Intima-Media Thickness of Brachial Artery, Vascular Function, and Cardiovascular Risk Factors

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Objective—Cardiovascular diseases are associated with impaired flow-mediated vasodilation (FMD) and increase in carotid intima-media thickness (IMT). Both FMD and IMT are independent predictors for cardiovascular outcomes. When measuring FMD and nitroglycerine-induced vasodilation in the brachial artery, IMT can also be simultaneously assessed in the same brachial artery. The purpose of this study was to determine the relationships between IMT of the brachial artery, vascular function, and cardiovascular risk factors.

Methods and Results—We measured brachial IMT, FMD, and nitroglycerine-induced vasodilation by ultrasound in 388 subjects who underwent health examination (mean age, 45±22 years; age range, 19–86), including patients with cardiovascular diseases. Univariate regression analysis revealed that brachial IMT significantly correlated with age (r=0.71; P<0.001), body mass index (r=0.27; P<0.001), systolic blood pressure (r=0.40; P<0.001), diastolic blood pressure (r=0.31; P<0.001), heart rate (r=0.15; P=0.002), glucose level (r=0.18; P=0.01), and smoking pack-years (r=0.42; P<0.001), as well as Framingham risk score, a cumulative cardiovascular risk index for heart attack (r=0.49; P<0.001). FMD and nitroglycerine-induced vasodilation were inversely associated with brachial IMT (r=−0.39, P<0.001; r=−0.32, P<0.001, respectively). In addition, there was a significant relationship between brachial IMT and carotid IMT (r=0.58; P<0.001). Multivariate analysis revealed that age, sex, hypertension, and brachial artery diameter were independent predictors of brachial IMT.

Conclusion—These findings suggest that brachial IMT may be a marker of the grade of atherosclerosis and may be used as a marker of vascular function, providing additive information for stratifying subjects with cardiovascular risk factors. (Arterioscler Thromb Vasc Biol. 2012;32:XX-XX.)

Key Words: endothelial function ■ risk factors ■ brachial artery ■ intima-media thickness

Recently, measurement of flow-mediated vasodilation (FMD) as an index of endothelium-dependent vasodilation and nitroglycerine-induced vasodilation as an index of endothelium-independent vasodilation in the brachial artery using high-resolution ultrasound has been widely used as a method for assessing vascular function.1–8 Endothelial function is initially impaired in the pathogenesis of atherosclerosis.9 Measurement of FMD is noninvasive and reflects NO production. In addition, growing evidence has shown that endothelial function assessed by FMD can serve as an independent predictor of cardiovascular events.10–14 It has been reported that nitroglycerine-induced vasodilation is impaired in subjects with cardiovascular risk factors and coronary heart disease.7,8 However, measurement of intima-media thickness (IMT) in the artery is established as an index of structural change in the artery. IMT is one of the manifestations of atherosclerosis and is usually assessed in the carotid and femoral arteries.15–17 Several lines of evidence suggest that IMT, especially carotid IMT, is associated with cardiovascular risk factors18 and is a predictor of cardiovascular outcomes.19–21 A previous study using an autopsy method clearly showed that atherosclerosis is a frequent finding even in the brachial artery and is associated with coronary and carotid atherosclerotic lesions.22 However, there is little information on whether IMT of the brachial artery can serve as a surrogate marker for progression of atherosclerosis. In addition, the impact of vascular function on structural arterial change has been investigated in several studies.23–26 Unfortunately, different vascular beds, the brachial artery for FMD, and common carotid artery for IMT were examined in these studies.

Ultrasound examination of the brachial artery may provide an opportunity for direct assessment of the interrelation

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between vascular function and structural arterial change in the same vessel. Indeed, measurements of FMD and nitroglycerine-induced vasodilation using ultrasound enables simultaneous assessment of IMT in the same vessel. We, therefore, investigated the relationships among brachial IMT, FMD, nitroglycerine-induced vasodilation, and conventional cardiovascular risk factors in a general population of subjects who underwent a health-screening examination, including healthy subjects and patients with cardiovascular diseases (CVDs).

