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Preserved acetazolamide reactivity in lacunar patients with severe white-matter lesions: ^{15}O-labeled gas and H_2O positron emission tomography studies

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Limited evidence exists on the relationships between severity of white-matter lesions (WMLs) and cerebral hemodynamics in patients without major cerebral artery disease. To examine changes of cerebral blood flow (CBF), oxygen metabolism, and vascular reserve capacity associated with severity of WML in patients with lacunar stroke, we used a positron emission tomography (PET). Eighteen lacunar patients were divided into two groups according to the severity of WMLs, assessed by Fazekas classification; grades 0 to 1 as mild WML group and grades 2 to 3 as severe WML group. Rapid dual autoradiography was performed with ^{15}O-labeled gas-PET followed by ^{15}O-labeled water-PET with acetazolamide (ACZ) challenge. Compared with the mild WML group, the severe WML group showed lower CBF (20.6 ± 4.4 versus 29.9 ± 8.2 mL/100 g per minute, $P = 0.008$), higher oxygen extraction fraction (OEF) (55.2 ± 7.4 versus $46.7 \pm 5.3\%$, $P = 0.013$), and lower cerebral metabolic rate of oxygen ($CMRO_2$) (1.95 ± 0.41 versus 2.44 ± 0.42 mL/100 g per minute, $P = 0.025$) in the centrum semiovale. There were no significant differences in the ACZ reactivity between the two groups ($48.6 \pm 22.6\%$ versus $42.5 \pm 17.2\%$, $P = 0.524$). Lacunar patients with severe WMLs exhibited reduced CBF and $CMRO_2$, and increased OEF in the centrum semiovale. The ACZ reactivity was preserved in both patients with severe and mild WMLs in each site of the brain.
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Introduction

White-matter lesions (WMLs), observed as white-mater hyperintensity in T2-weighted magnetic resonance imaging or fluid-attenuated inversion recovery (FLAIR) image, are commonly observed among elderly people (Hachinski *et al*, 1987). However, they are also associated with hypertension, diabetes, and other vascular risk factors (Murray *et al*, 2005; Pantoni and Garcia, 1997). Development of WMLs is known to be a cause of cognitive impairment, dementia, and disability (Prins *et al*, 2005). Recent studies showed that WMLs are not only a stroke risk factor (Streifler *et al*, 2002) but also a predictor of unfavorable stroke outcome (Koton *et al*, 2009). Despite accumulating evidence of the clinical significance of WMLs, the pathogenesis of WMLs has not been fully clarified.

Healthy elderly subjects with severe WMLs were reported to have reduced cerebral blood flow (CBF) and preservation of oxygen metabolism

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(Meguro *et al*, 1990). Patients with dementia of the Binswanger type have marked decrease of both CBF and oxygen metabolism in the white matter; however, patients without dementia have a lesser decrease in CBF with preservation of almost-normal oxygen metabolism (Yao *et al*, 1992). These findings indicated that chronic hypoperfusion due to the progression of small artery disease is associated with the development of WMLs. In addition, hemodynamic disturbance induced by internal carotid artery occlusive disease was suggested to contribute to the development of extensive WMLs (Yamauchi *et al*, 1999).

Limited evidence exists on the relationships between severity of WMLs and hemodynamic disturbance in patients without major cerebral artery occlusive disease. Some studies showed that vascular reactivity was not related to severity of WMLs (Birns *et al*, 2009; Turc *et al*, 1994). Other studies reported that vascular reactivity in patients with severe WMLs is impaired (Bakker *et al*, 1999; Chabriat *et al*, 2000; Fu *et al*, 2006; Isaka *et al*, 1994; Kozera *et al*, 2010; Mochizuki *et al*, 1997). These inconsistencies may be due to differences in modalities for evaluation of vascular reserve capacity; i.e., transcranial Doppler ultrasound (Bakker *et al*, 1999; Birns *et al*, 2009; Fu *et al*, 2006; Kozera *et al*, 2010), perfusion MRI (Chabriat *et al*, 2000), xenon inhalation computed tomography (Isaka *et al*, 1994; Mochizuki *et al*, 1997), and single photon emission computed tomography (Turc *et al*, 1994). There are also differences in the vasodilatory stimulus used; i.e., CO_2 inhalation (Bakker *et al*, 1999), breath holding, hyperventilation tests (Birns *et al*, 2009; Kozera *et al*, 2010), and acetazolamide (ACZ) challenge test (Chabriat *et al*, 2000; Fu *et al*, 2006; Isaka *et al*, 1994; Mochizuki *et al*, 1997; Turc *et al*, 1994). Although single photon emission computed tomography study with ACZ challenge can detect stage II hemodynamic failure (Powers, 1991) by positron emission tomography (PET) in patients with major cerebral artery occlusive disease (Hirano *et al*, 1994), the relationship between ACZ reactivity and oxygen metabolism in patients with WMLs without major artery disease has not been elucidated. We hypothesized that either impairment of vascular reserve capacity or chronic hypoperfusion in the white matter contributes to the development of WMLs without major artery disease.

