A Systematic Review of Dynamic Cerebral and Peripheral Endothelial Function in Lacunar Stroke Versus Controls

Susan F. Stevenson, BSc (Hons); Fergus N. Doublal, MB, ChB, MRCP; Kirsten Shuler, BSc; Joanna M. Wardlaw, FRCP, FRCR, FMedSci

Background and Purpose—The etiology of cerebral small vessel disease is unknown. An association with endothelial dysfunction has been suggested. We systematically assessed all relevant studies of dynamic endothelial function in patients with lacunar stroke as a marker of small vessel disease.

Methods—We searched for studies of cerebral or peripheral vascular reactivity in patients with lacunar or cortical (ie, large artery atheromatous) ischemic stroke or nonstroke control subjects. We calculated standardized mean difference (SMD) in vascular reactivity ± 95% CIs between small vessel disease and control groups.

Results—Sixteen publications (974 patients) were included. In lacunar stroke, cerebrovascular reactivity (n = 534) was reduced compared with age-matched normal (SMD −0.94, 95% CI −1.17 to −0.70), but not age + risk factor-matched control subjects (SMD 0.08, 95% CI −0.36 to 0.53) or cortical strokes (SMD −0.29, 95% CI −0.69 to 0.11); forearm flow-mediated dilatation (n = 401) was reduced compared with age-matched normal control subjects (SMD −1.04, 95% CI −1.33 to −0.75) and age + risk factor-matched control subjects (SMD −0.94, 95% CI −1.26 to −0.61), but not cortical strokes (SMD −0.23, 95% CI −0.55 to 0.08).

Conclusions—Endothelial dysfunction is present in patients with lacunar stroke but may simply reflect exposure to vascular risk factors and having a stroke, because a similar degree of dysfunction is found in cortical (large artery atheromatous) stroke. Current data do not confirm that endothelial dysfunction is specific to small vessel stroke. Future studies should include control subjects with nonlacunar stroke.

Key Words: atherosclerosis ■ endothelium ■ plethysmography ■ stroke ■ vascular diseases

Cerebral small vessel disease is common and has clinical (lacunar stroke, cognitive impairment, gait and movement disorders) and radiological (symptomatic small subcortical infarct, silent lacunar infarct, lacunes, white matter lesions, microbleeds) manifestations. The low early mortality of lacunar stroke masks a long-term risk of recurrent stroke and death similar to atherothromboembolic stroke, creating a massive public health burden.

Lacunar infarcts are small (<15 mm) subcortical lesions in the territory of a single deep perforating arteriole, most of which are associated with an intrinsic abnormality in the perforating arteriole wall of unknown etiology. An association between lacunar ischemic stroke and endothelial dysfunction has been suggested, but many patients with stroke have hypertension or diabetes or take medications that affect endothelial function. Atheromatous large artery disease is also associated with endothelial dysfunction. A recent systematic review identified endothelial dysfunction in lacunar ischemic stroke but did not control for risk factor exposure or other stroke subtypes. Therefore, it is unclear whether endothelial changes observed in patients with lacunar ischemic stroke might be specific to small vessel disease or simply reflect age, vascular risk factors, generalized (possibly coincidental) atheroma, or the effects of having a stroke. We performed a systematic review of all studies that assessed cerebral or peripheral vascular reactivity in patients with lacunar ischemic stroke.

Methods

We followed the general guidance for systematic reviews of observational and diagnostic studies (www.equator-network.org) modified to suit the type of study identified in this review.

We searched the published literature using MEDLINE and EMBASE from January 1, 1995 to February 15, 2008, using Ovid and a carefully devised search strategy (Appendix) developed with advice from the Cochrane Stroke Group (www.dcn.ed.ac.uk/csrg/). We updated the search using MEDLINE to January 6, 2010 (we did not research EMBASE because there was very little difference between it and MEDLINE in the initial search). We sought primary studies, in humans, in any language, which investigated patients with markers of cerebral small vessel disease and dynamic measures of endothelial function, for example, response to hypercapnia or acetazolamide or flow-mediated dilatation. We checked references in review and primary studies and hand-searched the journal Stroke.

