Retinal Vascular Caliber and Brachial Flow-Mediated Dilation
The Multi-Ethnic Study of Atherosclerosis

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Background and Purpose—Retinal vascular caliber changes have been shown to predict stroke, but the underlying mechanism of this association is unknown. We examined the relationship between retinal vascular caliber with brachial flow-mediated dilation (FMD), a measure of systemic endothelial function.

Methods—The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based study of persons 45 to 84 years of age residing in 6 US communities free of clinical cardiovascular disease at baseline. Brachial FMD data were collected at baseline (July 2000 to June 2002), and retinal vascular caliber was measured from digital retinal photographs at the second examination, immediately after the first (August 2002 to January 2004). Data were available for 2851 participants for analysis.

Results—The mean brachial FMD was 4.39 ± 2.79%. After adjusting for age and gender, brachial FMD was reduced in persons with wider retinal venular caliber (changes in FMD −0.25, 95% CI −0.36, −0.13; P < 0.001, per SD increase in venular caliber). This relationship persists after adjusting for systolic blood pressure, serum total cholesterol, use of lipid-lowering and antihypertensive medication, body mass index, current smoking status, and hemoglobinA1C (−0.18; 95% CI −0.30, −0.06; P = 0.004, per SD increase in venular caliber). Brachial FMD was not associated with retinal arteriolar caliber.

Conclusions—Persons with wider retinal venules have reduced brachial FMD, independent of other vascular risk factors. This suggests that retinal venular caliber, previously shown to predict stroke, may be a marker of underlying systemic endothelial dysfunction. (Stroke. 2010;41:1343-1348.)

Key Words: epidemiology ■ vasodilation ■ imaging ■ endothelial function

Precise measurement of retinal vascular caliber changes are now possible with digital retinal photography and new imaging software. Recent population-based studies have shown that changes in retinal vascular caliber, particularly wider venules, may predict stroke and other cardiovascular events. For example, wider retinal venules are associated with carotid artery disease, MRI-detected lacunar infarcts and white matter lesions, and incident clinical stroke, as well as incident coronary heart disease events and deaths.

Despite these data, the underlying mechanisms of these associations are unknown. It has been suggested that retinal vascular caliber changes are markers of systemic endothelial dysfunction, but the few studies that have examined this with indirect systemic markers of endothelial function have shown inconsistent results. Brachial flow-mediated dilation (FMD) is a validated, noninvasive physiological measure to quantify endothelial function. Brachial FMD has been shown previously to be abnormal in persons with type 1 and type 2 diabetes and in those with diabetic microvascular complications. Brachial FMD is linked with various cardiovascular risk factors and has been demonstrated to predict future cardiovascular disease events and mortality.

We hypothesize that retinal vascular caliber may reflect systemic endothelial dysfunction and that this may be a potential mechanism of the associations of retinal vascular caliber and stroke. A previous study in a small population (n = 256, with 52 having missing FMD) did not find an association between retinal vascular caliber and brachial FMD. In the current study, we assessed this association in a larger, multiethnic, population-based sample.
Methods

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) was a prospective cohort study of men and women 45 to 84 years of age comprising 4 racial/ethnic groups (whites, blacks, Hispanics, and Chinese). Participants had no history of clinical cardiovascular disease at baseline and were residents of 6 US communities. Tenets of the Declaration of Helsinki were followed, and institutional review board approval was granted at each study site. Written informed consent was obtained from each participant.

