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Endothelial Function and White Matter Hyperintensities in Older Adults With Cardiovascular Disease

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Background and Purpose—The presence of white matter hyperintensities on brain MRI is common among elderly individuals. Previous research suggests that cardiovascular risk factors are associated with increased white matter hyperintensities. Examining the role of direct physiological measures of vascular function will help to clarify the vascular mechanisms related to white matter hyperintensities. The aim of the present study was to examine the association between endothelial-dependent and endothelial-independent vasodilatation and white matter hyperintensity volume.

Methods—Twenty-five older adults with a range of cardiovascular diseases underwent brain MRI and completed assessments of blood vessel integrity using endothelial-dependent and independent flow-mediated dilation of the brachial artery. A semi-automated pixel-based method was used to quantify total brain volume and white matter hyperintensity volume, with white matter hyperintensity volume corrected for total brain volume. The association between measures of flow-mediated dilation and log-transformed white matter hyperintensities was examined.

Results—Correlation analysis revealed that endothelial-dependent vasodilatation was significantly and inversely associated with white matter hyperintensity volume. In contrast, endothelial-independent vasodilatation was not associated with white matter hyperintensities. Neither endothelial-dependent nor endothelial-independent vasodilatation was associated with total brain volume.

Conclusions—These data provide preliminary evidence that the integrity of the vascular endothelium is associated with white matter hyperintensities in older adults with cardiovascular disease. Impaired vascular function may be one mechanism that contributes to the development of white matter hyperintensities in the brain. Additional longitudinal research combining measures of vessel function, neuroimaging and cognition will be helpful in clarifying this potential mechanism. (*Stroke*. 2007;38:308-312.)

Key Words: cardiovascular disease ■ endothelium ■ magnetic resonance ■ white matter disease

Evidence of cerebrovascular disease on neuroimaging is common among elderly individuals,^{1,2} particularly regions of hyperintense signal in cerebral white matter observed on proton density T₂-weighted or fluid-attenuated inversion recovery images. Several studies have reported that increased white matter hyperintensities (WMH) are associated with cognitive impairment, even in the absence of dementia.³⁻⁵ Furthermore, WMH have been associated with depression and increased risk for stroke and death.⁶⁻⁸ The presence of traditional cardiovascular risk factors (eg, increasing age, smoking, hypertension) have a well-recognized impact on vascular function and have been associated with

increased WMH,⁹ suggesting that WMH may arise from an underlying vascular cause. Identifying specific vascular abnormalities associated with the presence of WMH will improve our understanding of the underlying physiology involved in the relationship between cardiovascular disease and cerebrovascular injury to the brain, reflected by WMH.

One potentially relevant aspect of vascular function in relation to WMH is the capacity of the cerebral blood vessels to regulate in response to physical and chemical stimuli by adjusting vascular tone and blood flow. Indeed, previous studies have documented a decrease in vasodilatory capacity in the cerebral cortex of individuals with WMH in response to

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CO₂ and acetazolamide challenges to cerebral circulation.^{10,11} Given the unique anatomical and physiological characteristics of cerebral blood vessels, regulation of vascular tone in the cerebral circulation is complex and is mediated by multiple factors.¹² One important influence on the regulation of vascular tone is the release of nitric oxide (NO) from the endothelium; this effect was initially established in the peripheral vasculature,¹³ although there is increasing evidence that NO plays an important role in cerebral circulation. For example, studies in animal models using inhibitors of NO synthase suggest that NO mediates basal cerebral blood flow and may be involved in the vasodilatory response to hypercapnia,^{14–18} although findings have been inconsistent within and across species.^{17,19,20} In vitro examination of human cerebral arteries suggests that NO mediates vessel relaxation^{21,22} and there is some in vivo evidence of a contribution of NO to cerebrovascular regulation among healthy adults.^{23,24} Given that impaired NO synthesis and release has been demonstrated in peripheral arteries of patients with risk factors for cerebrovascular disease (eg, hypertension and coronary artery disease^{25,26}) and increasing evidence of a role of NO in cerebrovascular regulation, a logical extension of these findings is to examine whether impairments of endothelial function and NO release affect the cerebrovasculature of individuals with cardiovascular disease.

