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# Forebrain-dominant deficit in cerebrovascular reactivity in Alzheimer's disease

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**Abstract**

Epidemiologic evidence and postmortem studies of cerebral amyloid angiopathy suggest that vascular dysfunction may play an important role in the pathogenesis of Alzheimer's disease (AD). However, alterations in vascular function under *in vivo* conditions are poorly understood. In this study, we assessed cerebrovascular-reactivity (CVR) in AD patients and age-matched controls using $CO_2$-inhalation while simultaneously acquiring Blood-Oxygenation-Level-Dependent (BOLD) MR images. Compared with controls, AD patients had widespread reduction in CVR in the rostral brain including prefrontal, anterior cingulate, and insular cortex ($p < 0.01$). The deficits could not be explained by cardiovascular risk factors. The spatial distribution of the CVR deficits differed drastically from the regions of cerebral blood flow (CBF) deficits, which were found in temporal and parietal cortices. Individuals with greater CVR deficit tended to have a greater volume of leukoaraiosis as seen on FLAIR MRI ($p = 0.004$). Our data suggest that early AD subjects have evidence of significant forebrain vascular contractility deficits. The localization, while differing from CBF findings, appears to be spatially similar to PIB amyloid imaging findings.


*Keywords:* Alzheimer's disease; Cerebral blood flow; Cerebrovascular reactivity; Magnetic resonance imaging; Vascular function

Alzheimer's disease (AD) is a neurodegenerative disease that affects more than four million people in the USA alone. Formation and accumulation of extracellular beta-amyloid (A$\beta$) containing plaques and intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau are thought to be the central process in AD (Hardy and Selkoe, 2002; Small, 2005). The possibility of a vascular contribution to AD has also received attention (de La Torre, 2004; Iadecola, 2004; Zlokovic, 2005). There is epidemiological evidence that many risk factors for AD relate to vascular disease (e.g., hypertension, hypercholesterolemia, diabetes) (Breteler, 2000; Meyer et al., 2000). Cerebral amyloid angiopathy is commonly observed in postmortem AD studies (Tian et al., 2004). Several studies have further shown that vascular dysfunction can stimulate A$\beta$ accumulation in the brain. Sun et al. (Sun et al., 2006) and Zhang et al. (Zhang et al., 2007) have independently reported that transient hypoxia can cause an increase in the gene expression of $\beta$-site of amyloid precursor protein cleaving enzyme (BACE1), the main form of $\beta$-secretase, and A$\beta$ production. Therefore, perfusion-induced hypoxia may have a significant impact on the homeostasis of A$\beta$.

While vascular abnormalities in AD have been studied in postmortem brain tissues (Chow et al., 2007) and in animal models (Meyer et al., 2008), *in vivo* investigation of vascular dysfunction in AD patients has not been systematically conducted. Measures of resting cerebral blood flow (CBF) (Yoshikawa et al., 2003) or CBF changes during brain activation (as used in functional MRI) (Xu et al., 2007) are not accurate indicators of vascular function, because these measures are highly sensitive to levels of neural activity and are thus not specific to vessel properties. *In vitro* studies have established that one of the best measures of vessel function is

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its contractility (Chow et al., 2007). Cerebral vessel reactivity (CVR) can be measured *in vivo* in humans by the use of $CO_2$ as a vasodilatory stimulus (Kastrup et al., 2001; Rostrup et al., 2000; Yezhuvath et al., 2009), and is the technique employed in our study of brain vasculature in AD.

In the present study, we hypothesized that AD patients would show diminished CVR compared with that of controls and that the degree of CVR impairment would correlate with the degree of leukoaraiosis, a commonly accepted surrogate for microvascular disease.

## 1. Methods

### 1.1. Participants

Participants were recruited from longitudinal cohorts maintained by the Alzheimer's Disease Center of University of Texas Southwestern Medical Center. The Health Insurance Portability and Accountability Act (HIPAA) compliant protocol was approved by the UT Southwestern Institutional Review Board and written informed consent was obtained from all participants. Inclusion/exclusion criteria for all subjects were a) No contraindication to MRI scanning (pacemaker, implanted metallic objects, renal/liver disease), b) general good health, with no serious or unstable medical conditions, c) able and willing to provide informed consent (caregivers cosigned consents for AD patients), d) age greater than 50, e) no evidence of stroke in clinical MRI, f) Hachinski (Hachinski et al., 1975) score $< 4$. Additional criteria for the control group were normal cognition and Clinical Dementia Rating (CDR) (Morris, 1993) score $= 0$. For AD group, this included a diagnosis of probable AD based on NINCDS/ADRDA criteria (McKhann et al., 1984) with CDR score $= 0.5$ or 1. This is a mild AD group (Mini-Mental State exam (MMSE) $22.8 \pm 4.1$) and they were able to follow instructions for the hypercapnia task. Table 1 lists demographic information for the participants.