We included papers that assessed endothelial function in patients with clinically evident lacunar ischemic stroke, with or without an

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Endothelial Dysfunction in Lacunar Stroke

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Characteristics of Included Studies

Some of the 16 papers contributed to >1 comparison: 14 of 16 papers compared 318 patients with recent lacunar ischemic stroke with 305 age-matched or age+risk factor matched control subjects; 5 of 15 papers compared 124 patients with recent lacunar ischemic stroke with 115 patients with recent cortical ischemic stroke; 4 of 15 papers compared patients with recent lacunar ischemic stroke of whom 71 had only 1 single small subcortical infarct and 71 had a single symptomatic lacunar infarct plus multiple silent infarctions on imaging (Table).

Most studies were small (mean 36 subjects, median 18 subjects per group) and gave the time interval between stroke and endothelial function assessment (range <72 hours to 3 months, median 26 days). The patients with lacunar and cortical ischemic stroke were not age-matched.

Characteristics of Included Patients and Control Subjects

All 16 papers defined lacunar ischemic stroke as “appropriate neurological features and a recent small subcortical infarct on imaging consistent with the symptoms.” Most papers excluded patients with carotid stenosis (except 130), 5 excluded cardiogenic sources of emboli, and 3 excluded middle cerebral artery (MCA) stenosis from the lacunar stroke group.

Assessment of Endothelial Function

Twelve papers assessed endothelial function in the cerebral circulation and 5 papers the systemic circulation; 1 assessed both. Several techniques (Table) were used to assess cerebral endothelial function, including the vascular response to hypercapnia or infusion of acetazolamide or L-arginine, expressing the response as a percentage increase in mean arterial blood velocity in the MCA or basilar artery. Change in blood oxygen level-dependent signal during hypercapnia detected using functional MRI, confirming the infarct subtype with CT or MRI. Four studies included patients with >1 lacunar infarct on CT, MR, or both.

Endothelial Function: Lacunar Stroke Versus Age-Matched Control Subjects

Thirteen studies compared lacunar ischemic stroke with healthy age-matched control subjects, 9 in the cerebral and 4 in the peripheral circulation. Vascular reactivity was reduced in the cerebral circulation in patients with lacunar stroke compared with age-matched healthy control subjects (8 of 9 studies, 324 patients, SMD = 0.94, 95% CI = 1.17 to −0.70, P < 0.00001; Figure 1) and in the forearm (4 of 4 studies, 211 patients, SMD = 1.04, 95% CI = 1.33 to −0.75, P < 0.00001; Figure 2). There was no significant heterogeneity between studies.
Table 1. Dynamic Endothelial Function in the (A) Cerebral and (B) Peripheral Circulation*