Endothelial function in humans is commonly assessed by measuring the degree of brachial artery dilatation in response to increased flow after forearm occlusion with a blood pressure cuff.^{27–29} This measure relies on the fact that an increase in blood flow causes shear stress stimulating vasodilators, resulting in endothelial-dependent dilatation within an intact vessel.³⁰ Flow-mediated dilatation measures in the brachial artery have been linked to vessel function in the coronary artery suggesting that it may be a noninvasive marker of “global” endothelial function.^{31,32} One previous study examined flow-mediated dilatation in relation to CO₂ vasoreactivity in the cerebral circulation;³³ however, to our knowledge, no study has examined flow-mediated dilatation in relation to neuroimaging findings among patients with cardiovascular disease.

The purpose of the current study was to examine the association between the integrity of the vascular endothelium and WMH among nondemented older adults with cardiovascular disease. To accomplish our goal, we measured brachial artery blood vessel function using a well-validated technique to assess endothelial-mediated and endothelial-independent vascular responses.³¹ In addition to completing the blood vessel measures, study participants underwent structural MRI of the brain, from which indices of WMH and total brain volume were quantified. We hypothesized that dysfunction of the vascular endothelium would be associated with greater WMH volume. Furthermore, because the participants were not demented and we anticipated that their total brain volume would be relatively normal, we hypothesized that endothelial function would not be significantly associated with total brain volume.

Materials and Methods

The study was approved by local institutional review boards and written informed consent was obtained from all participants.

TABLE 1. Demographic and Clinical Characteristics of Sample

Variable	Mean (SD)	No. of Participants
Age, y	72.2 (7.7)	
Sex, % female		10/25 (40%)
Mini-Mental State Exam Score	28.8 (1.1)	
Beck Depression Inventory	4.2 (2.5)	
Systolic BP, mm Hg*	134.4 (19.9)	
Diastolic BP, mm Hg*	68.5 (10.1)	
% Change in brachial artery diameter, reactive hyperemia, endothelial-dependent dilatation	5.99 (4.53)	
% Change in brachial artery diameter with nitroglycerin, endothelial-independent dilatation	15.64 (6.80)	
WMH, voxels (WMH/TBV×100)	0.68 (1.21)	
Total brain volume, cm ³	1226.77 (155.89)	
Hypertension		19/25 (76%)
Hypercholesterolemia		14/25 (56%)
Diabetes		4/25 (16%)
History of smoking		8/25 (32%)
Myocardial infarction		11/25 (44%)
Coronary artery bypass surgery		9/25 (36%)
Angioplasty/stents		2/25 (8%)
Heart failure		2/25 (8%)

*Blood pressure was collected before vascular assessment.

Twenty-five participants enrolled in an ongoing study examining the effects of cardiovascular disease on brain functioning in the elderly were included in the current analysis. Participants were recruited from cardiac rehabilitation programs, cardiology practices, and advertisements. All participants had a documented history of cardiovascular disease including at least 1 of the following: myocardial infarction, cardiac surgery, heart failure, coronary artery disease, or hypertension.

Demographic and clinical characteristics are presented in Table 1. None of the participants scored within the demented range on the Mini Mental State Exam.³⁴ Cardiovascular risk factors (ie, hypertension, hypercholesterolemia, tobacco use, and diabetes) were coded according to the number of factors present (ie, 0 to 4). Eight of 25 participants (32%) endorsed 1 risk factor, 10 of 25 (40%) endorsed 2, 3 of 25 (12%) endorsed 3, and 2 of 25 (8%) had all 4 factors. Medication information for the sample was as follows: 22 of 25 (88%) participants were currently being treated with antihypertensive medication, 17 of 25 (68%) with aspirin/antithrombotics, 17 of 25 (68%) with lipid-lowering agents, 12 of 25 (48%) with vitamins, 11 of 25 (44%) with gastric acid inhibitors, 3 of 25 (12%) with hypoglycemics, 2 of 25 (8%) with vasodilators, and 2 of 25 (8%) with psychoactive medications.