There were 6814 participants at the first/baseline examination. FMD data were available from the first examination (July 2000 to August 2002) for 5 of 6 centers. Retinal photography was done in 6176 persons at the second examination (August 2002 to January 2004), which followed the baseline examination by 18 months on average. Exclusion criteria included a systolic blood pressure (BP) of 150 mm Hg or BP ≥150 mm Hg if BP was ≥150 mm Hg. Images of the right brachial artery were captured continuously for 120 seconds after cuff deflation. Videotapes of the acquired images of the brachial artery were analyzed at the Wake Forest University cardiology image processing laboratory using a previously validated semiautomated system (by D.H.). The readings of these digitized images generated the baseline and maximum diameters of the brachial artery from which the absolute change from baseline diameter and percentage (%) brachial FMD was computed. FMD was computed with the formula: maximum diameter−baseline diameter×100%/baseline diameter. Intraobserver reproducibility for baseline diameter, maximum diameter, and %FMD was evaluated by comparing an original and a blinded quality control reread of ultrasounds from 40 MESA participants (32 male, 18 white, 2 Chinese, 10 black, and 10 Hispanic subjects). The intraclass correlation coefficients were 0.99, 0.90, and 0.93, respectively. Intrasubject variability was evaluated by comparing results from repeated examinations of 19 subjects on 2 days a week apart. The intraclass correlation coefficients for baseline diameter, maximum diameter, and %FMD were 0.90, 0.90, and 0.54, respectively. Percent technical error of measurement was 1.39% for baseline diameter measurement, 1.47% for maximum diameter measurement, and 28.4% for %FMD measurement.

Measurement of Retinal Vascular Caliber

Retinal photography was performed using a standardized protocol. Both eyes of each participant were photographed using a 45-degree 6.3 megapixel digital nonmydriatic camera. Two photographic fields (optic disc and macula) were taken of each eye. Images were sent from the 6 field centers to the Ocular Epidemiology Reading Center at the University of Wisconsin, Madison, for measurement of retinal vascular caliber.

Retinal vascular caliber was measured using a computer-based program by trained graders who were masked to participant characteristics, based on a detailed protocol. Photographs in the right eye were selected for measurement; the left eye was chosen if measurements could not be performed in the right eye. For each image, all arterioles and venules coursing through an area one-half to one-disc diameter from the optic disc margin were measured and summarized as the central retinal artery equivalent and central retinal

Table 1. Characteristics of the MESA Study Population (n=2851)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Excluded, n=3140</th>
<th>Included, n=2851</th>
<th>P*</th>
<th>White, n=981</th>
<th>Black, n=595</th>
<th>Hispanics, n=706</th>
<th>Chinese, n=569</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male</td>
<td>1438 (45.8%)</td>
<td>1428 (50.1%)</td>
<td>0.001</td>
<td>504 (51.4%)</td>
<td>288 (48.4%)</td>
<td>345 (48.9%)</td>
<td>291 (51.1%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hypertension, present</td>
<td>1584 (50.4%)</td>
<td>1331 (46.7%)</td>
<td>0.004</td>
<td>428 (43.6%)</td>
<td>354 (59.5%)</td>
<td>325 (46.0%)</td>
<td>224 (39.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, present</td>
<td>430 (13.7%)</td>
<td>358 (12.6%)</td>
<td>0.20</td>
<td>52 (5.3%)</td>
<td>106 (17.8%)</td>
<td>119 (16.9%)</td>
<td>81 (14.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current cigarette smoker</td>
<td>382 (12.2%)</td>
<td>310 (10.9%)</td>
<td>0.12</td>
<td>106 (10.8%)</td>
<td>103 (17.3%)</td>
<td>72 (10.2%)</td>
<td>29 (5.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.8 ± 10.1</td>
<td>62.6 ± 9.8</td>
<td>&lt;0.001</td>
<td>63.0 ± 9.6</td>
<td>62.7 ± 9.8</td>
<td>61.7 ± 9.7</td>
<td>62.7 ± 9.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>124.9 ± 21.3</td>
<td>123.2 ± 20.1</td>
<td>0.002</td>
<td>120.7 ± 18.7</td>
<td>128.8 ± 20.3</td>
<td>124.3 ± 20.5</td>
<td>120.5 ± 20.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>191.7 ± 36.6</td>
<td>191.3 ± 34.7</td>
<td>0.68</td>
<td>192.0 ± 34.5</td>
<td>187.7 ± 34.7</td>
<td>194.6 ± 37.5</td>
<td>189.6 ± 31.0</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.8 ± 5.6</td>
<td>27.9 ± 5.3</td>
<td>&lt;0.001</td>
<td>27.9 ± 5.0</td>
<td>29.6 ± 5.7</td>
<td>29.6 ± 5.2</td>
<td>24.0 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HemoglobinA1c, %</td>
<td>5.72 ± 0.98</td>
<td>5.70 ± 0.98</td>
<td>0.55</td>
<td>5.45 ± 0.60</td>
<td>5.90 ± 1.15</td>
<td>5.86 ± 1.22</td>
<td>5.74 ± 0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline brachial diameter, mm</td>
<td>. . . . . .</td>
<td>4.32 ± 0.83</td>
<td>. . . .</td>
<td>4.19 ± 0.85</td>
<td>4.48 ± 0.84</td>
<td>4.43 ± 0.83</td>
<td>4.27 ± 0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brachial FMD, %</td>
<td>4.75 ± 2.99</td>
<td>3.49 ± 2.46</td>
<td>4.38 ± 2.67</td>
<td>4.72 ± 2.72</td>
<td>4.01 ± 2.92</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were obtained during first examination, from July 2000 to August 2002.

