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Low carotid endothelial shear stress associated with cerebral small vessel disease in an older population: A subgroup analysis of a population-based prospective cohort study

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HIGHLIGHTS

- Low carotid wall shear stress was associated with increased risks of the progression of cerebral small vessel disease.
- Endothelial dysfunction induced by low wall shear stress might play a crucial role in this association.
- This study was a long-term and population-based prospective cohort study.

ARTICLE INFO

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Cerebral small vessel disease
Hemodynamic
Shear stress
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ABSTRACT

Background and aims: The association between carotid wall shear stress (WSS) and cerebral small vessel disease has yet to be fully elucidated. The major purpose of this study was to investigate this association in older subjects.

Methods: Common carotid artery WSS, endothelial function, white matter hyperintensities (WMH), lacunes, and microbleeds were assessed in 1396 older adults. Participants were followed-up for an average of 69.7 months.

Results: Mean (M) and peak (P) WSS and changes in endothelial function were independently associated with changes in WMH volume and fraction, lacune counts, and microbleed counts (all $p < 0.05$). The risks of new-incident Fazekas scale ≥ 2 [hazard ratio (HR) with 95% confidence interval (CI): 2.141 (1.469–3.119), $p = 0.005$ and 1.731 (1.197–2.505), $p = 0.004$, respectively], lacunes [HR (95% CI): 2.034 (1.369–3.022), $p < 0.001$ and 1.693 (1.151–2.490), $p = 0.003$, respectively], and microbleeds [HR (95% CI): 2.311 (1.509–3.541), $p < 0.001$ and 2.208 (1.299–3.751), $p < 0.001$, respectively] were significantly higher in the lowest quartile group than in the higher quartile group, as classified by either MWSS or PWSS, after adjustment for confounders.

Conclusions: Low carotid WSS is an independent risk factor for the progression of cerebral small vessel disease in older adults.

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1. Introduction

Cerebral small vessel disease is a common and prevalent vascular disease of the brain caused by white matter hyperintensities (WMH), lacunes, and microbleeds [1,2]. It contributes to more than 15% of all strokes, 30% of the future risk for stroke, and 45% of dementia cases in older populations. Cerebral small vessel disease, therefore, imposes high personal and societal healthcare burdens [1,2].

The etiology of cerebral small vessel disease is ischemia, affecting the perforating arterioles, capillaries, and venules [1,2]. Studies have demonstrated that vascular endothelial dysfunction plays a crucial role in the pathological process of cerebral small vessel disease [1,2]. Endothelial dysfunction leads to cerebral vessel wall stiffness, lumen narrowing, and a loss of hemodynamic autoregulation [1,2].

Wall shear stress (WSS) is one of the most important hemodynamic forces regulating vascular endothelial function [3]. WSS is the frictional force exerted by the movement of blood on the endothelial surface of the inner vessel wall, which modulates endothelial cell activity via regulating the release of vasoactive molecules such as nitric oxide (NO), endothelin (ET)-1, soluble intercellular cell adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1 [4]. Low or oscillatory WSS can lead to endothelial dysfunction and atherogenesis [5–7].

The common carotid artery supplies a precisely regulated volume and velocity of blood flow [8]. The common carotid artery is commonly thought of as an “observation window” into the systemic hemodynamic condition. Evidence has shown that the common carotid WSS is a measure that reflects the overall hemodynamic condition of the cerebral vessels, as it is strongly associated with ischemic and hemorrhagic strokes [2,8,9]. Previous studies have demonstrated that the common carotid WSS is inversely and independently related to WMH burden and cerebral infarcts in older adults [2,9,10]. Mutsaerts et al. reported that the diastolic common carotid WSS is correlated with both cerebral infarcts and periventricular WMH [9]. Similarly, Okada et al. found that the peak systolic and end diastolic common carotid WSS correlated with subcortical WMH [10]. However, the results of these aforementioned studies do not clearly elucidate the association between the common carotid WSS – and related endothelial function – and cerebral small vessel disease, mainly due to their cross-sectional study design. In addition, there are few studies investigating the association between the common carotid WSS and cerebral lacunes and microbleeds.

In this study, we followed up 1396 older adults aged ≥60 years from a community-based population for an average of 69.7 months to shed light on the association between the common carotid WSS – and related endothelial function – and the progression of cerebral small vessel disease.

2. Patients and methods

2.1. Subjects

This study used brain magnetic resonance imaging (MRI) to evaluate a subset of subjects from a population-based prospective cohort study (Registration number: ChiCTR-EOC-17013598) [11]. In the original cohort, we recruited 21,000 subjects aged 15 years and older, between April 2007 and November 2009, in the area of Shandong, China. The main goals were to investigate the determinants of chronic disease in the general population. In this sub-study, 1519 participants aged ≥60 years from the original cohort were deemed eligible for inclusion. The exclusion criteria were as follows: a Mini-Mental State Examination (MMSE) score ≤23 points, Alzheimer's disease, Parkinson's disease, seizures, schizophrenia, claustrophobia, bipolar disorder, stroke or transient ischemic attack, contraindication to MRI, myocardial infarction, decompensated heart failure, secondary hypertension, renal failure and dialysis treatment, drug and alcohol abuse, prior inclusion in other studies, and unwillingness to provide informed consent.

The study protocol was approved by the Research Ethics Committee of the Institute of Basic Medicine, Shandong Academy of Medical Sciences, and was in compliance with the Declaration of Helsinki. Written informed consent was obtained from each participant. The corresponding author has access to and responsibility for all the data supporting the findings of this study.

