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

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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 disease. 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 marks 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 papers 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...
acute subcortical infarct on brain imaging; lacunes (ie, rounded cerebral spinal fluid attenuation lesion <1.5 mm in the basal ganglia, hemispheric white matter, or brain stem) identified on brain imaging without clearly relevant symptoms; or leukoaraiosis (white matter lesions).

We excluded papers that only assessed endothelial function using plasma markers, animal studies, duplicate publications, small vessel disease caused by a single gene disorder, or had no control group.

Two reviewers independently extracted data using a standardized data extraction form. A third reviewer arbitrated on disagreements. We obtained translations of foreign language papers when possible.

We extracted data on study population (sample size, age, sex, presence of comorbidities, medications, previous strokes, and selection criteria for both patient and control groups), study design, endothelial function assessment method, vascular bed, blinding of investigators, and primary vascular reactivity results. We identified the method of stroke diagnosis, whether by a stroke specialist, if confirmed using imaging, the type of imaging, and the time interval after stroke. We assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) instrument (www.equator-network.org). We were careful to include each patient population only once at the same time as including all data available for that population.

The studies used different methods of assessing endothelial function, so we calculated the standardized mean difference (SMD) by the fixed-effects method (Review Manager 4 software) between the lacunar and control groups from the mean and standard deviation (SD) of the vascular reactivity results (when given). We used the endothelial function data from the cerebral circulation contralateral to the side of the symptomatic infarct in patients with cortical and lacunar stroke. Because most studies did not adjust for potential confounders, we stratified the analyses into lacunar versus age-lacunar stroke. Because most studies did not adjust for potential confounders, we stratified the analyses into lacunar versus age-matched control subjects; versus age-+risk-factor-matched control subjects; versus age-+risk-factor-matched control subjects; versus cortical stroke; and multiple versus single lacunar infarcts on imaging. We calculated heterogeneity between studies.

Results
We identified 1257 titles. Deduplication (n=467) and exclusion of irrelevant papers (n=739) resulted in 27 includable papers. Hand-searching identified 3 additional publications. Of the 30 for full text reading, 3 were excluded (unable to translate: Russian16 and Slovakian17,18) and 11 failed to meet the inclusion criteria (no control subjects,19,20 not assessing vascular reactivity, 3 assessed both19,20 not assessing dynamic endothelial function, or inappropriate patients21–29). Therefore, 16 papers were eligible, including 974 individuals.

Characteristics of Included Studies
Some of the 16 papers contributed to >1 comparison: 14 of 16 papers30–43 compared 318 patients with recent lacunar ischemic stroke with 305 age-matched or age-+risk-factor-matched control subjects; 5 of 15 papers35,37,38,42,44 compared 124 patients with recent lacunar ischemic stroke with 115 patients with recent cortical ischemic stroke; 4 of 15 papers30,37,40,45 compared 104 patients with recent lacunar ischemic stroke of whom 71 had only 1 small single 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 little detail about recruitment or methods of stroke diagnosis (in particular of how lacunar stroke was determined), did not state duration of hypertension or other risk factors, adjust for risk factors,37 or mention prescribed medications (only 2 stated that medications were discontinued before the study35,40). Only 2 studies explicitly stated that the analyses were blinded to subject group. Ten 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 neurologic 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,32,33,36,38,41 and 3 excluded middle cerebral artery (MCA) stenosis35,39,40 from the lacunar stroke group.

Thirty-six studies had age-matched medically confirmed normal control subjects; 5 of 13 also used imaging to exclude subjects with silent infarcts.30,32,39,40,42 Three studies recruited age- and risk-factor matched control subjects (with longstanding hypertension and hypercholesterolemia in 232,34, with hypertension only in 143). Five papers compared patients with lacunar ischemic stroke with patients with cortical ischemic stroke,55,37,38,42,44 confirming the infarct subtype with CT or MRI. Four studies included patients with >1 lacunar infarct on CT,30,37,40,45 MR,30,45 or both.37

Assessment of Endothelial Function
Twelve papers assessed endothelial function in the cerebral circulation30–32,34,37–42,44,45 and 5 papers the systemic circulation32,33,36,43; 1 assessed both.32 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,31 percent increase in regional cerebral blood flow in response to hypercapnia using stable Xenon CT,30 and “dynamic cerebral autoregulation” (the ability to restore cerebral blood flow after sudden changes in perfusion pressure)38 were also used. Peripheral endothelial function was assessed with brachial artery flow-mediated dilatation, expressed as the percentage change in arterial diameter, in 5 papers32,33,35,36,43; 1 also assessed endothelium-independent flow-mediated dilatation using response to sublingual nitroglycerine.43