**Patients and Methods**

**Subjects**

A total of 388 subjects (mean age, 45±22 years; age range, 19–86) were recruited from people who underwent health-screening examinations at Hiroshima University Hospital. All employees have an obligation to undergo health screening every year under the regulation of the society–managed health insurance union in Japan. In accordance with its regulation, we performed health-screening examinations at our institute. Hypertension was defined as systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg, in a sitting position, on at least 3 different occasions. Patients with secondary forms of hypertension were excluded in all patients with hypertension on the basis of complete history; physical examination; radiological and ultrasound examinations; urinalysis; plasma renin activity; plasma aldosterone and norepinephrine concentrations; serum creatinine, potassium, calcium, and free thyroxine concentrations; and 24-hour urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillylmandelic acid. Diabetes mellitus was defined according to the American Diabetes Association. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program. We defined smokers as those who had ever smoked. One pack-year was equivalent to 20 cigarettes per day for 1 year. Coronary heart disease, included angina pectoris, myocardial infarction, and unstable angina. Unstable angina was designated when a history of prolonged ischemic chest pain (>15 minutes in duration) was accompanied by transient ischemic ST segment and T-wave abnormality in the electrocardiographic tracing but not accompanied by development of Q-wave abnormality or by serum enzyme changes characteristic of myocardial necrosis. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. Healthy subjects had no history of cardiovascular and cerebrovascular diseases, liver diseases, renal diseases, autoimmune diseases, or malignant diseases and had no coronary risk factors, including hypertension, dyslipidemia, diabetes mellitus, and smoking. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, high-density lipoprotein cholesterol level, systolic blood pressure, and smoking status. The ethical committees in our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects.

**Figure 1.** A, Analysis displays show vessel images of B-mode and A-mode of the brachial artery. Intima-media thickness (IMT), defined as the distance between the starting point of the first curve, indicating the intimal interface, and that of the second curve, indicating the media-adventitia interface, on the A-mode image of the far wall depicted in the middle and right displays, was measured automatically. A total of 21 points over the 3-mm length of IMT were measured and the mean value of IMT in each of the 10 images was automatically calculated. The average of mean values, defined as brachial IMT, was automatically calculated with exclusion of the outliers (gray panels). B, The scheme shows a closeup of analysis displays and annotated anatomic landmarks for measurement of IMT.
Study Protocol

We measured IMT and vascular responses to reactive hyperemia in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 hours. The study began at 8:30 AM. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22°C–25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the prone position, basal brachial artery diameter and IMT were measured. Then FMD and nitroglycerine-induced vasodilation were measured. The observers were blind to the form of examination.

Measurement of Brachial IMT

Before FMD measurement, baseline longitudinal ultrasonographic images of the brachial artery, obtained at the end of diastole (defined as the R wave of an electrocardiogram) from each of 10 cardiac cycles, were automatically stored on a hard disk for off-line assessment of IMT with a linear, phased-array high-frequency (10-MHz) transducer using an UNEXEF18G ultrasound unit (UNEX Co, Nagoya, Japan) (Figure 1). Measurement of IMT was automatically performed on A-mode images of the far wall of the brachial artery. The analysis system automatically chose the measurement point where an image of the posterior intimal interface was clearly obtained. If the measurement point was inappropriate, another clear image site could be manually selected for measurement. A total of 21 points over a 3-mm length of IMT in the 10-mm longitudinal image depicted in the analysis display were measured and the mean value per image was automatically calculated. IMT was measured at the same point in each image. The average of mean values obtained from 10 cardiac cycles was defined as IMT of the brachial artery.

When measuring carotid IMT, we had an anatomical landmark, such as the carotid-artery bulb. Unfortunately, it is difficult to measure the same site of the brachial artery attributable to the lack of an anatomical landmark. Measurement of IMT in the brachial artery was performed at the proper site where the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained at 5 to 10 cm above the elbow. However, there was little influence of intra- and interpatient variability in the measurement location of the brachial artery at 5 to 10 cm above the elbow in the present study, because the interface on the intima media of brachial artery is relatively smooth and intima-media thickening is not localized or plaques are not presented, resulting in diffuse intima-media thickening. The coefficients of variation of intra- and interobserver brachial IMT measurements were 3.1% and 4.0%, respectively.

Measurement of Carotid IMT

The ultrasound unit Aloka-α7 (Aloka Co, Tokyo, Japan) equipped with a linear, phased-array high-frequency (13-MHz) transducer was used for scanning the common carotid artery. Ultrasound longitudinal images of the common carotid artery were acquired at the end of diastole (defined as the R wave of an electrocardiogram), in which the far wall intima-media interface was clearly defined. The leading edge of the intima and the media-adventitia interface were traced as continuous lines, and mean IMT values were calculated automatically. The mean IMT was measured over a segment of the common carotid artery that was 10 mm in length, located 5 mm proximal to the carotid-artery bulb and considered not to contain any plaque (Figure 2), as previously described.29–30

Measurement of FMD

The subjects remained supine throughout the study. The vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G; UNEX Co) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5 to 10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as a percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD (peak diameter–baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity corrected for heart rate and vessel cross-sectional area by (πr²). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

The response to nitroglycerine was used for assessment of endothelial-independent vasodilation. After acquiring baseline rest image for 30 seconds, a sublingual tablet (nitroglycerine 75 μg) was given and image of the artery was recorded continuously for 5 minutes. Nitroglycerine-induced vasodilation was automatically calculated as a percentage change in peak vessel diameter from the baseline value. Nitroglycerine percentage (peak diameter–baseline diameter/baseline diameter) was used for analysis.