2.2. Brain magnetic resonance imaging assessment

Brain MRI was performed on a 3.0-T GE Signa Horizon scanner (Signa Horizon LX, General Electric Medical Systems, USA) and on a 3.0-T Siemens Allegra scanner (Siemens Medical, Erlangen, Germany) by neuroradiologists blinded to the clinical data of participants. Scans were performed at baseline, at the first MRI follow-up (between April 2010 and November 2012), and at the second MRI follow-up (between April 2013 and November 2015). A uniform MRI protocol was used and consisted of T1-weighted 3D magnetization-prepared rapid gradient echo, T2-weighted 3D fast spin-echo, T2*-weighted gradient-echo type echoplanar, and fluid-attenuated inversion recovery (FLAIR) sequences. The WMH volume was computed on FLAIR axial images and assessed using the Fazekas scale (a score of 0–3 given for none, punctuate, early confluent, or confluent lesions, respectively) [12]. Lacunes, defined as cavities with a diameter of 3–15 mm with cerebrospinal-fluid-like signal, were assessed on T1-weighted, T2-weighted, and FLAIR images. Microbleeds, defined as smaller than 10 mm oval or round, hypointense, and homogeneous foci in the brain parenchyma, were assessed on T2*-weighted images. Mimics of microbleeds, such as calcifications and sulcal vessels, as well as signals averaging from bone, were systematically excluded [13]. Fazekas scale lesion assignments were dichotomized as 0 or 1 *vs.* 2 or 3, lacunes as 0 *vs.* 1 or more, and microbleeds as 0 *vs.* 1 or more [1]. After being tested in random samples of 140 patients, the weighted Cohen's kappa of inter-observer variability was 0.85 for the Fazekas scale lesions, 0.81 for lacunes, and 0.81 for microbleeds.

2.3. Common carotid artery ultrasonography and wall shear stress calculations

The common carotid artery ultrasound examinations were performed by experienced ultrasonographers who were blinded to the clinical details of participants. Measurement was performed using a high-resolution ultrasound with a 7.5-MHz linear array transducer (GE Medical Systems Ultrasound Israel Ltd, Tirat Carmel, Israel) and simultaneous electrocardiogram recording. The common carotid intima-media thickness (IMT) was assessed, and a plaque was defined as a localized IMT > 1.5 mm, with protrusion into the lumen or focal structure encroaching into the lumen by ≥ 0.5 mm, or 50% of the surrounding IMT [14]. Internal diameters of the common carotid artery at the R (ID_R) and peak T (ID_T) waves on the electrocardiogram were measured using the B-mode tracings, as previously described [2]. Mean velocity (V_M), end diastolic velocity, and peak systolic velocity (V_{PS}) were detected 1–2 cm below the bifurcation with the sample volume reduced to the smallest possible sample size (1 mm) in the center of the lumen time-averaged mean velocity over one cardiac cycle. The Doppler angle was kept between 44 and 55° and he sample volume box was placed in the mid-lumen parallel to the vessel wall. The mean of V_M and V_{PS} of three cardiac cycles were computed and used for further analysis. The weighted Cohen's kappa of the inter-observer variability was 0.94 for carotid plaques, and the coefficients of variation for blood flow velocity and carotid internal diameter were 0.84 and 0.81, respectively ($p < 0.001$), after being tested in random samples of 140 patients.

The following equations, described elsewhere [2,7,15], were used to calculate the mean (M) and peak (P) WSS: MWSS (Pa) is equal to $8 \times \eta \times V_M/ID_R$; PWSS (Pa) is equal to $8 \times \eta \times V_{PS}/ID_T$, where η is the blood viscosity, regarded as equal to 0.0035 Pa s in the present study [16]. Lower velocity from both sides was used for further analysis.

