate of WMHL includes both periventricular and subcortical WMHs.

We observed a negative correlation between WMHL and CBF as measured by GRE perfusion in the cingulate cortex, which was paralleled by a positive correlation with MTT and CTH measured by both GRE and SE. This positive correlation existed for RTH measured by SE in the left cingulate. However, the positive correlation between WMHL and RTH extended to the precuneus and large parts of the frontal, temporal and parietal lobes making SE RTH the only parameter demonstrating widespread correlation with WMHL (and survived FWE correction). Interestingly, the cluster surviving FWE correction was based on measurements from thalamus and caudate nucleus due to the nature of projecting volumetric measurements onto a closed surface. Others have reported increased cerebrovascular resistance index (ratio of mean arterial pressure to rCBF) in these regions and correlation with symptom severity and WMH volume in AD and MCI (Nation et al., 2013). The RTH correlation was accompanied by a strong, focal correlation with OEF^{max} in the cingulate cortex (surviving FWE correction). These findings suggest that progression of SVD is paralleled by increased cortical capillary dysfunction in several of the regions known to be affected in AD. However, the present data cannot reveal the temporal ordering of these microvascular abnormalities.

4.3. Capillary dysfunction and cognitive symptoms

Interestingly, in AD patients RTH was the only perfusion parameter to show widespread correlation with symptom severity as measured by MMSE (Figure 6). In passive, compliant networks, CTH tends to change in proportion to MTT, and RTH is therefore expected to remain constant in normal vasculature, independent

of any CBF changes. Therefore, RTH may represent a better marker of capillary dysfunction involved in the AD pathogenesis and may signal imminent hypoxia. In hypoxic states, neurons may not have sufficient oxygen to generate action potentials (by disrupting ATP synthesis (Zlokovic, 2011)), but may have sufficient oxygen to survive. Accordingly, functional impairment may be a consequence of initial hypoxia and explain the observed correlations between RTH and cognitive decline. Downstream, when hypoxia increases due to more severe capillary dysfunction and hypoperfusion, the neurons are susceptible to apoptosis and neurodegeneration occur. However, the temporal course of capillary dysfunction and the neuronal adaptation to the microvascular changes remain to be uncovered. Cognitive symptoms may follow closely the impaired oxygen availability caused by the capillary dysfunction, while neurodegeneration comes later. In a post hoc analysis we observed a negative correlation between RTH and cortical thickness confined to the precuneus and supramarginal gyrus (Supplementary Material).

4.4. Capillary dysfunction and blood brain barrier breakdown

The capillary dysfunction phenomenon arises from the fact that oxygen extraction from the capillary bed is most efficient if blood is homogeneously distributed along its parallel capillary pathways. In resting brain tissue, animal studies show that the distribution of blood is highly inhomogeneous across the capillary bed (Kleinfeld et al., 1998; Villringer et al., 1994), and capillary dysfunction is thus characterized by the inability to redistribute blood along different capillary paths over time, which may give rise to oxygen-starved 'lethal corners' in the tissue. Using GRE perfusion imaging and gold-standard PET ^{15}O in carotid stenosis patients, we have found preliminary evidence that knowledge of both CBF (or MTT) and CTH is necessary to explain actual oxygen extraction in brain tissue (Ostergaard et al., 2015). That is, the microvasculature seems to limit oxygen extraction in patient with cerebrovascular disease. During functional hyperaemia, homogenization of capillary flows is crucial for oxygen availability to meet the increasing metabolic demands (Jespersen and Ostergaard, 2012). The homogenization of capillary transit times during increases in CBF (reductions in MTT) is seemingly an intrinsic property of passive, compliant microvascular network. In addition, an active mechanism seems to provide additional homogenization and thus more efficient oxygen extraction (Gutierrez-Jimenez et al., 2016). Interestingly, such flow homogenization antedates the increase in flow (Lee et al., 2016), as does the dilation of capillary pericytes compared to upstream arterioles (Hall et al., 2014). The identity of the cells which engage in microvascular flow regulation has been debated, some pointing to pericytes (Hall et al., 2014; Peppiatt et al., 2006; Yemisci et al., 2009), others to terminal smooth muscle cells (Hill et al., 2015), possibly due to differences in terminology (Attwell et al., 2015). At any rate, the capillary pericyte plays a number of crucial roles for the integrity, morphology and barrier function of capillaries and the blood brain barrier (BBB) (Armulik et al., 2005; Armulik et al., 2010).