The aim of this study was to examine the changes of CBF, oxygen metabolism, and vascular reserve capacity associated with the severity of WMLs in patients with lacunar stroke.

Materials and methods

Patients

This study was a single-center hospital-based prospective study. The study protocol was governed by the guidelines of national government based on the Helsinki Declaration revised in 1983, and it was approved by the Institutional Research and Ethics Committee of our hospital. All patients gave written informed consent to participate in the study. Patients with lacunar stroke, at least 3 weeks after the onset, were enrolled between April 2009 and April 2010. All patients underwent PET studies with ^{15}O-labeled gas ($C^{15}O_2$, $^{15}O_2$, $C^{15}O$) inhalation and ^{15}O-water with ACZ challenge autoradiography as described previously (Kudomi *et al*, 2005, 2007), as well as MRI studies. Lacunar stroke was defined as a typical clinical syndrome associated with a small infarct, <15 mm in diameter on MRI, restricted to the territory of a perforating artery without adjacent major artery occlusive lesions. Patients with stenosis ($>50\%$ in diameter) or occlusion of the internal carotid artery or the trunk of the middle cerebral artery on magnetic resonance angiography or ultrasonography were excluded from the study. The median time interval between the onset of stroke and PET studies was 1,017 days (interquatile range 519 to 1,856).

Baseline clinical characteristics including age, sex, hypertension, diabetes mellitus, dyslipidemia, and current smoking were recorded. Information of risk factors and medical history was collected from a self-reported medical history or inferred from prescribed medication by the primary physicians. Criteria for hypertension, diabetes mellitus, and dyslipidemia were as previously defined (Yokota *et al*, 2009). Cognitive function was evaluated in all patients by the mini-mental state examination (Folstein *et al*, 1975) and clinical dementia rating (Hughes *et al*, 1982). Dementia was defined as clinical dementia rating ≥ 1, and patients with dementia met the criteria proposed by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA Alzheimer's Criteria) (Roman *et al*, 1993).

Magnetic Resonance Imaging

Magnetic resonance imaging was performed on a 1.5-T scanner (Magnetom Vision or Magnetom Sonata; Siemens Medical Systems, Erlangen, Germany). The imaging protocol consisted of a T1-weighted spin-echo, a T2-weighted spin-echo, and FLAIR image. Severity of WMLs was assessed using the FLAIR (repetition time 900 ms, echo time 119 ms, field-of-view 230×201 mm^2, matrix 256×210, 4 mm slice thickness, and 2 mm gap between slices).

Two investigators (CY and TN), who were unaware of all clinical data, graded the degree of severity of WMLs by visual inspection using the Fazekas classification of WMLs as follows: none (grade 0), punctate (grade 1), early confluent (grade 2), and confluent lesions (grade 3) (Fazekas *et al*, 1987). The patients with grades 0 to 1 were defined as the mild WMLs group and those with grades 2 to 3 were defined as the severe WMLs group. Additionally, WMLs volume was measured manually based on FLAIR imaging (20 slices) using Dr View/LINUX software (AJS, Ver R2.5, Tokyo, Japan).