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Lacunar Subject Details</th>
<th>Lacunar No. (age)</th>
<th>Control No. (age)</th>
<th>Clinical Classification</th>
<th>Imaging Modality</th>
<th>Endothelial Function Method</th>
<th>Endothelial Function Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Cerebral circulation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pretnar-Oblak et al (a)32</td>
<td>2006</td>
<td>Lacunar stroke versus normal age-matched controls</td>
<td>18 (61.1 ± 7.6)</td>
<td>19 (59.2 ± 7.1)</td>
<td>TOAST</td>
<td>CT</td>
<td>Percent increase in mean MCA blood flow velocity on TCD after L-arginine</td>
<td>13.1 ± 8.4</td>
</tr>
<tr>
<td>Pretnar-Oblak et al (c)34</td>
<td>2006</td>
<td>Lacunar stroke with hypercholesterolemia</td>
<td>20 (60.9 ± 6.2)</td>
<td>21 (59.5 ± 7.3)</td>
<td>Not stated</td>
<td>CT</td>
<td>Percent increase in mean MCA blood flow velocity on TCD after L-arginine</td>
<td>13.4 ± 9.1</td>
</tr>
<tr>
<td>Mochizuki et al30</td>
<td>1997</td>
<td>Single lacunar stroke</td>
<td>15 (63.5)</td>
<td>16 (58.4)</td>
<td>Clinical</td>
<td>CT</td>
<td>Absolute percent increase in cCBF of white matter after acetazolamide</td>
<td>7.3 ± 6.0</td>
</tr>
<tr>
<td>Molina et al40</td>
<td>1999</td>
<td>First-ever lacunar stroke</td>
<td>46 (56.6 ± 13.4)</td>
<td>46 (58.3 ± 12)</td>
<td>Based on imaging</td>
<td>MRI</td>
<td>Percent increase in mean MCA blood flow velocity on TCD after acetazolamide</td>
<td>50 ± 12.7</td>
</tr>
<tr>
<td>Panczel et al41</td>
<td>1999</td>
<td>Brain stem lacunar stroke</td>
<td>20 (62.2 ± 13.9)</td>
<td>10 (64.4 ± 4.3)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>Increase in mean BA blood flow velocity on TCD after acetazolamide (%)</td>
<td>47.3 ± 21.9</td>
</tr>
<tr>
<td>de Leeuw et al39</td>
<td>2003</td>
<td>Single lacunar stroke</td>
<td>12 (58.2 ± 16.8)</td>
<td>12 (52 ± 12.1)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>Percent increase in mean MCA blood flow velocity on TCD per mm Hg CO₂ increase CO₂</td>
<td>3.4 ± 5.0</td>
</tr>
<tr>
<td>Maeda et al42</td>
<td>1993</td>
<td>Lacunar stroke</td>
<td>20 (59.6 ± 6.8)</td>
<td>25 (57.3 ± 6.5)</td>
<td>NINDS</td>
<td>CT</td>
<td>Mean spatial Doppler frequency—A exp/k PET CO₂ hypercapnia</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>Cupini et al37</td>
<td>2001</td>
<td>Lacunar stroke</td>
<td>14 (61.4 ± 9.2)</td>
<td>15 (56.8 ± 12.7)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>ΔMFV/baseline MFP × 100/s</td>
<td>1.36 ± 0.39</td>
</tr>
<tr>
<td>Hund-Georgiadis et al31</td>
<td>2003</td>
<td>Lacunar infarction and leukoaraiosis</td>
<td>5 (61.8)</td>
<td>6 (57)</td>
<td>Not stated</td>
<td>MRI</td>
<td>Measured using fMRI: BOLD signal volume decrease (cm³) change normalized to ET-CO₂</td>
<td>290 ± 190</td>
</tr>
<tr>
<td>Immink et al38</td>
<td>2005</td>
<td>Lacunar stroke</td>
<td>10 (63 ± 3)</td>
<td>10 (57 ± 2)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>Delay (in seconds) of MCA Vmean counterregulation during changes in MAP increments in seconds</td>
<td>5.3 ± 0.5</td>
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<tr>
<td>Single lacunar stroke versus age and risk factor-matched control subjects</td>
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<td>Pretnar-Oblak et al (a)32</td>
<td>2006</td>