To assess the effect of vascular factors, we made a scale by summing cardiovascular risk factors (Breteler, 2000; de La Torre, 2004) of the participants (history of cardiovascular disease, stroke or transient ischemic attack, hypertension, hypercholesterolemia, diabetes mellitus and smoking status): each receiving a value of 1 or 0, with a maximum total score of 6. In addition, medications that may affect the vascular risk factors are also listed in Table 1. These include cholesterol-lowering drugs such as statins and antihypertensive drugs such as $\beta$-blockers, calcium channel blockers, angiotensin inhibitors, angiotensin converting enzyme inhibitors, and diuretics.

Table 1
Characteristics of AD subjects and controls

| Characteristic | AD group | Control group |
|---|---|---|
| Number of subjects | 17 | 17 |
| Mean age ± SD | 70.5 ± 8.3 | 68.7 ± 8.4 |
| Gender | | |
| Male | 13 | 7 |
| Female | 4 | 10 |
| Education (years) ± SD | 15.9 ± 2.5 | 16.5 ± 2.5 |
| MMSE[a] score ± SD | 22.8 ± 4.1 | 29.6 ± 0.7 |
| ApoE4 carrier | 10/17 | 6/17 |
| Cardiovascular risk factors[b] ± SD | 2.2 ± 0.9 | 1.6 ± 1.3 |
| Cholesterol lowering medications | 14/17 | 8/17 |
| Antihypertensive medications[c] | 14/17 | 7/17 |

[a] Mini-mental State examination.
[b] See text for definition of the risk factor score.
[c] Includes $\beta$-blocker, calcium channel blocker, angiotensin inhibitor, angiotensin converting enzyme inhibitor, and diuretics.

A total of 17 AD patients and 17 elderly controls were recruited. Some participants had incomplete data because they declined the $CO_2$ breathing task or were unable to stay still during the MRI. As a result, the actual sample sizes for CVR, CBF and FLAIR data were 12/13 (AD/control), 15/14 and 15/15, respectively. While this caused the reported vascular parameters to be based on different samples, no differences were seen in the severity across the subgroups as measured by MMSE (Folstein et al., 1975), CDR and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Morris et al., 1989) battery tests.

### 1.2. Experiments

MRI investigations were performed on a 3 Tesla MR system (Philips Medical System, Best, The Netherlands). A body coil was used for radiofrequency (RF) transmission and an 8-channel head coil with parallel imaging capability was used for signal reception. CVR measurement followed protocols established in our previous studies (Yezhuvath et al., 2009). Region-specific CVR was assessed using hypercapnia induced by 5% $CO_2$-breathing (mixed with 21% $O_2$ and 74% $N_2$). Hypercapnia was administered via a Douglas bag with a valve to switch between room air and $CO_2$ air. A mouth piece and nose clip was used to achieve mouth-only breathing. A research assistant was inside the magnet room throughout the experiment to switch the valve and monitor the subject. Physiologic parameters, including end-tidal (Et) $CO_2$, breathing rate, heart rate and arterial oxygenation ($SO_2$), were recorded during experiments (MEDRAD, Pittsburgh, PA; Novametrix Medical Systems, Wallingford, CT). The type of air breathed in was switched every minute similar to a block design fMRI experiment, while Blood-Oxygenation-Level-Dependent (BOLD) MR images were acquired for 7 min. Other imaging parameters were FOV $=$ $220 \times 220$ mm$^2$, matrix size $= 128 \times 128$, 25 axial slices, thickness $= 6$ mm, TR/TE/flip angle $= 3{,}000$ ms/30 ms/90°, single-shot echo-planar-imaging (EPI).

For comparison with CVR, resting CBF was measured using Arterial-Spin-Labeling (ASL) MRI (Garcia and Alsop, 2005; Wong, 2007; Wu et al., 2007). Scan parameters were FOV $= 240 \times 240$ mm$^2$, matrix $= 80 \times 80$, 17 axial slices, thickness $= 7$ mm, TR/TE $= 4{,}000$ ms/14 ms, pseudocontinuous labeling with a duration of 1.6 sec, delay $=$ 1.5 sec, single-shot EPI, 30 pairs of label and control images, duration $= 240$ sec. For assessment of brain volume,