Positron Emission Tomography Imaging

We used an ECAT47 PET scanner (Siemens Medical Systems), which provided an intrinsic spatial resolution of 4.5 mm full-width at half-maximum at the center of the field-of-view. Data were acquired in 2D mode, and corrected for scatter compensation. A catheter was placed in the brachial artery for continuous monitoring of the arterial blood radioactivity concentration and arterial input function using a scintillator block detector system (BeCON; Molecular Imaging Labo, Suita, Japan) (Kudomi *et al*, 2003).

Quantitative images of CBF and oxygen extraction fraction (OEF) were obtained from a series of PET scans with ^{15}O-labeled gas ($C^{15}O_2$, $^{15}O_2$, and $C^{15}O$) inhalation after a rapid dual autoradiography protocol as reported in a series of publications by Kudomi *et al* (2005, 2007). Briefly, after a 10-minute transmission scan for the attenuation correction and an ^{15}O-labeled carbon monoxide ($C^{15}O$) scan for the blood volume assessment, a single dynamic scan was performed for 8 minutes, during which 4,000 MBq of oxygen ($^{15}O_2$) and 5,000 MBq of ^{15}O-labeled carbon dioxide ($C^{15}O_2$) gases were inhaled each >1 minute, sequentially at an interval of 5 minutes. Time to complete the whole dual autoradiography protocol was ~40 minutes. Cerebral metabolic rate of oxygen ($CMRO_2$) was calculated by multiplying the arterial oxygen content to the product images of OEF times CBF.

Additionally, two sets of PET scans were performed, each followed with ^{15}O-labeled water injection to assess regional CBF images using ^{15}O-water autoradiography (Kanno *et al*, 1987). The first scan was initiated without any pharmacological or physiological stress (at rest) and the second scan was performed at 10 minutes after an intravenous injection of ACZ titrated to 17 mg/kg. Physiological and laboratory data such as blood pressure, heart rate, and blood gas analysis (Siemens RAPIDLab 1265; Siemens Medical Systems) were obtained during the PET study.

Data Analysis

The small circular regions of interest (ROIs) (10 mm in diameter) were placed in the frontal cortex, parietal cortex, occipital cortex, basal ganglia, and centrum semiovale based on automatic registration of MRI to PET by using PVElab (the PVEOut Consortium) (Quarantelli *et al*, 2004; Svarer *et al*, 2005). The program is followed by automatic segmentation (running with Statistical Parametric Mapping 5 (SPM5) Software (Institute of Neurology, University College of London, London, UK) and correction of PET counts for fractional volume as determined from the segmentation. The ROIs were manually placed on the FLAIR images and transferred to the CBF images for analysis (Figure 1). We defined the ACZ reactivity as the percentage increase in CBF after ACZ administration relative to baseline CBF. In each subject, the mean measures were obtained by averaging the values for both hemispheres.

Statistical Analysis

Statistical analysis was performed using JMP 7.0 software (SAS Institute, Cary, NC, USA). The statistical significance of intergroup differences was assessed by χ^2 tests, unpaired *t*-tests, and the Mann–Whitney *U*-test, as appropriate. Logarithmic transformation was performed on WMLs volumes, which was a skewed variable. The relationship between each parameter of PET and log-WML was examined by Pearson's correlation. A value of $P<0.05$ was considered statistically significant.

Results

Patients were divided into two groups of severe WMLs ($n=9$) and mild WMLs ($n=9$) on the basis of MRI findings. There were no significant differences in age, sex, and vascular risk factors between the two groups (Table 1). Three patients with dementia defined as clinical dementia rating ≥1 were enrolled in the severe WMLs group; however, the rating of mini-mental state examination was not significantly different between the two groups. There were no significant differences in baseline CBF values between the gas-PET and H_2O-PET results. Compared with patients in the mild WMLs group, the patients