*P value based on χ² (categorical), independent sample t test (quantitative) to compare characteristics of excluded and included participants and across the races.
vein equivalent. These equivalents were projected calibers for the central retinal vessels, measured away from the optic disc. Reproducibility of these measurements had been reported, with intragrade and intergrader intraclass–correlation coefficients ranging from 0.78 to 0.99.

**Assessment of Cardiovascular Risk Factors**

A detailed questionnaire was used to obtain participant information, including past medical history, alcohol intake, and cigarette smoking. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/L (126 mg/dL) or history of diabetes and the use of insulin or oral hypoglycemic medications (13). Hypertension was defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or current use of antihypertensive medications. Height and weight were measured to determine BMI, defined as kilograms per meter squared (weight/height). Fasting (>8 hours) blood samples were drawn from participants, and aliquots were prepared for central analysis. We used total cholesterol, high-density and low-density lipoprotein cholesterol, and high sensitive C-reactive protein, and hemoglobinAIC in this analysis.

**Statistical Analysis**

Retinal vascular caliber was categorized into quartiles for analysis and also analyzed as a continuous variable (per 1-SD changes in retinal arteriolar and venular caliber). We used ANCOVA to estimate mean FMD for quartiles of arteriolar and venular caliber and linear regression to determine the change in FMD per SD change in arteriolar and venular caliber, adjusting for age, gender, race/ethnicity, and cardiovascular risk factors, including total cholesterol, systolic BP, use of lipid-lowering and antihypertensive medication, BMI, current smoking status, and hemoglobinAIC. We examined potential interaction by age (>65 years and <65 years), gender, race/ethnicity, diabetes, and hypertension. In models for arteriolar caliber, we adjusted for venular caliber (and vice versa) to account for potential confounding from fellow vascular caliber. All analyses were performed in SPSS version 16.0.1 (SPSS Inc).

**Results**

Selected characteristics and risk factors for each of 4 races/ethnicity: whites (n=981), blacks (n=595), Hispanics (n=706), and Chinese (n=569), among participants who have brachial FMD and retinal photographs (n=2851) are shown in Table 1. Whites have lower prevalence of diabetes, higher brachial FMD, and lower baseline brachial artery diameter compared with nonwhites. In addition, those who have FMD performed have a lower proportion of hypertension, and a higher proportion is male, as well as lower systolic BP and BMI (Table 1).