<td>Lacunar stroke with hypercholesterolemia</td>
<td>18 (61.1 ± 7.6)</td>
<td>20 (62.7 ± 5.3)</td>
<td>TOAST</td>
<td>CT</td>
<td>Percent increase in mean blood velocity on TCD after L-arginine</td>
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<tr>
<td>Pretnar-Oblak et al (c)34</td>
<td>2006</td>
<td>Lacunar infarction</td>
<td>20 (60.9 ± 7.3)</td>
<td>21 (61.0 ± 6.2)</td>
<td>Not stated</td>
<td>CT</td>
<td>Percent increase in mean blood velocity on TCD after L-arginine</td>
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<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Lacunar Subject Details</th>
<th>Lacunar No. (age)</th>
<th>Control No. (age)</th>
<th>Clinical Classification</th>
<th>Brain Imaging Modality</th>
<th>Endothelial Function Method</th>
<th>Subjects</th>
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<tbody>
<tr>
<td>Single lacunar versus cortical ischemic stroke</td>
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<tr>
<td>Gur et al\cite{44}</td>
<td>2007</td>
<td>Lacunar infarction</td>
<td>24 (60.5 ± 13.7)</td>
<td>23 (72 ± 13.7)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>Percent increase in mean blood velocity after acetazolamide 1 gram IV</td>
<td></td>
</tr>
<tr>
<td>Maeda et al\cite{45}</td>
<td>1993</td>
<td>Lacunar infarction</td>
<td>20 (59.6 ± 6.8)</td>
<td>8 (53 ± 9.5)</td>
<td>NINDS</td>
<td>CT/MRI</td>
<td>Mean spatial Doppler frequency—A exp(k PET CO2) response to CO2</td>
<td></td>
</tr>
<tr>
<td>Cupini et al\cite{37}</td>
<td>2001</td>
<td>Lacunar infarction</td>
<td>14 (61.4 ± 9.2)</td>
<td>13 (53.9 ± 11.8)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>BHI: ΔMFV/baseline MFr×100/s</td>
<td></td>
</tr>
<tr>
<td>Imminik et al\cite{34}</td>
<td>2005</td>
<td>Lacunar infarction</td>
<td>10 (63 ± 3)</td>
<td>10 (59 ± 5)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>dCA: delay of MCA Vmean counterregulation during changes in MAP increments in seconds</td>
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<tr>
<td>Single versus multiple lacunar infarcts on imaging</td>
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<tr>
<td>Chamorro et al\cite{45}</td>
<td>1996</td>
<td>Lacunar stroke ± single or multiple infarcts on imaging</td>
<td>21 (NA)</td>
<td>22 (NA)</td>
<td>Stroke Data Bank MRI</td>
<td></td>
<td>Percent increase in mean MCA flow velocity after acetazolamide</td>
<td></td>
</tr>
<tr>
<td>Molina et al\cite{40}</td>
<td>1999</td>
<td>Lacunar stroke ± single or multiple infarcts on imaging</td>
<td>26 (NA)</td>
<td>20 (NA)</td>
<td>Based on imaging MRI</td>
<td></td>
<td>Percent increase in mean MCA flow velocity after acetazolamide (contralateral only)</td>
<td></td>
</tr>
<tr>
<td>Mochizuki et al\cite{30}</td>
<td>1997</td>
<td>Lacunar stroke ± single or multiple infarcts on imaging</td>
<td>10 (61.6)</td>
<td>15 (63.5)</td>
<td>Not stated</td>
<td>CT</td>
<td>Stable xenon CT method increase in rCBF of white matter after acetazolamide (contralateral only) absolute increase:</td>
<td></td>
</tr>
<tr>
<td>Cupini et al\cite{37}</td>
<td>2001</td>
<td>Lacunar stroke ± single or multiple infarcts on imaging</td>
<td>14 (60.5 ± 10.5)</td>
<td>14 (61.4 ± 9.2)</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>BHI: ΔMFV/baseline MFr×100/s Breath holding</td>
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</tbody>
</table>