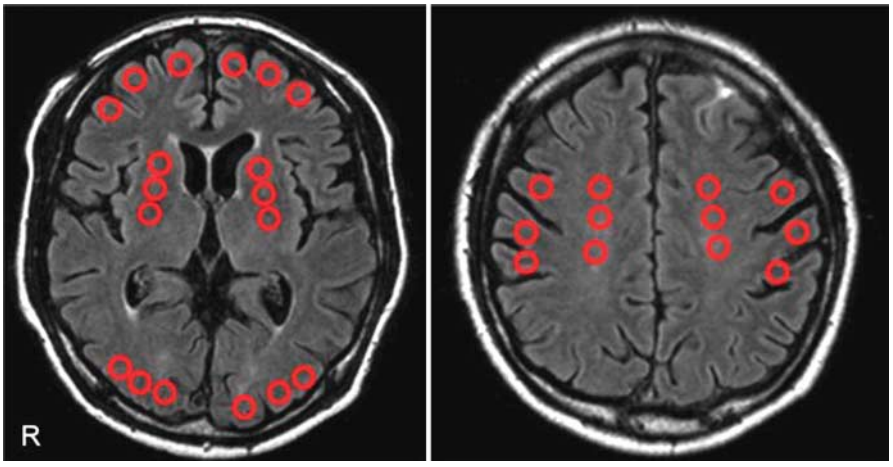

Figure 1 Regions of interest (ROIs) on fluid-attenuated inversion recovery (FLAIR). The small circular ROIs (10 mm in diameter) were placed on the frontal cortex, parietal cortex, occipital cortex, basal ganglia, and the centrum semiovale based on FLAIR image.

Table 1 Baseline characteristics

	Severe WMLs group (n = *9*)	*Mild WMLs group* (n = *9*)	P
Age (years)	76 (73–78)	74 (70–77)	0.329
Male	6 (67)	8 (89)	0.577
Current smoker	7 (78)	7 (78)	0.999
Hypertension	9 (100)	8 (88)	0.999
Diabetes mellitus	3 (33)	3 (33)	0.999
Dyslipidemia	6 (67)	6 (67)	0.999
WMLs (cm^3)	33.3 (21.5–90.9)	3.1 (1.3–4.4)	0.003
History of stroke	3 (33)	2 (22)	0.999
Time interval between stroke onset and PET study (days)	953 (445–1,958)	1,017 (519–1,623)	0.847
MMSE	24.0 (20.5–28.5)	28.0 (24.5–29.5)	0.140
CDR	0.5 (0–1)	0 (0–0.5)	0.185
Dementia	3 (33)	0 (0)	0.206

WMLs, white-matter lesions; PET, positron emission tomography; MMSE, mini-mental state examination; CDR, clinical dementia rating.
Data are number of patients (%), median (interquartile range) for discontinuous variables.

in the severe WMLs group had lower CBF (20.6 ± 4.4 versus 29.9 ± 8.2 mL/100 g per minute, $P = 0.008$), higher OEF (55.2 ± 7.4 versus 46.7 ± 5.3%, $P = 0.013$), and lower $CMRO_2$ (1.95 ± 0.41 versus 2.44 ± 0.42 mL/100 g per minute, $P = 0.025$) in the centrum semiovale, by gas-PET study (Table 2). There were no significant differences in any other parameters of the gas-PET in other ROIs between the two groups. Cerebral blood flow and $CMRO_2$ had a negative correlation with the severity of WMLs, and OEF had a positive correlation with the severity of WMLs (Figure 2). There were no significant differences in ACZ reactivity between the severe and mild WMLs groups in each site of the brain by H_2O-PET examination (Table 3). The results of physiological data and blood gas analysis during ACZ challenge were comparable between the two groups (data not shown). The ACZ reactivity was not correlated with the OEF or with the severity of WMLs ($P = 0.422$ and $P = 0.316$, respectively) (Figure 3).

Table 2 Comparison of each parameter of the gas-PET study between patients with severe or mild WMLs in the brain