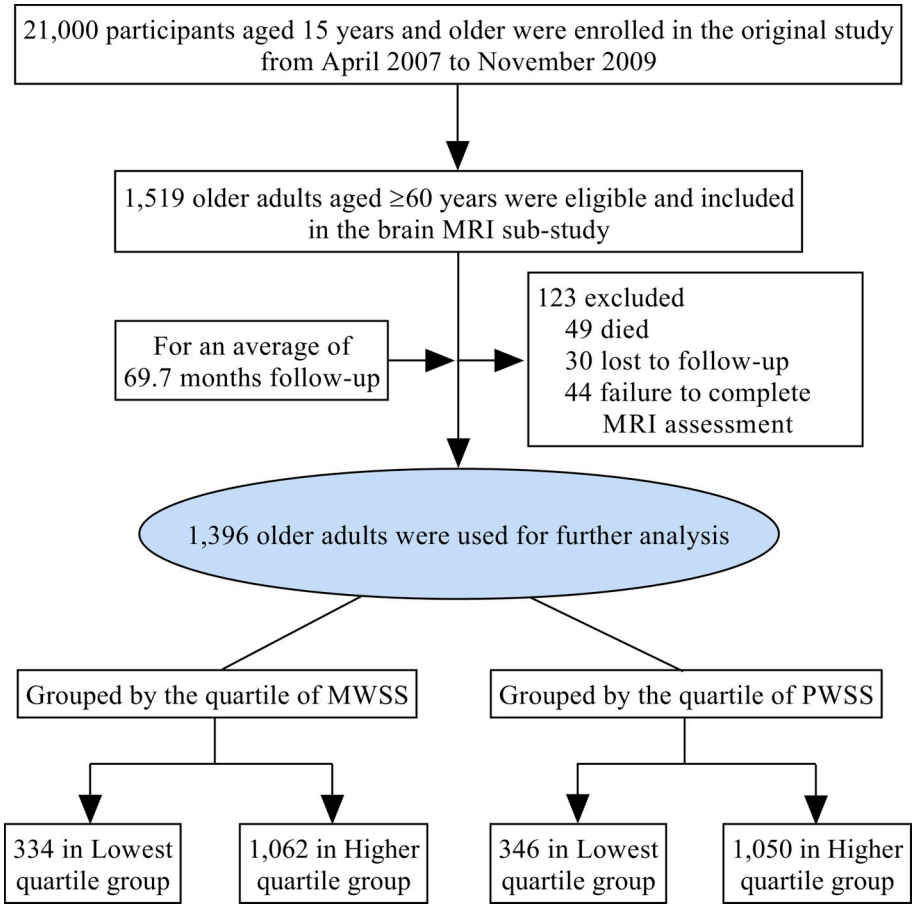

Fig. 1. The protocol flowchart.
MRI

2.4. Measurement of biomarkers of endothelial function

Vascular endothelial function was assessed using the serum levels of NO, ET-1, sICAM-1, and sVCAM-1, at baseline and biennial follow-up. Serum levels of NO were assessed by the quantification of nitrite levels, a stable metabolite of NO, using the Griess assay [17]. Serum levels of ET-1, sICAM-1, and sVCAM-1 were determined by enzyme-linked immunosorbent assay kits, according to the manufacturer's instructions. Reagents used for NO measurement were purchased from Sigma-Aldrich (St. Louis, MO, USA). All ELISA kits were purchased from Bender (Bender MedSystems, Vienna, Austria). Each serum sample was tested in duplicate and the mean was used for further analysis.

2.5. Covariates

The possible covariates included age; sex; smoking; alcohol intake; history of hypertension, diabetes, and dyslipidemia; medication for hypertension, diabetes, and dyslipidemia; body mass index; blood pressure; fasting plasma glucose; and lipids.

2.6. Outcomes

The primary outcomes included the changes in WMH volume and fraction, lacune and microbleed counts, as well as new-incident Fazekas scale ≥2 lesions, lacunes, and microbleeds during the follow-up period. New incidents were defined as ≥1 newly diagnosed Fazekas scale ≥2 lesions, lacunes, or microbleeds during the follow-up period [18].

2.7. Statistical analysis

Statistical analyses were performed using SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). The normality of data was determined using the Kolmogorov-Smirnov test. Continuous data are presented as the mean ± standard deviation (SD), or the median with interquartile range (IQR). Categorical data are presented as numbers with percentages. Participants were categorized into two groups, namely, the lowest WSS quartile (Q_1) group and the higher quartile WSS (Q_{2+3+4}) group, according to the IQR of the mean and peak WSS separately. A Student's *t*-test or a Mann-Whitney *U* test was used to assess the differences in continuous data depending on their normality. A chi-square test was performed to assess differences in categorical data. A Kruskal-Wallis test with a Wilcoxon rank-sum test was performed to compare the differences in NO, ET-1, sICAM-1, sVCAM-1, WMH volume and fraction, and the counts of lacunes and microbleeds between baseline and follow-up assessments. A linear mixed model was performed to compare changes in NO, ET-1, sICAM-1, sVCAM-1, WMH volume and fraction, and counts of lacunes and microbleeds during the follow-up period between groups. Multiple linear backward stepwise regression analyses were used to assess whether any factors were independently associated with the changes in WMH volume and fraction, lacune counts, and microbleed counts. The changes were defined as the difference in value between the baseline and the second MRI follow-up assessment. The changes in NO, ET-1, sICAM-1, and sVCAM-1 levels were also defined as the difference in value between the baseline and the second MRI follow-up assessment, and were used as independent variables in the models. A Kaplan-Meier with log-rank test was performed to compare the differences in the risks over time for new-