Exclusion criteria included neurological disease (eg, stroke, traumatic head injury with loss of consciousness >10 minutes, dementia), major psychiatric illness (ie, schizophrenia, bipolar disorder), substance abuse (current abuse or previous hospitalization for abuse), and MRI contraindications. Participants underwent cardiovascular assessment of blood vessel functioning using Doppler ultrasound flow-mediated dilatation and brain MRI on separate visits.

Brachial Artery Flow-Mediated Dilatation

Peripheral vascular autoregulation and associated endothelial function were assessed by flow-mediated brachial artery vasodilatation. Participants fasted and held vasoactive medications (eg, calcium

TABLE 2. Correlations (*r*) between Flow-Mediated Dilatation and MRI Measures

	WMH*		Total Brain Volume	
	Flow-Induced % Δ in Vessel Diameter	Nitroglycerin-Induced % Δ in Vessel Diameter	Flow-Induced % Δ in Vessel Diameter	Nitroglycerin-Induced % Δ in Vessel Diameter
No adjustments	-0.63 ($P=0.001$)	-0.25 ($P=0.26$)	0.26 ($P=0.20$)	0.04 ($P=0.87$)
Adjusting for age	-0.53 ($P=0.01$)	-0.16 ($P=0.49$)	0.23 ($P=0.32$)	-0.01 ($P=0.97$)
Adjusting for age and risk factors	-0.53 ($P=0.02$)	-0.19 ($P=0.42$)	0.21 ($P=0.37$)	-0.02 ($P=0.93$)

*WMH values are ratios (WMH/TBV \times 100) normalized via log transformation.

channel blockers, angiotensin-converting enzyme inhibitors), caffeine, and smoking for 6 hours before the vascular assessment. Before initiating vascular interrogation, participants remained supine for 15 minutes in a quiet room. High-frequency B-mode ultrasound was used to visualize the brachial vessel. A Hewlett Packard 5500 ultrasound system, equipped with a linear array vascular (7.5 MHz) transducer was used to acquire 2-dimensional and Doppler flows of each participant's left arm. Images were obtained in longitudinal orientation \approx 5 cm above the antecubital fossa; straight segments were obtained at least 10 mm and were targeted for optimal assessments. Blood pressure was measured with an automated Datascope accutor 3SAT (Paramus, NJ) in the contralateral arm.

To assess endothelial function, hyperemic (flow-mediated) vascular responses were assessed. First, baseline images of brachial artery diameter and blood flow velocity were recorded for 1 minute (sequential images, captured and digitized on each R-wave). Thereafter, a 4-cm cuff positioned on the mid-forearm was inflated to 40 mm above the baseline systolic blood pressure for 5 minutes. The same brachial segment was interrogated for 3 minutes after the cuff was deflated (the period of hyperemic flow). Analyses of the digital images were performed by an investigator who was blinded to subject characteristics. Arterial diameter was determined using a validated software algorithm that automatically calculates the average diameter over the selected segment.⁸ Flow-mediated vasodilation was calculated as the percentage change in diameter from baseline to the maximum diameter induced by reactive hyperemia.

Ten minutes after the hyperemic brachial assessment was completed, endothelial-independent vascular function was assessed by measuring the same portion of the brachial artery before and 5 minutes after the administration of 0.4 mg sublingual nitroglycerin, a time corresponding to peak vasodilatory responses. Nitroglycerin-mediated vasodilation was calculated as the percentage change in arterial diameter from the pre-nitroglycerin baseline to the diameter after nitroglycerin.