Our study provides further evidence to support the hypothesis that hypoperfusion and/or hypoxia may be involved in the pathophysiology of AD. It has recently been proposed that hypoperfusion and BBB breakdown may act in concert to accelerate neuronal injury (Zlokovic, 2011). In this study, we did not measure vascular permeability and therefore cannot address whether hypoperfusion and BBB breakdown co-exist in the cortex. However, accumulating evidence show increased vascular permeability in aging and even more so in vascular dementia and AD (Farrall and Wardlaw, 2009). So far, imaging evidence of compromised BBB in AD and MCI has been limited to WMHs (Shindo et al., 2005; Taheri et al., 2011) and hippocampus (Montagne et al., 2015; Wang et al., 2006). Unfortunately, our imaging protocol did not allow us to assess

hippocampal perfusion. In future studies we will address the question of possible co-existence of hypoperfusion, capillary dysfunction and BBB breakdown.

4.5. Limitations

The patients studied here were recruited prospectively from the local memory clinic, diagnosed with possible or probable AD. The diagnosis has not been confirmed by autopsy, nor did we acquire amyloid imaging to assess the presence of A β retention in the brain. The cortical thinning pattern revealed prominent frontal lobe atrophy in the cohort, which may indicate presence of additional dementia related pathologies and not pure AD. Nevertheless, we were able to detect signs of capillary disturbances in this cohort compared to healthy controls as well as a correlation with cognitive impairment. Even though the cohort may represent a mix of dementias, the results emphasize the involvement of changes to the microcirculation.

Our estimates of capillary flow are derived from macroscopic measurements of the passage of an intravascular bolus. It is well-known that the signal changes detected during the intravascular passage of paramagnetic contrast agent cannot be attributed to the micro-vasculature alone. Accordingly, bolus-based transit time estimates represent contributions from all vessels within a particular image voxel. However, for spin-echo sequences, the proportion of contrast contributed by capillaries is significantly larger than the signal originating from arteries and arterioles (Boxerman et al., 1995; Simonsen et al., 2000). Indeed, our SE based measurements of flow disturbances correlated strongly with WMHL, which is ascribed to microvascular, and particularly capillary, pathology (Ostergaard et al., 2016), and SE-based RTH was the only perfusion parameter which correlated widely with symptom severity.

Another technical limitation imposed on the study is the trade-off between whole brain coverage and spatial and temporal resolution. We aimed at acquiring pixel sizes at a scale less than typical cortical thickness (<2mm) and at a temporal resolution to capture the bolus passage with high precision. These requirements come at a cost of limited coverage, which is why the most inferior parts of the temporal lobes and cerebellum are missing in both GRE and SE images and additionally the most superior part of the frontal lobes in SE images. The statistical maps of perfusion changes are further limited as only regions with data from all study participants can be included. Areas outside this consensus region are marked dark grey in figures 3-6 and in Supplementary Material. This limitation also prevented analyses of subcortical GM regions.

Direct comparison between GRE and SE sequences should be made with caution, since AIFs are chosen independently for each sequence. Quality of the AIF has major impact on the resulting perfusion parameter estimates. However, similar criteria (spatial location and shape characteristics) were applied in both sequences and the same operator selected the AIF voxels in all subjects and sequences. This ensured consistency and diminished the impact of potential differences in AIF selection.

Finally, most of the statistical maps did not survive family-wise error correction. Conservative thresholds are useful when seeking to confirm results. However, in this explorative study we chose to present the uncorrected maps in order to interpret patterns across parameters. The statistical power is limited by the relatively small sample size and the number of covariates in the regressions.

Strength of the study is that all our perfusion results are corrected for cortical thickness, WMH, age and gender. There was no systematic effect of cortical thickness on our perfusion estimates, which indicate that partial volume effects do not affect our measurements at the central cortical layer.