Statistical Analysis

Results are presented as means±SD. All reported probability values were 2-sided, and a probability value of <0.05 was considered statistically significant. Categorical variables were compared by means of χ² test. Relations between variables were determined by Spearman correlation coefficient analysis. Multivariate regression analyses
Table 1. Clinical Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=388)</th>
<th>Men (n=288)</th>
<th>Women (n=100)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45±22</td>
<td>43±21</td>
<td>51±21</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.5±4.3</td>
<td>22.9±4.3</td>
<td>21.2±4.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129.9±21.4</td>
<td>130.6±19.8</td>
<td>127.8±25.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74.3±14.2</td>
<td>74.8±14.2</td>
<td>72.7±14.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68.4±12.6</td>
<td>67.4±12.7</td>
<td>70.9±11.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.89±1.00</td>
<td>4.84±1.00</td>
<td>5.04±0.99</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.62±1.19</td>
<td>1.69±1.27</td>
<td>1.44±0.92</td>
<td>0.15</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.46±0.48</td>
<td>1.38±0.43</td>
<td>1.66±0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.77±0.87</td>
<td>2.78±0.88</td>
<td>2.75±0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.8±2.6</td>
<td>6.7±2.7</td>
<td>6.7±2.7</td>
<td>0.95</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>154 (40.0)</td>
<td>108 (37.8)</td>
<td>46 (46.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>114 (29.7)</td>
<td>75 (26.3)</td>
<td>39 (39.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>77 (19.8)</td>
<td>47 (16.3)</td>
<td>30 (30.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>168 (44.3)</td>
<td>155 (54.4)</td>
<td>15 (15.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>28 (7.2)</td>
<td>22 (7.6)</td>
<td>6 (6.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Cerebrovascular disease, n (%)</td>
<td>21 (5.4)</td>
<td>15 (5.2)</td>
<td>6 (6.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>FMD, %</td>
<td>5.12±3.45</td>
<td>5.14±3.37</td>
<td>5.08±3.72</td>
<td>0.87</td>
</tr>
<tr>
<td>Nitroglycerine-induced vasodilation, %</td>
<td>13.0±5.8</td>
<td>13.3±5.9</td>
<td>12.2±5.5</td>
<td>0.22</td>
</tr>
<tr>
<td>IMT of brachial artery, mm</td>
<td>0.29±0.10</td>
<td>0.29±0.10</td>
<td>0.27±0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>3.97±0.64</td>
<td>4.13±0.60</td>
<td>3.53±0.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FMD, flow-mediated vasodilation; IMT, intima-media thickness.

were performed to identify factors associated with brachial IMT in risk factors and laboratory data. The receiver operating characteristic (ROC) curve analyses were carried out to assess the sensitivity and specificity of measurement of brachial IMT and carotid IMT for diagnosing patients without clinical evidence of CVDs (at-risk group) or patients with CVDs. The data were processed using the software package Stata version 9 (Stata Co, College Station, TX).

Results

Baseline Clinical Characteristics

The baseline clinical characteristics are summarized in Table 1. Of the 388 subjects, 288 (74.2%) were men and 100 (25.8%) were women. One hundred fifty-four (40%) had hypertension, 77 (19.8%) had diabetes mellitus, 114 (29.7%) had dyslipidemia, and 168 (44.3%) were smokers. The mean value of brachial IMT was 0.29±0.10 mm (median, 0.27 mm; interquartile range, 0.22–0.34 mm; range, 0.12–0.59 mm). There was no significant difference in brachial IMT and FMD between men and women (0.29±0.10 versus 0.27±0.08 mm and 5.1±3.4% versus 5.1±3.7%; P=0.11 and P=0.87, respectively). Brachial artery diameter was significantly larger in men than in women (4.13±0.60 versus 3.53±0.55 mm; P<0.001). Brachial IMT positively correlated with brachial artery diameter (r=0.41; P<0.001) for both sexes (men: r=0.42; P<0.001; women: r=0.39; P<0.001).

Relationship Between Brachial IMT and FMD

There was a significant relationship between brachial IMT and FMD in all subjects (r=−0.39; P<0.001) (Figure 3A). Significant relationships between brachial IMT and FMD were found in both men (r=−0.41; P<0.001) and women (r=−0.36; P<0.001).