Brain MRI and WMH Quantification

The brain MRI and WMH quantification techniques used in the current study have been described elsewhere.³⁵ MRI was obtained using a Siemens Symphony 1.5-T unit. Fluid-attenuated inversion recovery-weighted (repetition time/echo time=6000/105) images (5-mm thickness with a 2-mm gap) were obtained for each participant and used to quantify WMHs via a semi-automated threshold technique in ANALYZE (Biomedical Imaging Resource, Mayo Foundation). The total number of voxels representing WMH from each slice was summed to calculate the total WMH for each participant. Total brain volume was calculated using threshold histogram values consistent with brain parenchyma. Total brain volume was used as a correction factor for WMH values (ie, WMH pixel total/TBV \times 100), and this ratio was used as the primary dependent variable. Given that WMH volume was positively skewed, log transformation was used to normalize this variable. Nonparametric correlations were considered; however, given the relatively normal distribution of the blood vessel functioning measures, transformation of the WMH variable was deemed to be more appropriate.

Data Analysis

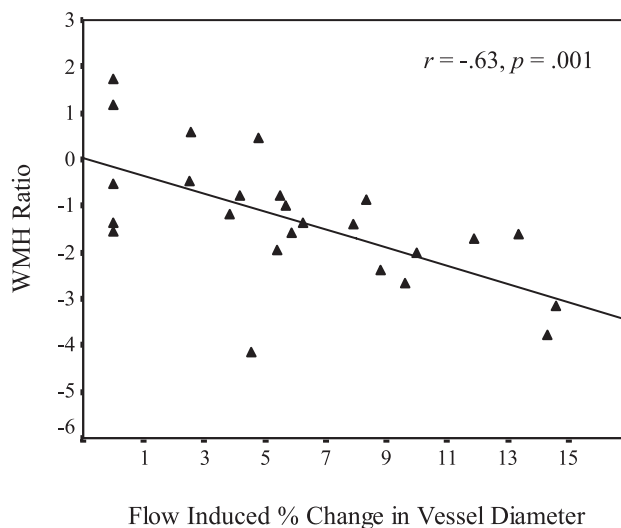
Four bivariate correlations were calculated to examine the association between blood vessel functioning and WMH. Correlations were calculated between endothelial-dependent vasodilation (percent change in brachial artery diameter, reactive hyperemia), endothelial-independent vasodilation (nitroglycerin-induced percent change in brachial artery diameter), and the 2 MRI variables (ie, log-transformed WMH volume and raw TBV). These correlations were then recalculated as partial correlations adjusting for age and level of cardiovascular risk, coded as described. An α level of 0.05 was retained for all analyses given the exploratory nature of the study and the fact that only 4 main correlations were calculated with a priori hypotheses.

Results

As shown in Table 2, endothelial-dependent flow-mediated dilatation was significantly and inversely associated with WMH ($r=-0.63$, $P<0.01$; Figure), but not total brain volume ($r=0.26$, not significant), suggesting that as endothelial-dependent flow-mediated dilatation decreases, WMH significantly increase. In contrast, endothelial-independent dilatation was not significantly correlated with either WMH volume ($r=-0.25$, not significant) or total brain volume ($r=0.04$, not significant). These relationships remained consistent after adjusting for age and level of cardiovascular risk (Table 2).

Discussion

The present study provides preliminary evidence suggesting that impaired endothelial function is associated with increased cerebral WMH volume in older adults with cardio-



Scatterplot of flow-mediated vessel dilatation and WMH ratio.

vascular disease. Endothelial dysfunction, as measured by flow-mediated dilatation of the brachial artery, was significantly associated with greater volume of WMH on brain MRI. Endothelial-independent vasodilatation, measured after administration of nitroglycerin, was not significantly associated with WMH volume. Importantly, the relationship between endothelial function and WMH remained significant after adjusting for the effects of age and level of cardiovascular risk, calculated based on the presence of traditional cardiovascular risk factors (ie, hypertension, diabetes, hypercholesterolemia, and smoking). This suggests that endothelial dysfunction may be associated with WMH independent of these factors. Thus, flow-mediated dilatation may provide an integrated measure of large conduit blood vessel regulation, reflecting the cumulative deleterious effects of multiple risk factors on vascular function.^{26,27}