	Severe WMLs group (n = *9*)	*Mild WMLs group* (n = *9*)	P
Frontal cortex			
CBF (mL/100 g per minute)	35.7 ± 9.0	37.8 ± 8.5	0.630
CBV (mL/100 g)	3.0 ± 0.9	3.0 ± 0.6	0.969
OEF (%)	54.1 ± 14.7	48.3 ± 5.2	0.275
$CMRO_2$ (mL/100 g per minute)	3.24 ± 0.49	3.26 ± 0.73	0.946
Parietal cortex			
CBF	40.2 ± 6.9	44.1 ± 11.6	0.403
CBV	2.8 ± 0.7	3.1 ± 0.5	0.284
OEF	50.6 ± 6.9	46.3 ± 4.9	0.146
$CMRO_2$	3.53 ± 0.35	3.62 ± 0.80	0.743
Occipital cortex			
CBF	40.4 ± 8.6	47.4 ± 16.1	0.266
CBV	3.5 ± 0.9	3.7 ± 1.5	0.745
OEF	55.8 ± 8.8	50.4 ± 4.5	0.116
$CMRO_2$	3.88 ± 0.63	4.22 ± 1.16	0.442
Basal ganglia			
CBF	45.1 ± 9.4	49.5 ± 13.1	0.426
CBV	2.3 ± 0.7	2.5 ± 0.5	0.521
OEF	52.8 ± 7.9	50.5 ± 6.3	0.505
$CMRO_2$	4.14 ± 0.66	4.43 ± 0.90	0.441
Centrum semiovale			
CBF	20.6 ± 4.4	29.9 ± 8.2	0.008
CBV	1.2 ± 0.4	1.4 ± 0.3	0.217
OEF	55.2 ± 7.4	46.7 ± 5.3	0.013
$CMRO_2$	1.95 ± 0.41	2.44 ± 0.42	0.025

CBF, cerebral blood flow; CBV, cerebral blood volume; $CMRO_2$, cerebral metabolic rate of oxygen; OEF, oxygen extraction fraction; PET, positron emission tomography; WMLs, white-matter lesions.
P value by Mann–Whitney *U*-test.

Discussion

This study showed reduced CBF, reduced $CMRO_2$, and increased OEF in patients with severe WMLs compared with those with mild WMLs in the centrum semiovale. All patients in this study had lacunar stroke without major cerebral artery disease. The study also showed that ACZ reactivity was not impaired in either the cortex or the white matter of the patients of both groups.

Hatazawa *et al* (1997) found asymptomatic WMLs subjects exhibited reduction of CBF in the white matter and basal ganglia without decrease in $CMRO_2$. They also observed an increase in OEF in these areas, suggesting a chronic hypoperfusion in these territories. The present study provided additional information of reduction of both CBF and $CMRO_2$ with an increase in OEF in the WML in the patient groups with severe WMLs. Centrum semiovale is located at the border of an area supplied by deep perforating arteries and the terminal branches of the middle cerebral artery. A decrease in CBF with reduction of $CMRO_2$ in the centrum semiovale in the present study should indicate a consequence of a reduced tissue metabolism in this terminal zone.

In the present study, patients with severe WMLs without major artery disease had increased OEF showed by gas-PET; however, their ACZ reactivity by H_2O-PET was preserved. The vascular reserve capacity evaluated by ACZ reactivity was preserved in both patients with severe and mild WMLs. Reduction of both CBF and $CMRO_2$ in the white matter was previously shown in patients with the Binswanger type dementia (Yao *et al*, 1990), being consistent with our results. Postmortem neuropathologic studies have shown decreased neuronal connectivity in the white matter in progressive subcortical vascular encephalopathy of Binswanger type (Yamanouchi *et al*, 1989, 1990). Functional reduction in cortical neuronal activity due to disruption of connections between the cortex and subcortex, as indicated previously (Pozzilli *et al*, 1987; Sette *et al*, 1989), is likely to be associated with a reduction of $CMRO_2$ in the centrum semiovale