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Table. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Lacunar Subject Details</th>
<th>Lacunar No. (age)</th>
<th>Control No. (age)</th>
<th>Clinical Classification</th>
<th>Brain Imaging Modality</th>
<th>Endothelial Function Method</th>
<th>Endothelial Function Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al(^{23})</td>
<td>2006</td>
<td>Lacunar stroke</td>
<td>56 (67.7 ± 10.2)</td>
<td>40 (66.7 ± 8.3)</td>
<td>TOAST</td>
<td>CT/MRI</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>4.3 ± 6.1; 8.8 ± 6.0</td>
</tr>
<tr>
<td>Lavallee et al(^{34})</td>
<td>2006</td>
<td>Lacunar stroke</td>
<td>17 (61 ± 21)</td>
<td>20 (60 ± 25)</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>4.2 ± 1.1; 6.7 ± 2.5</td>
</tr>
<tr>
<td>Lavallee et al(^{36})</td>
<td>2006</td>
<td>Lacunar stroke</td>
<td>17 (61 ± 21)</td>
<td>20 (60 ± 25)</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Flow-mediated dilatation: percent increase in carotid artery diameter after CO(_{2})</td>
<td>4.0 ± 1.0; 11.4 ± 2.7</td>
</tr>
</tbody>
</table>

**Single lacunar versus age + risk factor-matched control subjects**

<table>
<thead>
<tr>
<th>Year</th>
<th>Lacunar stroke with hypercholesterolemia</th>
<th>Control</th>
<th>Modality</th>
<th>Endothelial Function Method</th>
<th>Endothelial Function Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>18 (61.1 ± 7.6)</td>
<td>20 (62.7 ± 5.3)</td>
<td>TOAST</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>0.06 ± 4.9; 3.1 ± 4.8</td>
</tr>
<tr>
<td>2006</td>
<td>20 (60.9 ± 7.3)</td>
<td>20 (61.0 ± 6.2)</td>
<td>Scan-based</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>0.4 ± 5.0; 3.8 ± 4.8</td>
</tr>
<tr>
<td>2009</td>
<td>45 (60 ± 7)</td>
<td>44 (59 ± 8)</td>
<td>&quot;Typical clinical features + MRI&quot;</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>6.6 ± 4.5; 12.2 ± 4.6</td>
</tr>
</tbody>
</table>

**Single lacunar versus cortical ischemic stroke**

<table>
<thead>
<tr>
<th>Year</th>
<th>Lacunar stroke versus large artery atheroma stroke</th>
<th>Control</th>
<th>Modality</th>
<th>Endothelial Function Method</th>
<th>Endothelial Function Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>56 (67.6 ± 10.2)</td>
<td>40 (67.8 ± 8.2)</td>
<td>TOAST</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>4.3 ± 6.1; 5.7 ± 5.4</td>
</tr>
<tr>
<td>2006</td>
<td>“Chen 2” in Figure 2</td>
<td></td>
<td>TOAST</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation + deflation</td>
<td>4.3 ± 6.1; 5.6 ± 5.0</td>
</tr>
</tbody>
</table>

**Lacunar Ischemic Stroke Versus Age + Risk Factor-Matched Control Subjects**

Four papers compared vascular reactivity in patients with lacunar stroke with age + risk-factor-matched control subjects, 2 in the cerebral\(^{32,34}\) and 3 in the peripheral circulation.\(^{32,33,43}\) There was no significant difference in cerebrovascular reactivity (2 of 2 studies, 79 patients, SMD 0.08, 95% CI −0.36 to 0.53, \(P=0.71\); Figure 1) but significantly impaired peripheral vascular reactivity (3 of 3 studies, 167 patients, SMD −0.94, 95% CI −1.26 to −0.61, \(P<0.00001\); Figure 2). There was no significant heterogeneity between studies.

**Lacunar Versus Cortical Ischemic Stroke**

Four of the 16 studies (\(n=127\))\(^{37,38,42,44}\) compared cerebral vascular reactivity in patients with lacunar stroke (\(n=68\)) with patients with cortical stroke (\(n=54\)), of which 2 studies contributed to >1 comparison (Figure 1). One study (\(n=117\)) compared peripheral vascular reactivity between patients with lacu-
nar (n=56) stroke and those with cortical (n=61) stroke (Figure 2). For the cerebral comparisons, we used the test results from the asymptomatic side of the brain, because vascular reactivity was reduced in ipsilateral versus contralateral arteries in patients with cortical stroke due to tissue damage resulting from the stroke (Table). Although individual studies showed differences in endothelial function between patients with lacunar stroke and those with cortical stroke, the combined data showed no difference in vascular reactivity between lacunar and cortical stroke in either cerebral (SMD 0.29, 95% CI 0.49 to 0.19, P=0.16) or in peripheral (SMD 0.23, 95% CI 0.55 to 0.08, P=0.15) circulation. There was no significant heterogeneity between studies.

**Lacunar Ischemic Stroke With Single Versus Multiple Silent Lacunar Infarcts**

Four of the 16 papers compared cerebral vascular reactivity in patients with lacunar stroke with 1 single lacunar infarction (n=71) with those with additional multiple silent infarctions (n=71) on imaging (Figure 1). Patients with lacunar ischemic stroke plus multiple silent lacunar infarcts had reduced cerebral vascular reactivity compared with patients without silent lacunar infarcts on imaging (SMD -0.68, 95% CI -1.02 to -0.34, P=0.0001) with no significant heterogeneity between studies.