Endothelial Function: Lacunar Stroke Versus Age-Matched Control Subjects
Thirteen studies (n=534) compared lacunar ischemic stroke with healthy age-matched control subjects, 9 in the cerebral (n=360)30–32,34,37,39,42 and 4 in the peripheral circulation (n=211).32,33,35,36 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>Control No. Details</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>
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<tr>
<td>Single lacunar stroke versus normal age-matched control subjects</td>
<td>2006</td>
<td>Lacunar stroke with hypercholesterolemia</td>
<td>18 (61.1 ± 7.6)</td>
<td>Age-matched</td>
<td>TOAST</td>
<td>CT</td>
<td>Percent increase in mean MCA blood flow velocity on TCD after L-arginine</td>
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<tr>
<td>Pretnar-Oblak et al (a)32</td>
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<tr>
<td>Pretnar-Oblak et al (c)34</td>
<td>2006</td>
<td>Lacunar stroke</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>
</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>Not stated</td>
<td>CT</td>
<td>Absolute percent increase in rCBF of white matter after acetazolamide</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 flow velocity on TCD after acetazolamide</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 flow velocity on TCD after acetazolamide (%)</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>Response to hypercapnia cm/sec/kPA</td>
</tr>
<tr>
<td>Cupini et al37</td>
<td>2001</td>
<td>Lacunar stroke</td>
<td>14 (61.4 ± 9.2)</td>
<td>15 (57.6 ± 12.7)</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>
</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>
</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>
</tr>
<tr>
<td>Single lacunar stroke versus age and risk factor-matched control subjects</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>
</tr>
<tr>
<td>Pretnar-Oblak et al (a)32</td>
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</tr>
<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. Continued

<table>
<thead>
<tr>
<th>Study 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>Lacunar Subjects</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Laminal stroke versus normal age-matched control</td>
<td>24 (60.5 ± 13.7)</td>
<td>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>25.6 ± 12.2</td>
<td>12.2 ± 15.9</td>
</tr>
<tr>
<td>1993</td>
<td>Lacunar infarction</td>
<td>20 (59.6 ± 5.8)</td>
<td>8 (53.5 ± 9.5)</td>
<td>NINDS</td>
<td>CT/MRI</td>
<td>Mean spatial Doppler frequency—A exp(k frequency/Hz) response to CO2</td>
<td>0.072 ± 0.004</td>
<td>0.027 ± 0.004</td>
</tr>
<tr>
<td>2001</td>
<td>Lacunar infarction</td>
<td>14 (61.4 ± 9.2)</td>
<td>53.9 ± 11.8</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>dCA: delay of MCA vmean counterregulation during changes in rCBF in seconds</td>
<td>1.36 ± 0.39</td>
<td>1.45 ± 0.51</td>
</tr>
<tr>
<td>2005</td>
<td>Lacunar infarction</td>
<td>10 (63 ± 3)</td>
<td>59 ± 5</td>
<td>Not stated</td>
<td>CT/MRI</td>
<td>Passive followed MAP</td>
<td>Not detectable</td>
<td>4.6 ± 0.7 (%)</td>
</tr>
<tr>
<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>Percent increase in mean MCA flow velocity after acetazolamide</td>
<td>29.6 ± 28.2</td>
<td>35.5 ± 17.6</td>
<td></td>
</tr>
<tr>
<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>Percent increase in mean MCA flow velocity after acetazolamide</td>
<td>46.36 ± 12.6</td>
<td>54.83 ± 11.58</td>
<td></td>
</tr>
<tr>
<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</td>
<td>5.0 ± 3.4</td>
<td>7.3 ± 6.0</td>
</tr>
<tr>
<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>Breath holding</td>
<td>0.90 ± 0.36</td>
<td>1.36 ± 0.39</td>
</tr>
</tbody>
</table>