Brachial FMD is reduced in persons with wider retinal venular caliber (Table 2). Brachial FMD is 0.25% lower (95% CI, −0.36, −0.13; P<0.001) per SD increase in venular caliber. This relationship persists after adjusting for serum total cholesterol, systolic BP, use of lipid-lowering and antihypertensive medication, BMI, current smoking, and hemoglobinAIC: brachial FMD is lower by 0.18% (95% CI, −0.30, −0.06; P=0.004) per SD increase in venular caliber. Additional adjustment for time between examinations, diabetes status, alcohol consumption, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol, or C-reactive protein does not change the associations (data not shown). There is no association between arteriolar caliber and brachial FMD after multivariable adjustment.

Table 2 also shows an association between wider venular caliber and baseline brachial artery diameter (0.049 mm; 95% CI, 0.020 to 0.077; P=0.001) in models of retinal arteriolar caliber (CRAE); and CRAE in models of CRVE; †Model 2: adjusted for variables in model 1 plus total cholesterol, systolic blood pressure, use of lipid-lowering and antihypertensive medication, BMI, current smoking status, and hemoglobinAIC in this analysis.

<table>
<thead>
<tr>
<th>CRAE</th>
<th>Brachial FMD, % Mean±SD (n=2851)</th>
<th>Baseline Brachial Artery Diameter, mm Mean±SD (n=2851)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>†</td>
</tr>
<tr>
<td>First quartile, &lt;135 µm</td>
<td>4.24±2.94 (0.02)</td>
<td>4.35±4.80 (0.03)</td>
</tr>
<tr>
<td>Second quartile, 135–144 µm</td>
<td>4.29±2.67 (0.02)</td>
<td>4.31±4.81 (0.03)</td>
</tr>
<tr>
<td>Third quartile, 144–153 µm</td>
<td>4.50±2.66 (0.02)</td>
<td>4.31±4.79 (0.03)</td>
</tr>
<tr>
<td>Fourth quartile, &gt;153 µm</td>
<td>4.48±2.89 (0.02)</td>
<td>4.34±4.73 (0.03)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>Per SD decrease</td>
<td>−0.13 (−0.25, 0.02)</td>
<td>−0.054 (−0.13, 0.07)</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.38</td>
</tr>
<tr>
<td>CRVE</td>
<td>First quartile, &lt;200 µm</td>
<td>4.44±2.92 (0.02)</td>
</tr>
<tr>
<td>Second quartile, 200–214 µm</td>
<td>4.41±2.69 (0.02)</td>
<td>4.48±3.50 (0.03)</td>
</tr>
<tr>
<td>Third quartile, 214–228 µm</td>
<td>4.47±2.66 (0.02)</td>
<td>4.50±3.46 (0.03)</td>
</tr>
<tr>
<td>Fourth quartile, &gt;228 µm</td>
<td>4.17±2.90 (0.02)</td>
<td>4.15±3.43 (0.03)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Per SD increase</td>
<td>−0.25 (−0.36, −0.13)</td>
<td>−0.18 (−0.30, −0.06)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>
increase in brachial artery diameter per SD decrease in arteriolar caliber). The significant associations disappear after adjustment for baseline brachial diameter (data not shown).

Table 3 shows stratified analysis of the association of retinal venular caliber with brachial FMD, stratified by diabetes and hypertension status, and ethnicity/race, adjusting for serum total cholesterol, systolic BP, use of lipid-lowering and antihypertensive medication, BMI, current smoking status, and hemoglobinA1C. Brachial FMD is generally lower in persons with wider venular caliber quartile (fourth quartile compared with other 3 quartiles), in those with and without diabetes or hypertension and in the 4 ethnic/racial groups. Interaction terms for diabetes, hypertension, and ethnicity/race are not significant (data not shown). The associations between brachial FMD or baseline brachial diameter and retinal arteriolar caliber are no longer statistically significant, except between arteriolar caliber and brachial FMD in those with hypertension (3.83% ± 3.48 versus 4.16% ± 4.25; P = 0.04; comparing narrowest first quartile versus second with fourth quartiles; data not shown).