**Discussion**

Endothelial dysfunction is common in patients with symptomatic large artery atheromatous disease and has also been postulated as a mechanism underlying the development of lacunar stroke. This systematic review suggests that lacunar ischemic stroke is associated with impaired vascular reactivity compared with normal age-matched control subjects, but the association is less clearcut when compared with control subjects matched for vascular risk factors or patients with cortical ischemic stroke. One interpretation of this is that endothelial dysfunction may be a general response of the vascular system to the vascular risk factors that predispose to stroke or other circumstances associated with stroke such as secondary prevention medications rather than being specific to small or large artery disease.

A previous systematic review identified associations between lacunar stroke and altered vascular reactivity but did not perform a meta-analysis or make direct comparisons between lacunar
stroke and patients with vascular risk factors or cortical stroke control subjects.12 Endothelial function is altered in the presence of vascular risk factors9 and by drugs used for risk factor reduction and stroke prevention.10 Patients with cortical stroke control for use of medications and presence of vascular risk factors so are the most valid comparison.46 It is worth noting that the presence of an infarct in the brain was associated with reduced ipsilateral vascular reactivity, for example, patients with cortical stroke had reduced reactivity ipsilateral to the ischemic cortical stroke. Hence, any reduction in cerebral vascular reactivity in patients with multiple silent lacunar infarcts on imaging in both hemispheres (compared with patients with only a single lacunar infarct) is unsurprising and is likely to be a consequence of more brain damage.

The strengths include following well-established guidance for conducting systematic reviews of observational and diagnostic data (www.equator-network.org) and Cochrane Stroke Group search advice. We used standard prespecified criteria for study assessment. We carefully avoided duplicate data. Some studies provided comparison, but we were careful to avoid double counting the total number of subjects. In patients with stroke, we only used the cerebral vascular reactivity results from the asymptomatic side of the brain to avoid simply measuring the effects of brain damage resulting from the index stroke. We meta-analyzed the data thereby effectively increasing sample size and precision. Note that because several studies contributed to >1 comparison, some of the same patient/subject groups appear more than once in the figure; hence, the “totals” are more than the number of individuals in the whole review.

The limitations include the small number of relevant studies, their small sample size, the varied and often poorly described diagnosis of lacunar stroke, and the various and poorly standardized endothelial function tests used. In general, the papers gave little detail about how the diagnosis of lacunar stroke had been made clinically and/or with imaging, so inevitably there will be some “noise” due to imprecise diagnoses. However, given the relative lack of literature on this topic, we decided that it would be better to include studies that appeared to have included patients with symptomatic lacunar stroke because any attempt to exclude studies on the basis of their lacunar stroke diagnosis could have resulted in further bias. Studies that used suboptimal imaging, either insensitive or applied too late after the acute symptoms, may have confused up to 20% of lacunar strokes as cortical strokes and vice versa.47 It was often unclear if the investigators were blind to study group; unblinding may increase investigator bias. The endothelial function data were not adjusted for potential confounders such as blood pressure, diabetes, hypercholesterolemia, smoking, prior stroke, white matter hyperintensities on imaging, old infarcts or hemorrhages on imaging, age, or medication. Although studies generally matched with healthy control subjects for age, the lacunar and cortical stroke groups were not well age-matched. Despite many antihypertensive and stroke prevention medications being known to influence endothelial function, there was little information about current medications, most studies did not indicate if medications had been stopped before the study, and where this was mentioned, it was a very short time (eg, 12 hours35) before the endothelial function studies. Although some studies used hospital control subjects, recruitment procedures (source, mechanism) were unclear for many studies. Other limitations reflect the
limited resources available for this review, for example, we were unable to obtain translations for 3 papers that might have contained relevant data. The cerebral circulation studies used several different endothelial function tests. However, it is important to realize that the meta-analysis does not directly compare studies with each other, but rather the magnitude of association within each study with that in other studies. Therefore, the grouping of apparently different methods of assessing endothelial function is more valid than attempting to combine data from different studies that used different methods in an individual patient data meta-analysis. There is also likely to be publication bias, meaning that the present analyses are overpositive.