#### (B) Peripheral circulation

<table>
<thead>
<tr>
<th>Study 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>Lacunar Subjects</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Lacunar stroke + single or multiple infarcts on imaging</td>
<td>18 (61.1 ± 7.6)</td>
<td>59.2 ± 7.1</td>
<td>TOAST</td>
<td>CT</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation</td>
<td>0.06 ± 4.9</td>
<td>8.1 ± 6.0</td>
</tr>
<tr>
<td>2006</td>
<td>Lacunar stroke</td>
<td>20 (69.0 ± 7.3)</td>
<td>59.5 ± 7.3</td>
<td>Scan-based CT</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter, after cuff inflation + deflation</td>
<td>0.4 ± 5.0</td>
<td>7.9 ± 6.0</td>
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</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 Results</th>
<th>Endothelial Function Method</th>
<th>Lacunar</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al(^{35})</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</td>
<td>8.8±6.0</td>
<td></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</td>
<td>6.7±2.5</td>
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</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 carotid artery diameter after CO(_{2})</td>
<td>4.0±1.0</td>
<td>11.4±2.7</td>
<td></td>
</tr>
<tr>
<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>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation - deflation</td>
<td>0.06±4.9</td>
<td>3.1±4.8</td>
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<tr>
<td>Pretnar-Oblak et al (b)(^{33})</td>
<td>2006</td>
<td>Lacunar stroke</td>
<td>20 (60.9±7.3)</td>
<td>20 (61.0±6.2)</td>
<td>Scan-based</td>
<td>CT</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation - deflation</td>
<td>0.4±5.0</td>
<td>3.8±4.8</td>
<td></td>
</tr>
<tr>
<td>Kim et al(^{43})</td>
<td>2009</td>
<td>Lacunar ischemic stroke</td>
<td>45 (60±7)</td>
<td>44 (59±8)</td>
<td>MR</td>
<td>MR</td>
<td>Flow-mediated dilatation: percent increase in brachial artery diameter after cuff inflation - deflation</td>
<td>6.6±4.5</td>
<td>12.2±4.6</td>
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</tr>
<tr>
<td>Stevenson et al Endothelial Dysfunction in Lacunar Stroke 5</td>
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<td></td>
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<td>14.3±4.9()†</td>
<td>13.8±4.9()‡</td>
</tr>
<tr>
<td>Single lacunar versus age + risk factor-matched control subjects</td>
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<tr>
<td>Single lacunar versus cortical ischemic stroke</td>
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<tr>
<td>Chen et al(^{35})</td>
<td>2006</td>
<td>Lacunar stroke versus large artery atheroma stroke</td>
<td>56 (67.6±10.2)()§</td>
<td>40 (67.8±8.2)</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</td>
<td>5.7±5.4</td>
<td></td>
</tr>
<tr>
<td>Chen et al(^{35}) \“Chen 2\” in Figure 2</td>
<td>2006</td>
<td>Lacunar stroke versus cardioembolic stroke</td>
<td>56 (67.6±10.2)()§</td>
<td>21 (67.6±9.2)</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</td>
<td>5.6±5.0</td>
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</tbody>
</table>

*Includes studies evaluating dynamic cerebral and peripheral endothelial function in patients with lacunar stroke and control subjects.
†For back to back blood pressure changes.
‡For flow-mediated dilatation in response to sublingual nitroglycerine (endothelium-independent dilatation).
§Note that there are 56 patients with lacunar stroke in total in Chen 2006, not 56×3.
NA indicates not available; TOAST, Trial Of Org 10172 In Acute Stroke Treatment; NINDS, National Institute of Neurological Diseases and Stroke; TCD, transcranial Doppler; rCBF, regional cerebral blood flow; BA, basilar artery; PET, positron emission tomography; MFF, mean flow velocity; fMRI, functional MRI; BOLD, blood oxygen level-dependent; MAP, mean arterial pressure; IV, intravenous; BHI, breath holding index; dCA, dynamic cerebral autoregulation.

### 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.\(^{32,33,34}\) 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-
Table 1. Forest plot showing SMD in cerebrovascular reactivity in patients with lacunar ischemic stroke versus age-matched, age + risk factor matched and cortical ischemic stroke control subjects and in patients with lacunar ischemic stroke with versus without multiple silent lacunar infarcts. Squares represent the ratio of the lacunar response divided by the control response with the solid black lines representing the 95% CI. The diamond represents the summary result. Note that because some 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.

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 clear cut 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 hours15) 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 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 leukoaraiosis, but the results of genetic association studies are awaited.

In addition to including a control group with a pathophysiologically different 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 assess methods, ensure adequate blinding of endothelial subtype, provide clear descriptions of their recruitment and should ensure optimal clinical and imaging diagnosis of stroke. Because 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.

Appendix: Search Strategy

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
2. ischemi$ adj6 (stroke$ or apoplexi$ or cerebral vasc$ or cerebrovasc$ or eva or attack$).tw.
3. brain or cerebr$ or cerebell$ or vertebrobas$ or hemisphere$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circula$) adj5 (ischemi$ 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 microvascular$ 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. endotheli$ adj5 (contraction or relaxation).tw
11. vascular tone/ or arterial stiffness.mp. [mp=title, original title, abstract, name of substance word, subject heading word]
12. vascul$ tone or neurovascul$ coupl$ or arterial stiff$ or vascul$ remodel$ or cerebrovascular reactiv$ or cerebral autoregulation).tw.
14. exp Ultrasonography, Doppler, Transcranial/
15. pulse wave analysis.tw.
16. strain gauge plethysmography.tw.
17. brachial artery or radial artery or popiteal artery or posterior tibial artery.tw.
18. or/7 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.tw.
26. (dorsal hand vein technique or aellig technique).tw.
27. stimulated tPA release.tw.
28. or/24 to 27
29. or/18 or 28
30. 6 and 29

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Disclosures

None.

References

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