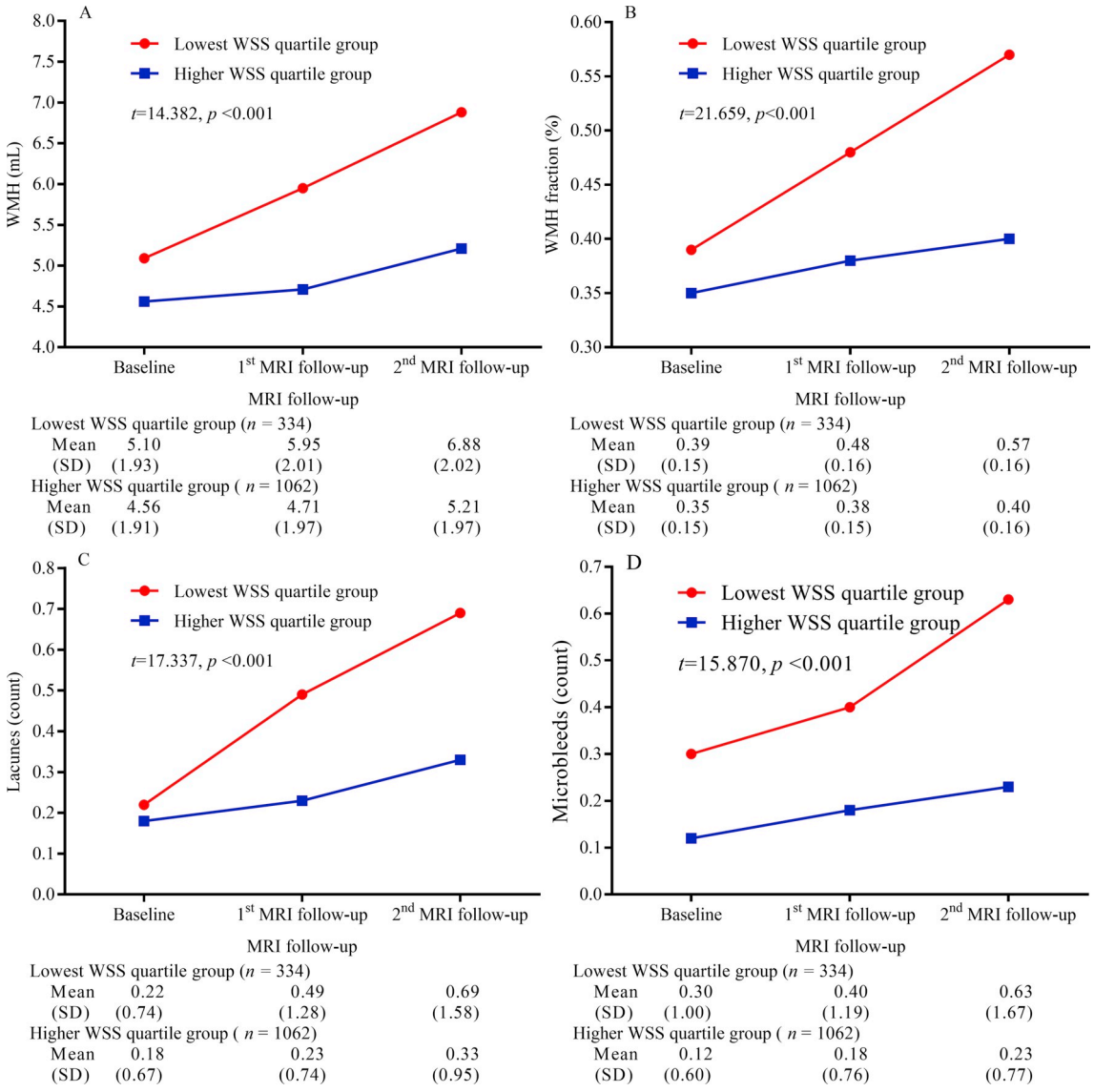

Fig. 2. Differences in the progression of cerebral small vessel disease lowest quartile group and higher quartile groups classified by the quartile of mean wall shear stress.
(A) Changes in WMH volume. (B) Changes in WMH fraction. (C) Changes in lacune counts. (D) Changes in microbleed counts. WSS.

incident Fazekas scale ≥2 lesions, lacunes, and microbleeds between groups. The hazard ratio (HR) with 95% confidence intervals (CIs) was assessed using a Cox proportional hazards model. The contributions of the changes in NO, ET-1, sICAM-1, and sVCAM-1 levels to new-incident Fazekas scale ≥2 lesions, lacunes, and microbleeds were assessed using logistic regression analysis. All models were adjusted for age; sex; current smoking; alcohol consumption; history of hypertension, diabetes, and dyslipidemia; medication for hypertension, blood glucose levels, dyslipidemia, and platelet aggregation; common carotid IMT and plaques; profiles of blood pressure and lipids; and the differences in time between the baseline MRI and the follow-up visit. A two-sided p value less than 0.05 was considered statistically significant.

3. Results

3.1. Baseline demographic and clinical characteristics

The protocol flowchart of the study is summarized in Fig. 1. After an average of 69.7 (range: 66 to 75) months of follow-up, 123 participants were excluded, leaving 1396 participants eligible for analysis. The IQR of the MWSS was < 0.89, 0.89 to 1.03, 1.04 to 1.25, and ≥1.26 Pa. The IQR of the PWSS was < 1.71, 1.71 to 2.03, 2.04 to 2.37, and ≥2.38 Pa. Supplementary Table 1 shows the details of the baseline demographic and clinical characteristics, grouped by mean and peak WSS.

3.2. Changes in white matter hyperintensities volume and fraction, and counts of lacunes and microbleeds

Compared to the baseline, at the first and second follow-up visits, WMH volumes were increased by 0.78 and 1.32 mL and the fraction was increased by 0.06 and 0.11%, lacune counts were increased by 0.24 and 0.53, and microbleed counts were increased by 0.16 and 0.40 (all $p < 0.001$). The increasing tendencies of WMH volume ($p < 0.001$ and < 0.001, respectively) and fraction ($p < 0.001$ and < 0.001, respectively), lacunes ($p < 0.001$ and < 0.013, respectively), and microbleeds ($p < 0.001$ and < 0.001, respectively) were all significantly higher in the lowest quartile group than in the higher quartile group, as classified by the quartile of mean and peak WSS (Fig. 2 and Supplementary Fig. 1), separately. These significant differences remained after adjustment for confounders.