### Discussion

In this population-based study of persons free of clinical cardiovascular disease, we show that wider retinal venular caliber is associated with reduced brachial FMD, independent of traditional cardiovascular risk factors. Our study suggests that retinal venular caliber may reflect underlying systemic endothelial dysfunction, and that this may provide a novel explanation of why retinal venular caliber predicts incident stroke and other cardiovascular events.

To our best knowledge, there has only been one published study with which to compare our findings. An analysis from the Hoorn study of 256 persons 60 to 85 years of age (of 631 eligible, with 6 missing retinal photographs and 52 missing FMD) showed that after controlling for age, sex, glucose tolerance, baseline diameter, and increase in peak systolic velocity, wider venules were associated with reduced brachial FMD, although this was not statistically significant.

Our study findings may provide additional insights into previously demonstrated associations between retinal venular caliber and a range of cardiovascular risk factors and diseases. Wider venules have been shown to associate with carotid artery disease, MRI-detected lacunar infarcts and white matter lesions, and incident clinical stroke, as well as incident coronary heart disease events and deaths. Further, wider retinal venules have been linked with the metabolic syndrome, serum markers of inflammation, and other markers of atherosclerosis, such as lower ankle–arm index, higher carotid plaque score, and increased aortic calcification, as well as reduced small–artery compliance, which have been suggested to also reflect endothelial dysfunction.

Our study suggests that variation in retinal venules may reflect underlying systemic endothelial dysfunction.

In addition, we also found association of larger baseline brachial artery diameter with wider retinal venules. Larger baseline brachial artery diameter has been found to be predictive of cardiovascular events in the Cardiovascular Health Study, and its predictive value is similar to that of brachial FMD. In addition, larger baseline brachial artery is associated with narrower retinal arterioles in our study. Narrower retinal arterioles are associated with risk of type 2 diabetes, hypertension, incident coronary heart disease events, and deaths. Additional research is needed to clarify the significance of these relationships with brachial artery diameter.

The strengths of this study include a large population-based sample and the use of quantitative measures of retinal vascular caliber and FMD. Limitations of this study should also be noted. First, the cross-sectional nature of the study limits our ability to judge temporal sequence of associations. Second, FMD and retinal photography were not done at the same visit. This is important because brachial FMD and retinal vascular caliber may fluctuate and be influenced by physiological factors. Further, this could have distorted the associations between FMD and retinal vessel measurements, and this may explain why no relationship was found between FMD and arteriolar caliber. Adherence to guidelines for measuring brachial artery reactivity minimizes but does not eliminate the variability of brachial FMD. Third, there were a proportion of MESA participants with no FMD data, and
may bias our results because those who had FMD performed have a lower proportion of hypertension, there was a higher proportion of males, and lower systolic BP and BMI. Fourth, in our analysis, there was a much greater difference of FMD between retinal venular categories in whites than for other racial/ethnic groups. Therefore, we are uncertain whether the association between wider retinal venules and reduced FMD would be apparent with a larger sample of the nonwhite ethnic groups. In addition, the significant relationships disappear after additional adjustment of baseline brachial FMD. From our analysis, we are unable to determine whether this is attributable to statistical overadjustment because the percentage of brachial FMD is also computed from brachial diameter or changes in brachial diameter being an inverse predictor of FMD. Finally, it was not possible to perform endothelium-independent vasodilation with nitroglycerin in a population-based study, so although brachial FMD is associated with retinal vascular caliber, we cannot be certain that the relationship is entirely attributable to endothelium-dependent vasodilation.

Summary

In conclusion, in this large population-based study, wider retinal venular caliber is associated with reduced brachial FMD, independent of traditional cardiovascular risk factors. Our study provides the first line of evidence to suggest that retinal venular caliber may reflect systemic endothelial function, and that this may explain the relationship between changes in retinal venular caliber and incident stroke and other cardiovascular disease. If supported by additional research, quantitatively measured retinal vascular caliber may be a novel, noninvasive measure of systemic endothelial function.

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Disclosures

None.

References


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