5. Conclusion

The primary findings in this study are (i) RTH and OEF^{max} is elevated in patients with AD compared to age-matched controls and (ii) RTH correlates with cognitive symptoms as measured by MMSE and SVD load as measured by white matter hyperintensities. These results indicate that capillary flow disturbances play a role in early AD and add to the growing evidence of microvascular involvement in the pathogenesis of the disease.

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Disclosure statement

The method for estimating microvascular transit times is the subjects of US Patent Application 14/008,487. FACE is the subject of European Patent No. EP04011386.2. and US Patent No. 060/570,493. SNIPE is the subject of a US provisional patent application (US 61/535,720 / P1310USPR).

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Table 1. Participants characteristics. MMSE = mini mental state examination, WMH = white matter hyperintensities (milliliter and % of whole brain volume), HC = hippocampus (milliliter), n.s. = not significant. Data are presented as mean $\pm$ SD or number of subjects (%) of the sample. Ranges are provided in hard brackets. P values are derived from Student *t* tests for continuous variables or Pearson χ^2 tests for categorical variables (p-value estimated by Monte Carlo simulation).

		Patients (n=18)	Controls (n=19)	p Value
Age, y		71.4 $\pm$ 6.7 [54 - 84]	67.5 $\pm$ 7.2 [54 - 79]	n.s.
Gender, M		5 (28%)	10 (53%)	n.s.
Hypertension ($\leq$2 years)		1 (6%)	2 (11%)	n.s.
Smoker		4 (22%)	6 (32%)	<0.01
	- Past	2 (11%)	5 (26%)	
	- Current	2 (11%)	1 (5%)	
Hypercholesterolemia		2	0	<0.01
First degree relative with dementia		9 (50%)	6 (32%)	0.01
MMSE score		24.4 $\pm$ 2.7 [20 - 28]	29.4 $\pm$ 0.7 [28 - 30]	<0.01
Whole brain volume, mL		1376 $\pm$ 148 [1098 - 1620]	1470 $\pm$ 120 [1298 - 1695]	0.02
WMH	mL	11.7 $\pm$ 9.6 [1.9 - 32.9]	7.4 $\pm$ 6.6 [1.4 - 25.0]	0.05
	Load (%)	0.83 $\pm$ 0.64 [0.13 - 2.22]	0.51 $\pm$ 0.45 [0.10 - 1.67]	0.04
HC volume, mL	Left	2.14 $\pm$ 0.32 [1.51 - 2.75]	2.44 $\pm$ 0.26 [1.86 - 2.92]	<0.01
	Right	2.18 $\pm$ 0.32 [1.53 - 2.78]	2.46 $\pm$ 0.32 [1.98 - 3.09]	0.01

Figures

Figure 1. Mapping perfusion values onto the middle cortical surface. Left: Surfaces which approximate the white matter surface (white), middle cortical surface (blue), and outer cortical surface (gray) are shown as overlays on a T1w image. Right: Perfusion image from the same subject shown with the surfaces as overlays after co-registration to the T1w image.

Figure 2. Statistical map showing significant ($p < 0.05$) thinner cortex in patients compared to controls adjusted for gender and age.

Figure 3. Perfusion differences in patients compared to controls. Statistical maps corrected for cortical thickness, WMHL, gender and age, thresholded at $p < 0.05$. Top row: spin echo based measurements. Bottom row: gradient echo based measurements. Left to right: Significant reductions in CBF, increases in CTH and increases in RTH in patients when compared to controls.

Figure 4. Oxygenation differences in patients compared to controls. Statistical maps corrected for cortical thickness, WMHL, gender and age, thresholded at $p < 0.05$. Top row: spin echo based measurements. Bottom row: gradient echo based measurements. Left: Significant increases in OEFmax in patients. Right: significant reductions in CMRO2max in patients when compared to controls.

Figure 5. White matter hyperintensities load correlated with SE based perfusion parameters in patients. Statistical maps corrected for cortical thickness, gender and age, thresholded at $p < 0.05$. Plots show mean values for clusters surviving family-wise error correction.

Figure 6. Relationship between MMSE and spin echo based RTH. Left: example maps of RTH from two controls and two patients with different MMSE scores. Right: statistical map showing significant ($p < 0.05$) negative correlation between MMSE and RTH. Statistical map corrected for cortical thickness, WMHL, gender and age. Plot shows mean values for highlighted cluster.