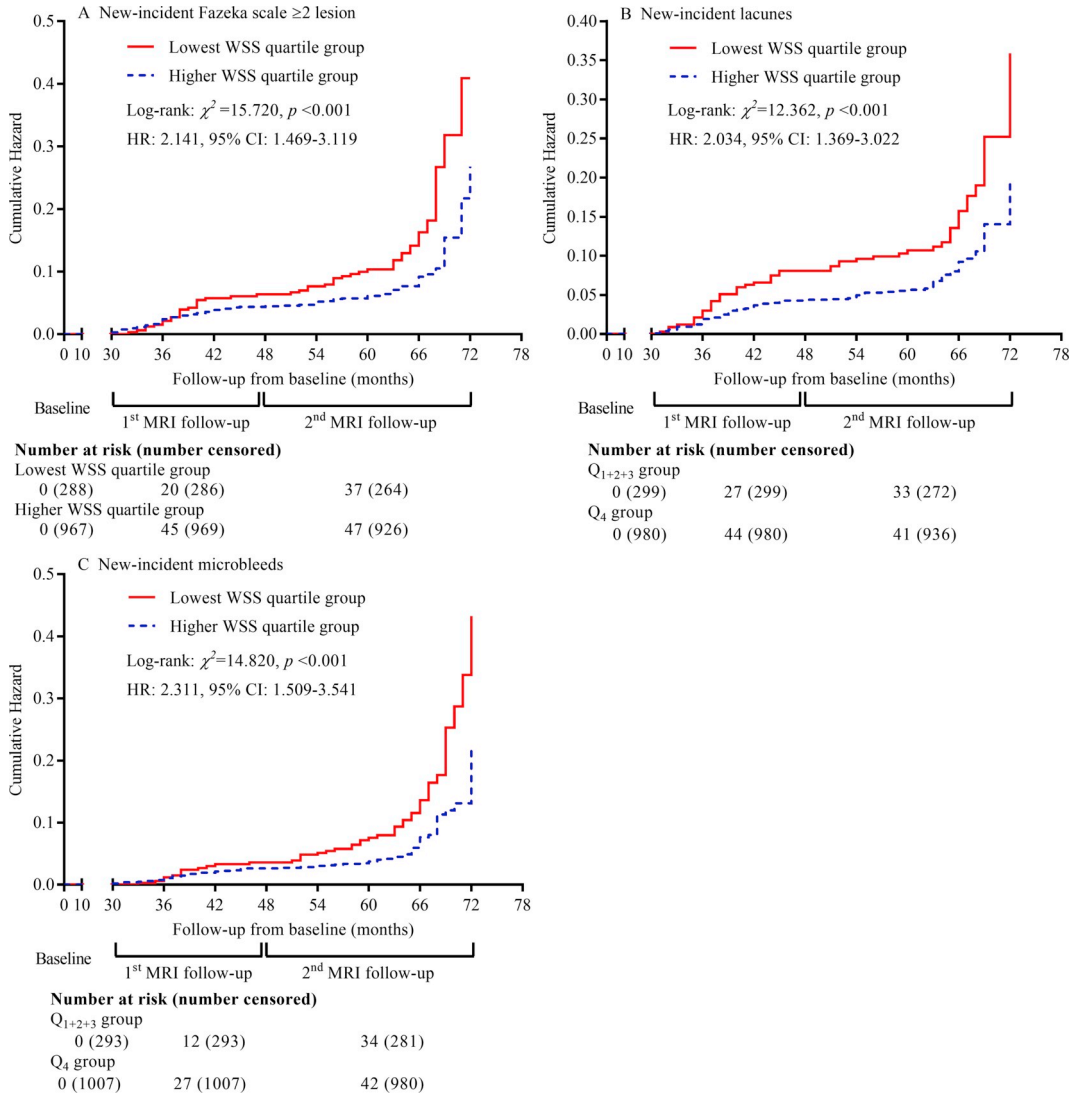

Fig. 3. Difference in the cumulative new-incidents of cerebral small vessel disease between lowest quartile group and higher quartile groups classified by the quartile of mean wall shear stress.
(A) Difference in the cumulative new incidents of Fazekas scale ≥2 lesions. (B) Difference in the cumulative new incidents of lacunes. (C) Difference in the cumulative new incidents of microbleeds. WSS = wall shear stress; MRI = magnetic resonance imaging.

3.3. The contribution of wall shear stress and endothelial function to changes in white matter hyperintensities volume and fraction, and lacune and microbleed counts

A multiple linear regression analysis was conducted to investigate possible factors independently associated with the progression of cerebral small vessel disease (Supplementary Table 2). Changes in WMH volume and fraction, and lacune and microbleed counts between the baseline assessment and the second MRI follow-up assessment were treated as separate dependent variables in their corresponding model. The mean and peak WSS and hanges in NO, ET-1, sICAM-1, and sVCAM-1 levels were separately included as independent variables in every model. The results indicated that mean and peak WSS, and changes in NO levels were significantly and independently associated with changes in WMH volume and fraction, as well as changes in lacune and microbleed counts, after adjustment for covariates (all $p < 0.05$). Changes in ET-1, sICAM-1, and sVCAM-1 levels were also significantly associated with changes in WMH volume and fraction, as well as changes in lacune and microbleed counts. However, the association between changes in ET-1, sICAM-1, and sVCAM-1 levels and change in microbleed counts was no longer observed after adjustment for common carotid IMT and plaques, baseline WMH volume, and the differences in time between the baseline and MRI follow-up visits.

3.4. The contribution of wall shear stress and endothelial function to new-incidents of Fazekas scale ≥2 lesions, lacunes, and microbleeds

Of 1396 participants, 1255 participants had a Fazekas scale scoring of less than two, 1279 participants had no lacunes, and 1300 participants had no microbleeds. Over the follow-up period, 146 participants (2.3% per year) developed Fazekas scale ≥2 lesions, 135 (2.1% per year) participants developed lacunes, and 115 (1.7% per year) participants developed microbleeds among the 1396 participants.