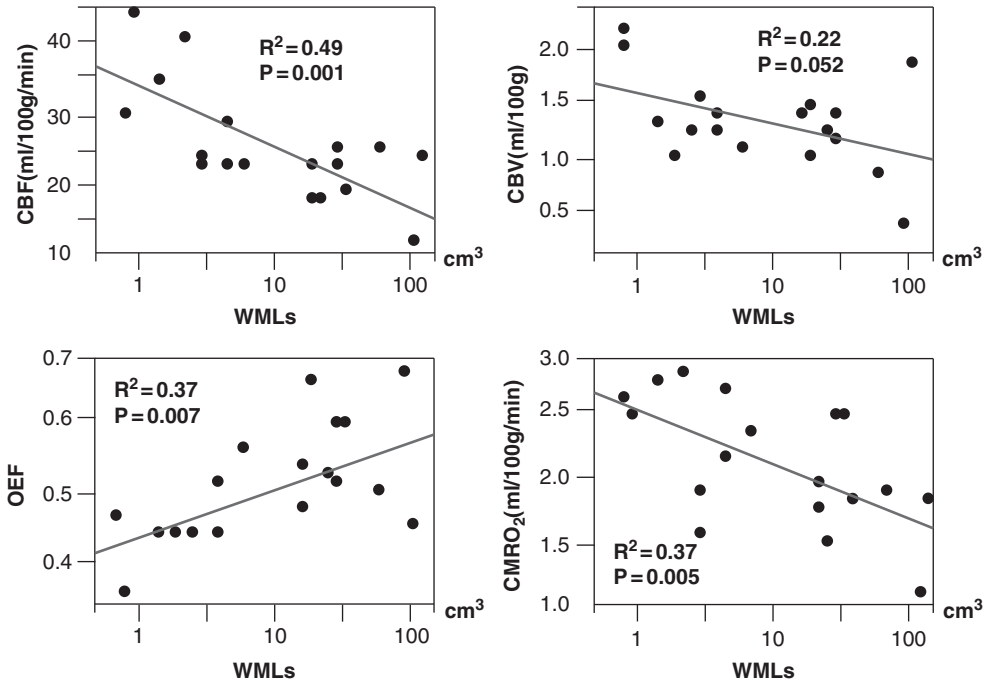

Figure 2 Correlation between WML volume and each gas-PET parameter in the centrum semiovale. CBF and $CMRO_2$ had a negative correlation with the severity of WMLs, while OEF was positively correlated with the severity of WMLs. CBF, cerebral blood flow; CBV, cerebral blood volume; OEF, oxygen extraction fraction; $CMRO_2$, cerebral metabolic rate of oxygen; WMLs, white-matter lesions; PET, positron emission tomography.

Table 3 Comparison of CBF between patients with severe or mild WMLs in the brain by H_2O-PET

	Severe WMLs group (n = 9)	*Mild WMLs group* (n = 9)	P
Frontal cortex			
CBF baseline	36.1 ± 7.2	40.2 ± 7.3	0.244
CBF ACZ	58.5 ± 10.2	59.9 ± 10.3	0.770
ACZ reactivity (%)	64.6 ± 28.5	49.7 ± 14.9	0.183
Parietal cortex			
CBF baseline	39.7 ± 4.8	45.7 ± 10.5	0.136
CBF ACZ	62.0 ± 7.1	66.9 ± 14.6	0.387
ACZ reactivity (%)	57.2 ± 17.1	47.1 ± 13.5	0.181
Occipital cortex			
CBF baseline	38.1 ± 7.1	45.7 ± 11.5	0.109
CBF ACZ	61.7 ± 13.3	70.1 ± 17.0	0.259
ACZ reactivity (%)	62.2 ± 21.5	54.2 ± 16.6	0.392
Basal ganglia			
CBF baseline	47.1 ± 9.8	54.6 ± 11.3	0.148
CBF ACZ	73.7 ± 10.5	85.7 ± 24.6	0.200
ACZ reactivity (%)	60.9 ± 31.0	55.7 ± 22.9	0.694
Centrum semiovale			
CBF baseline	19.0 ± 4.1	29.8 ± 9.2	0.005
CBF ACZ	28.5 ± 5.9	41.8 ± 10.9	0.005
ACZ reactivity (%)	48.6 ± 22.6	42.5 ± 17.2	0.524

ACZ, acetazolamide; CBF, cerebral blood flow; PET, positron emission tomography; WMLs, white-matter lesions.
P value by Mann–Whitney *U*-test.

in the patients with severe WMLs. Furthermore, the cerebral vessels would not dilate during fluctuations in systemic arterial pressure in daily life in these conditions of disruption of connections. Chronic hypoperfusion with a reduction of $CMRO_2$ in accordance with a disconnection between the cortex and subcortex may be the cause of development of WMLs without major artery disease.