Is there a need for further research on endothelial function and lacunar stroke? The existing data do not exclude a specific association between endothelial dysfunction and lacunar stroke. Based on the modest difference in the cerebral circulation between lacunar and cortical patients identified in this review and the large SD of the endothelial function measurement methods, a future study would require a total sample size of 570 patients (half lacunar and half cortical) to confirm a difference in cerebral vascular reactivity of 22%, SD 20%, with 80% power at the P<0.05 level, particularly if there were to be any adjustment for even a few key potential confounding variables. If the SD could be reduced, for example, by increasing the precision of the endothelial function measurements (although ±20% is biologically very plausible and any less would be unlikely), then the sample sizes would be smaller. On the other hand, a difference of 22% is optimistic, the differences in the present studies being nearer 6%, in which case a sample size of 752 would be required. The studies to date were much smaller than this. Numerous studies report plasma markers of endothelial function (eg, asymmetrical dimethylarginine$^{12,48}$) and lacunar disease, but the identification and meta-analyses of these studies was beyond the remit of this review. There may be an association between angiotensin-converting enzyme insertion/deletion polymorphism (influencing endothelial function) and leukoaarosis, but the results of genetic association studies are awaited.

In addition to including a control group with a pathophysiological subtype of ischemic stroke, future studies should ensure optimal clinical and imaging diagnosis of stroke subtype, provide clear descriptions of their recruitment and assessment methods, ensure adequate blinding of endothelial assessments, have appropriate control subjects drawn from a relevant and comparative population, record medications, try to balance study groups for medications, preferably discontinue vasoactive drugs before study, adjust for differences in vascular risk factors, and match for age. The peripheral circulation provides a valuable method of examining systemic endothelial dysfunction outside the territory affected by the recent stroke. Cerebral small vessel disease may be a systemic small vessel problem affecting multiple organs$^{50}$ in the same way that large artery atheroma is rarely a disease of only 1 large artery, and therefore it is legitimate and necessary to study small vessel disease in multiple organs, not just the brain.

**Appendix: Search Strategy**
1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
2. (isch?emi? adj6 (stroke$^*$ or apoplex$^*$ or cerebral vasc$^*$ or cerebrovasc$^*$ or cva or attack$^*$)).tw.
3. ((brain or cerebr$^*$ or cerebell$^*$ or vertebrobasil$^*$ or hemisphere$^*$ or intracran$^*$ or intracerebral or infratentorial or supratentorial or middle cerebr$^*$ or mca$^*$ or anterior circulation$^*$) adj5 (isch?emi? or infarct$^*$ or thrombo$^*$ or emboli$^*$ or occlus$^*$ or hypoxi?$^*$)).tw.
4. 1 or 2 or 3
5. (lacun$^*$ or small vessel$^*$ or small infarct$^*$ or microinfarct$^*$ or subcortical lesion$^*$ or subcortical infarct$^*$ or microvascular$^*$ or microcirculation$^*$)).tw.
6. 4 and 5
7. blood–brain barrier/ or endothel$^*$, vascular/ or tunica intima/ or microcirculation/.
8. (endothel$^*$ adj5 (function$^*$ or dysfunction$^*$ or impairment$^*$)).tw.
9. ((vascular or capillary) adj5 endothel$^*$)).tw
10. (endothel$^*$ adj5 (contraction or relaxation$^*$)).tw
11. vascular tone/ or arterial stiff$^*$ness.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
12. (vascul$^*$ tone or neurovascul$^*$r or arterial stiff$^*$ness or vascul$^*$ remodel$^*$ or cerebrovascular reactiv$^*$ or cerebral autoregulation$^*$)).tw.
13. (Flow mediated adj3 (dilat$^*$ or vasodilat$^*$)).tw
14. exp Ultrasonography, Doppler, Transcranial/.
15. pulse wave analysis.tw.
16. strain gauge plethysmography.tw
17. (brachial artery or radial artery or popliteal artery or posterior tibial artery$^*$).tw.
18. or7 to 17
19. 6 and 18
20. limit 19 to yr="1995 to 2008"
21. limit 19 to humans
22. limit 21 to humans
23. from 22 keep 1 to 376
24. (strain gauge plethysmography or venous occlusion plethysmography$^*$).tw
25. forearm blood flow$^*$
26. (dorsal hand vein technique or aellig technique$^*$).tw
27. stimulated tPA release.tw
28. or7/24 to 27
29. 18 or 28
30. 6 and 29

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None.

**References**
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The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/41/6/e434