The lowest quartile group had a significantly higher risk for new incidents of Fazekas scale ≥2 lesions, lacunes, and microbleeds, relative to the higher quartile group, after participants were grouped by quartile of MWSS ($p < 0.001$, < 0.001, and < 0.001, respectively, Fig. 3). The cumulative HR with 95% CI was 2.141 (1.469–3.119) for new incidents of Fazekas scale ≥2 lesions, 2.034 (1.369–3.022) for new incidents of lacunes, and 2.311 (1.509–3.541) for new incidents of microbleeds, after adjustment for confounders. Similarly, after participants were grouped by quartile of PWSS, the risks of new incidents of

Table 1
Contribution of the changes in endothelial function to new incidents of cerebral small vessel disease.

	New-incident Fazekas scale ≥2 lesion		New-incident lacunes		New-incident microbleeds	
	Exp (B) (95% CI)	*p* value	Exp (B) (95% CI)	*p* value	Exp (B) (95% CI)	*p* value
Changes in NO (μmol/L)*						
Model 1	0.551 (0.484–0.628)	< 0.001	0.574 (0.498–0.661)	< 0.001	0.598 (0.518–0.691)	< 0.001
Model 2	0.550 (0.483–0.627)	< 0.001	0.575 (0.499–0.662)	< 0.001	0.597 (0.517–0.690)	< 0.001
Model 3	0.546 (0.479–0.622)	< 0.001	0.563 (0.483–0.657)	< 0.001	0.587 (0.504–0.683)	< 0.001
Changes in ET-1 (pg/mL)*						
Model 1	1.786 (1.558–2.046)	< 0.001	1.779 (1.527–2.073)	< 0.001	1.693 (1.449–1.978)	< 0.001
Model 2	1.777 (1.549–2.038)	< 0.001	1.771 (1.521–2.062)	< 0.001	1.689 (1.453–1.985)	< 0.001
Model 3	1.747 (1.516–2.013)	< 0.001	1.771 (1.521–2.062)	< 0.001	1.686 (1.441–1.972)	< 0.001
Changes in sICAM-1 (ng/mL)*						
Model 1	1.052 (1.039–1.065)	< 0.001	1.052 (1.037–1.066)	< 0.001	1.053 (1.038–1.068)	< 0.001
Model 2	1.047 (1.033–1.062)	< 0.001	1.051 (1.036–1.065)	< 0.001	1.052 (1.037–1.067)	< 0.001
Model 3	1.044 (1.028–1.060)	< 0.001	1.045 (1.031–1.059)	< 0.001	1.051 (1.036–1.066)	< 0.001
Changes in sVCAM-1 (ng/mL)*						
Model 1	1.036 (1.027–1.046)	< 0.001	1.032 (1.023–1.041)	< 0.001	1.033 (1.023–1.043)	< 0.001
Model 2	1.033 (1.025–1.041)	< 0.001	1.029 (1.020–1.039)	< 0.001	1.031 (1.022–1.040)	< 0.001
Model 3	1.028 (1.019–1.037)	< 0.001	1.025 (1.017–1.034)	< 0.001	1.030 (1.021–1.039)	< 0.001

*The changes were defined as the differences between the second MRI follow-up assessment and baseline.
NO = nitric oxide; ET-1 = endothelin-1; sICAM-1 = soluble intercellular cell adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1.
In models, the changes in NO, ET-1, ICAM-1, and VCAM-1 were used as continuous data.
Model 1: adjusted for baseline age and sex.
Model 2: model 1 + smoking; alcohol intake; history of hypertension, diabetes, and dyslipidemia; medication for hypertension, blood glucose levels, dyslipidemia, and platelet aggregation; and baseline body mass index, systolic and diastolic blood pressure, fasting blood glucose, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.
Model 3: model 2 + carotid artery intima-media thickness, carotid artery plaques, baseline white matter hyperintensities, and differences in time between the baseline and MRI follow-up visits.

Fazekas scale ≥2 lesions (HR: 1.731, 95% CI: 1.197 to 2.505; $p = 0.004$), lacunes (HR: 1.693, 95% CI: 1.151 to 2.490; $p = 0.003$), and microbleeds (HR: 2.208, 95% CI: 1.299 to 3.751; $p < 0.001$) were higher in the lowest quartile group than in the higher quartile group, after adjustment for covariates (Supplementary Fig. 2).

We assessed the contribution of changes in endothelial function to new incidents of Fazekas scale ≥2 lesions, lacunes, and microbleeds using a logistic regression analysis (Table 1). The results showed that changes in NO levels were negatively associated with an increased risk for new incidents of Fazekas scale ≥2 lesions, lacunes, and microbleeds, whereas changes in ET-1, sICAM-1, and sVCAM-1 levels were positively associated, after adjustment for confounders (all adjusted $p < 0.001$).

3.5. Association between wall shear stress and change in endothelial function

During the follow-up period, NO levels were significantly decreased, whereas ET-1, sICAM-1, and sVCAM-1 levels were increased compared to the baseline assessment ($p < 0.05$). The median changes were as follows: NO, −5.02 (IQR: 6.61 to −3.44) μmol/L; ET-1, 4.07 (IQR: 2.70 to 5.50) pg/mL; sICAM-1, 22.47 (IQR: 10.67 to 37.03) ng/mL; sVCAM-1, 27.44 (IQR: 14.64 to 39.51) ng/mL. The tendencies for NO to decrease and for ET-1, sICAM-1, and sVCAM-1 to increase were significantly higher in the lowest quartile group than in the higher quartile group, classified by the quartile of MWSS (all $p < 0.001$, Fig. 4). These significant differences remained after adjustment for confounders (all adjustments $p < 0.001$). Similar results were found between the lowest quartile and higher quartile groups as classified by the quartile of PWSS, even after adjustment for confounders ($p < 0.001$, $= 0.001$, < 0.001, and $= 0.013$, respectively, Supplementary Fig. 3).