The current study included a clinically heterogeneous sample with regard to type of cardiovascular disease. Such heterogeneity maximized our ability to examine endothelial function as an integrated measure of vascular health among a clinical sample with a broad range of cardiovascular risk factors known to contribute to endothelial dysfunction. The current study extends previous research that has demonstrated a relationship between cardiovascular risk factors and WMH by examining a more direct physiological measure of blood vessel function, particularly involvement of NO, potentially reflecting the combined effects of various vascular risk factors.⁹

Our initial evidence of an association between peripheral endothelial function and WMH supports the notion that impaired regulation of vascular tone and the vasodilatory response may be one mechanism underlying the development of WMH in the brain. Previous studies have reported that decreased cerebrovascular dilatory capacity in response to CO₂¹⁰ and acetazolamide¹¹ challenges are associated with increased white matter lesions. For example, Bakker et al examined the association between vasomotor reactivity, using CO₂ transcranial Doppler, and white matter lesions on MRI in a population-based cohort and concluded that their data supported the hypothesis that some white matter lesions might be secondary to hemodynamic ischemic injury to the brain.¹⁰ Furthermore, neuropathological studies have documented that chronic vascular injury in the blood vessels of the brain leads to atrophic changes immediately surrounding these damaged vessels.^{36,37} These atrophic spaces become filled with extracellular fluid that then appears as WMH on MRI. Thus, it is possible that chronic impairment of the vasodilatory response may contribute, in part, to the development of WMH over time. Furthermore, the measure that we used to assess endothelial function (ie, flow-mediated dilatation) is thought to be a noninvasive measure of endothelium-derived NO activity.²⁹ This observation, coupled with evidence from studies examining the role of NO in cerebral circulation,^{14–18} raises the possibility that NO may be one mediator of the relationship.

It is important to note that our sample consisted of a relatively small number of patients and, as a result, the findings should be considered preliminary. However, the correlations were consistent with our expectations and re-

mained significant after adjusting for the effects of age and level of cardiovascular risk, providing some evidence of robust effects despite the small sample size. Given the correlational nature of the study, the observed association does not directly link impaired endothelial function to WMH, because WMH likely result from multiple complex mechanisms. It will be important to examine these associations longitudinally to clarify the potential causal role of endothelial dysfunction in the development of WMH.

The current findings suggest several directions for future studies. First, an important extension of these findings would be to examine relationships among endothelial functioning, WMH, and neuropsychological performance within the same sample of patients. Previous research has demonstrated a relationship between an invasive measure of peripheral endothelial function and global neuropsychological performance.³⁸ The literature on vascular cognitive impairment suggests that the frontal subcortical networks implicated in complex attention, executive functioning, and processing speed are particularly susceptible to ischemic damage resulting from blood flow abnormalities.^{39,40} Thus, it is reasonable to hypothesize that executive function and processing speed deficits may be observed in relation to endothelial dysfunction and WMH. Second, in future larger studies it would be interesting to examine whether there is a specific quantitative relationship between measures of endothelial function and WMH, such that a certain level of cardiovascular impairment is necessary before there is a significant impact on the brain. Finally, in the current study we included only individuals who had cardiovascular disease. The idea that endothelial function and WMH are associated would be strengthened by research examining the relationship between brachial response and WMH in healthy individuals, also, to determine whether the association differs among elderly people free of cardiovascular risk.

Summary

We observed an inverse association between endothelial-dependent flow mediated dilation and WMH volume. Overall, the present findings suggest the possibility that endothelial dysfunction is associated with increased WMH. This observation raises the prospect that impaired regulation of vascular tone and the vasodilatory response may be one mechanism that contributes to the development of WMH in the brain. Although this could not be directly tested given the cross-sectional and correlational nature of the current study, future large-scale prospective studies will be important to extend the current findings and determine the causal nature of this relationship. The current findings also underscore the importance of potential interventions to improve vessel function, given the possible deleterious effects of cerebrovascular changes.

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Disclosures

None.

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