To our knowledge, this is the first report of alterations in CBF, $CMRO_2$, and OEF, with preservation of ACZ reactivity in patients with mild or severe WMLs, with careful consideration of possible methodological errors. Indeed, quantitation of physiological parameters using PET is still a challenging issue, particularly in the white-matter area. As shown in earlier studies (Herscovitch and Raichle, 1983; Huang *et al*, 1987), the absolute values of both CBF and $CMRO_2$ could be biased because the spatial resolution of PET devices is limited compared with the physical size of the brain tissue component, or the partial volume effects. Oxygen extraction fraction is relatively stable and is less affected by partial volume effects. Our observation of increased OEF could not be explained by partial volume effects alone. Scatter is smaller in 2D mode in PET as compared with 3D acquisition. In this study, scatter correction was applied to minimize the contribution of radioactivity from the surrounding tissue components due to scatter. The ROIs were placed carefully with a guide of anatomical MRI to

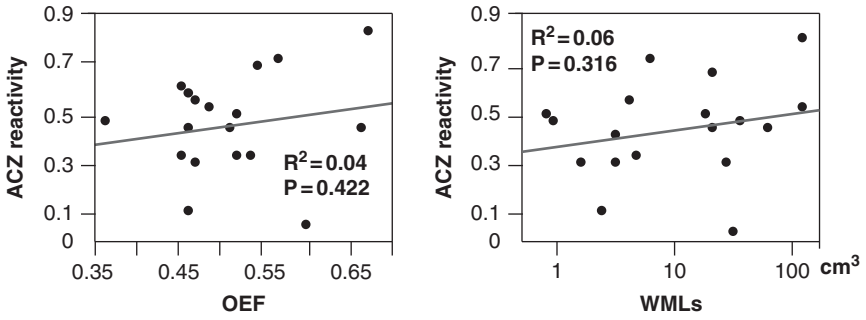

Figure 3 The correlation between ACZ reactivity and OEF or WML volume in the centrum semiovale. Neither OEF nor WML volume was correlated with ACZ reactivity. ACZ, acetazolamide; OEF, oxygen extraction fraction; WMLs, white-matter lesions.

minimize the errors arising from radioactivity counts of surrounding tissues. These factors remain concerns to be dealt with in future investigations.

There are several issues that need to be addressed, as follows. First, we intended to avoid possible bias in the patient selection, but a relatively small number of subjects could cause selection bias despite our efforts. Second, three patients with dementia were enrolled in the severe WMLs group. Because oxygen metabolism in demented patients was reported to be different from that in non-demented patients (Yao *et al*, 1992), a reduced $CMRO_2$ with reduced CBF in the severe WMLs group could be attributed to secondary effects arising from decreased cognitive function. Third, we examined the vascular reserve capacity by ACZ challenge. Recently, ACZ-induced vasodilation was reported not to inhibit the visually evoked flow response (Yonai *et al*, 2010), which indicates that the vasodilatory mechanism during neurovascular coupling may be different from the mechanism of ACZ-induced vasodilation. Acetazolamide at a dose of 17 mg/kg would not cause maximal cerebral vasodilatation. However, there were no significant differences in ACZ reactivity between the two groups, and ACZ reactivity was preserved in all patients in the present study. Fourth, PET imaging in the present study was a single scatter subtraction technique based on the Klein–Nishina formulation which was implemented in the reconstruction software (Watson, 2000). This technique was shown to provide reasonable accuracy in several phantom experiments. It should also be noted that the data were acquired in 2D mode, which has much smaller amount of scatter as compared with recently available 3D mode. Further, the filtered-back projection technique was applied for the image reconstruction. In this procedure, the scatter contribution is likely reduced in the reconstructed images. However, limited spatial resolution of PET devices is a significant source of errors that causes possible contamination of radioactivity counts of cortical grey matter tissue. Exact magnitude of errors in the calculated parameters in the WML cannot be well defined. In addition, PET scanning in the present study has not been applied to age-matched normal subjects. Further systematic study is needed.

In conclusion, we showed that there is reduced CBF and $CMRO_2$, and increased OEF in the centrum semiovale of patients with severe WMLs compared with patients with mild WMLs. The ACZ reactivity was preserved in both patients with severe and mild WMLs. Further studies will be needed to clarify the pathogenesis of WMLs.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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