4. Discussion

In this longitudinal study, the major findings were as follows: (1) low carotid WSS was independently associated with WMH volume and fraction, lacune, and microbleed progression in older adults; (2) decreased NO levels and increased ET-1, sICAM-1, and sVCAM-1 levels, markers of endothelial dysfunction, were associated with WMH volume and fraction, lacune, and microbleed progression; (3) low carotid WSS was significantly correlated with the progression of endothelial dysfunction. Together, these results indicated that endothelial dysfunction may act as a crucial mediator between low carotid WSS and cerebral small vessel disease progression.

In addition to systemic risk factors such as age and genetic variation [19,20], vascular pathophysiological factors such as arterial WSS are increasingly thought to be important contributors to chronic cerebrovascular disease [2,9,21,22]. A few cross-sectional studies have demonstrated that carotid WSS is related to ischemic stroke and WMH [2,9,21]. However, the results of these studies were vague, and the nature of the association between carotid WSS and cerebral small vessel disease remains inconclusive. In this study, we found that the lowest carotid WSS, either mean or peak WSS, was strongly associated with an increased risk of WMH, lacune, and microbleed progression. The risk of new incidents of Fazekas scale ≥2 lesions, lacunes, and microbleeds was significantly higher in the lowest MWSS quartile group relative to those in the higher MWSS quartile group. Similar associations were observed between PWSS and WMH, lacune, and microbleed progression. Furthermore, we found that WMH and the prevalence of Fazekas scale ≥2 lesions and microbleeds at baseline were significantly higher in the lowest WSS quartile group than in the higher quartile WSS group, classified by either mean or peak WSS. These results are in agreement with and further complement a previous study that revealed an independent association between carotid WSS and cerebral small vessel disease [2]. Furthermore, these results indicated that older adults with lower carotid WSS may be more prone to developing cerebral small vessel disease than those with higher carotid WSS.

It is well-known that low WSS is closely associated with endothelial failure [3,5,6]. Low carotid WSS predicting cerebral small vessel disease might be linked to impaired lumen diameter adaption (endothelial dysfunction per se) or also to higher resistance of cerebral vessels. In this study, consistent with previous studies [3,5,6], we found that low common carotid WSS was closely associated with the progression of

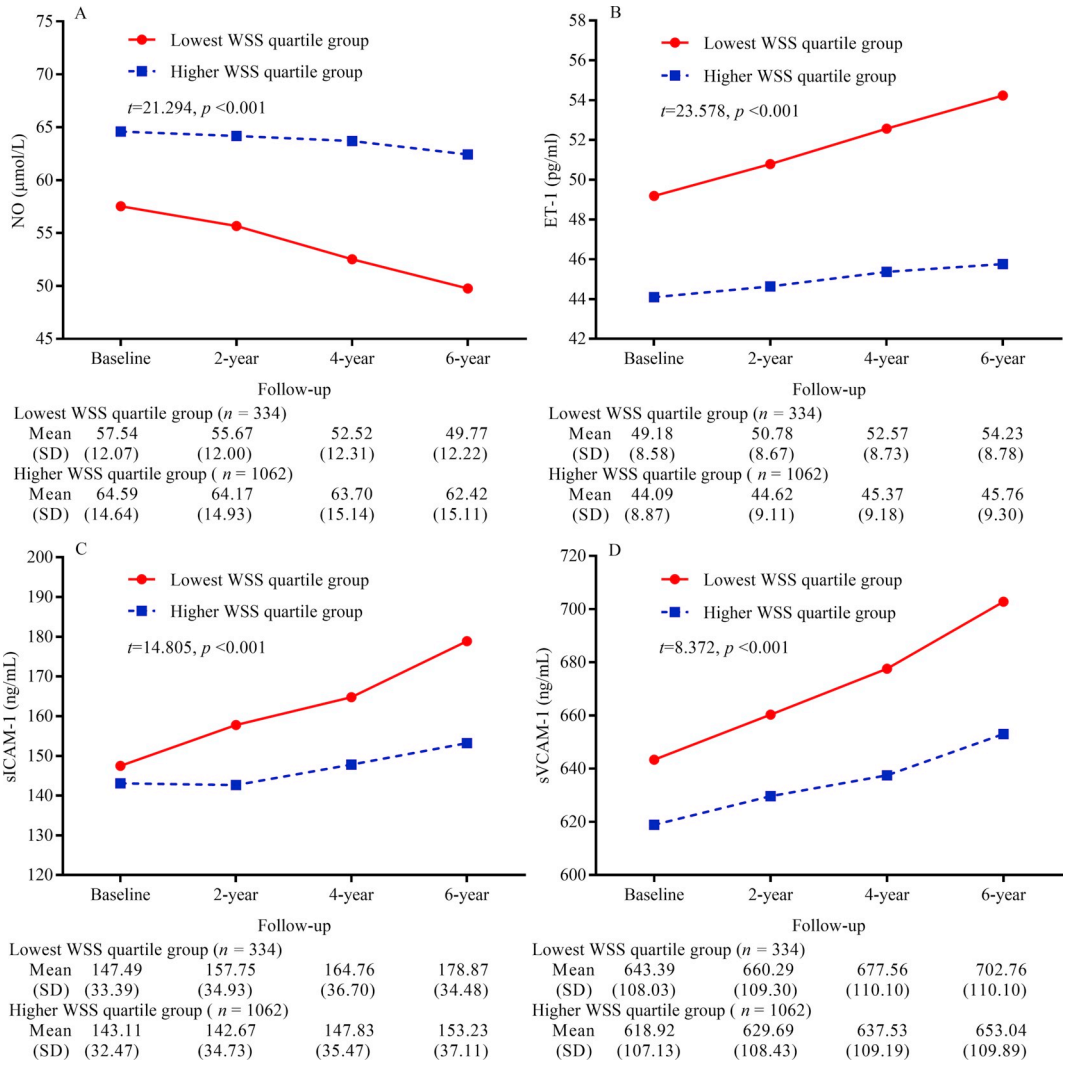

Fig. 4. Differences in the changes in endothelial function between lowest quartile group and higher quartile groups classified by the quartile of mean wall shear stress.

(A) Changes in NO. (B) Changes in ET-1. (C) Changes in sICAM-1. (D) Changes in sVCAM-1. NO = nitric oxide; ET-1 = endothelin-1; sICAM-1 = soluble intercellular cell adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; WSS = wall shear stress; SD = standard deviation.

endothelial dysfunction. At the capillary level, the endothelium forms the most important part of the blood-brain barrier [23,24]. Endothelial failure may result in damage to endothelial tight junction proteins such as claudins and occludins, leading to significantly increased permeability of the blood-brain barrier [23,24]. In arterioles, endothelial disruption may lead to arteriolar thrombosis, luminal occlusion, and impaired autoregulation [23,25]. Finally, endothelial failure leads to ischemic changes in brain tissue [23]. The underlying cause of cerebral small vessel disease is usually assumed to be ischemia, resulting from either arteriolar occlusion or the structural or functional narrowing of vessels [23]. Our study demonstrated that decreases in NO levels and increases in ET-1, sICAM-1, and sVCAM-1 levels were significantly associated with an increased risk of new incidents of Fazekas scale ≥ 2 lesions, lacunes, and microbleeds, even after adjustment for confounders.

Previous studies have demonstrated that WMH is associated with lacunes and microbleeds [23,26]. A baseline WMH burden might be an important contributor to WMH progression and incidents of lacunes and microbleeds. Besides a baseline WMH burden, we observed significant differences in smoking, common carotid IMT and plaques among groups classified by either MWSS or PWSS. Smoking has been found to be associated with worse brain microstructure [27], endothelial dysfunction [28], and arterial stiffness [29]. To reduce the bias of our results, we introduced these variables into the models using logistic regression and Cox proportional regression analyses, and found that common carotid WSS was still independently associated with the progression of WMH and new incidents of lacunes and microbleeds.

In multiple linear models, we found that there were no significant associations between changes in microbleed counts with changes in ET-1, sICAM-1, and sVCAM-1 levels after adjustment for confounders, including common carotid IMT and plaques, baseline WMH, and the differences in time between the baseline and MRI follow-up visits. Possible causes for this lack of association may be the presence of different underlying etiologies and pathophysiological mechanisms in WMH, lacunes, and microbleeds, despite the fact that these features share some common mechanisms and risk factors, as well as associate with each other [13,23,26,30]. Red blood cell leakage, bleeding vessels, and amyloid angiopathy may be some of the primary mechanisms responsible for microbleeds [19,23,30]. WMH and lacunes, however, might result from ischemic damage to brain blood vessels, and generally manifests as increases in endothelial dysfunction and proinflammatory mediators [23,30].

A population-based prospective cohort study design with a nearly 6-year follow-up time was the major strength of this study. This reduced

the amount of bias in the results and enabled us to assess the association between common carotid WSS and cerebral small vessel disease, an association which could not be elucidated by cross-sectional or short-term follow-up studies.

There are some limitations that must be considered in our study. First, carotid WSS did not exactly represent the WSS in cerebral vascular vessels, especially in small cerebral vessels [31]. In cerebral vessels, the response to WSS was thought to be dependent on vessel size, or vessel location within the vascular tree [32]. Second, genetic factors of participants were not considered in this study. It has been reported that WMH is strongly genetically influenced [33,34]. Thirdly, we assessed the vascular endothelial function using the serum levels of NO, ET-1, sICAM-1, and sVCAM-1 in peripheral venous blood. It may not correspond to the local level of endothelial function in the common carotid artery where shear stress was calculated. In addition, as an observational cohort study, we were unable to control for medication schedules, such as treatment for hypertension, blood glucose levels, or dyslipidemia. All data were observational in nature with known limitations, which may have led to the introduction of bias in the results.

In conclusion, our findings demonstrated that low common carotid WSS was an independent risk factor for cerebral small vessel disease in older adults. Endothelial dysfunction might act as a crucial mediator between low common carotid WSS and cerebral small vessel disease. These results indicated that interventions that improve hemodynamics, such as enhanced external counterpulsation [35,36], may be effective preventative measures and/or treatments for cerebral small vessel disease. However, larger sample sizes, as well as multinational and multiracial studies are needed to further validate the association between common carotid WSS and cerebral small vessel disease.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Z.L. and Y.G. had full access to all the data in the study and had final responsibility for the decision to submit for publication. Z.L., Y.G., and H.Z. planned and initiated the trial. H.Z. and Y.C. contribute to the management of data. Z.L., Y.G., Y.C., and Q.C. contribute to the analysis and interpretation of data. Y.C., H.Y., J.Z., and Z.L. contribute to the drafting of the manuscript. Z.L., Y.G., G.G. and Q.C. contribute to the critical revision of the manuscript for important intellectual content. All authors contribute to the data collection. All authors interpreted data, critically reviewed the report, and approved the final version of the report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.07.006.

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