Brachial IMT and Cardiovascular Risk Factors

There was a significant positive correlation between brachial IMT and Framingham risk score (r=0.49; P<0.001) (Figure 4A). Univariate regression analysis revealed that brachial IMT significantly correlated with age (r=0.71; P<0.001), body mass index (r=0.27; P<0.001), systolic blood pressure (r=0.40; P<0.001), diastolic blood pressure (r=0.31; P<0.001), heart rate (r=0.15; P=0.002), glucose (r=0.18; P=0.01), and smoking pack-year (r=0.42; P<0.001), as well as Framingham risk score (Table 2). Subjects were classified into 3 groups based on Framingham risk scores: low Framingham risk score group (≤4), intermediate Framingham risk score group (5–8), and high Framingham risk score group (≥9). Brachial IMT increased in relation to increase in the Framingham risk score (Figure 4B).

We next categorized subjects into 3 tertiles based on brachial IMT (Table 3). There were significant increases in the prevalence of hypertension, dyslipidemia, diabetes mellitus, smoking, coronary heart disease, and cerebrovascular disease with increase in brachial IMT (Figure 5A and 5B). Multivariate analysis revealed that age, sex, hypertension, and brachial artery diameter were independent predictors of brachial IMT (Table 4).
Relationship Between Brachial IMT and Carotid IMT

There was a significant relationship between brachial IMT and carotid IMT in 60 of the 388 subjects ($r=0.58; P<0.001$) (Figure 6). Significant correlations were also found in men ($r=0.59; P<0.001$) and women ($r=0.65; P<0.001$). Figure 7 shows brachial IMT and carotid IMT in subjects with no cardiovascular risk factors (no risk group), subjects with at least 1 coronary risk factor, including hypertension, dyslipidemia, diabetes mellitus, and smoking, but without established CVD (at-risk group), and subjects with cardiovascular disease (CVD group). Brachial IMT in the CVD group was significantly larger than that in either the no risk group or at-risk group (no risk group, 0.21±0.05 mm; at-risk group, 0.32±0.09 mm; CVD group, 0.37±0.09 mm; $P<0.001$, respectively, Figure 7A). Brachial IMT in the at-risk group was significantly larger than that in the no risk group ($P<0.001$, Figure 7A). There was a significant difference in carotid IMT among groups (no risk group, 0.50±0.05 mm; at-risk group, 0.72±0.16 mm; CVD group, 0.88±0.16 mm; respectively, $P<0.001$ for CVD group or at-risk group versus no risk group; $P=0.01$ for CVD group versus at-risk group, Figure 7B).

Diagnostic Accuracy of Brachial IMT and Carotid IMT

The ROC curves of brachial IMT and carotid IMT for diagnosing the at-risk group or CVD group are shown in Figure 8. Area under the curve values of ROC curves for brachial IMT and carotid IMT to diagnose the at-risk group were 0.89 and 0.94, respectively (Figure 8A). The sensitivity and specificity of brachial IMT to diagnose the at-risk group were 0.74 and 0.81, respectively, at an optimal cutoff of 0.26 mm, and those for carotid IMT were 0.77 and 0.89, respectively, at an optimal cutoff of 0.60 mm. Area under the curve values of ROC curves for brachial IMT and carotid IMT to diagnose CVD group were 0.81 and 0.82, respectively (Figure 8B). The sensitivity and specificity of brachial IMT to diagnose the CVD group were 0.93 and 0.60, respectively, at an optimal cutoff of 0.28 mm, and those for carotid IMT were 0.86 and 0.71, respectively, at an optimal cutoff of 0.77 mm.

Discussion

In the present study, we demonstrated that brachial IMT was inversely correlated with FMD and nitroglycerine-induced vasodilation and that brachial IMT was increased in relation to cumulative cardiovascular risk factors and significantly correlated with cardiovascular risk factors. In addition, we confirmed that there was a significant relationship between brachial IMT and carotid IMT. To our knowledge, this is the first report

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Scatter plots show the relationship between intima-media thickness (IMT) of the brachial artery, flow-mediated vasodilation (FMD) (A) and nitroglycerine-induced vasodilation (B).

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** A, Scatter plots show the relationship between intima-media thickness (IMT) of the brachial artery and Framingham risk score. B, Bar graphs show brachial IMT of subjects with low, intermediate, and high Framingham risk score.
showing an association of brachial IMT with cardiovascular risk factors and incidence of CVD in a general population.

The relationship between IMT, an index of vascular structure, and FMD and nitroglycerine-induced vasodilation, an index of vascular function, has been controversial.23–25 In previous studies, different vascular beds were used for the assessment, ie, common carotid artery for IMT and brachial artery for FMD and nitroglycerine-induced vasodilation. Measurement of IMT,

**Table 2. Univariate Analysis of the Relation Between IMT of the Brachial Artery and Variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
<td></td>
<td>r</td>
<td>P Value</td>
<td></td>
<td>r</td>
<td>P Value</td>
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<tr>
<td>Age, y</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td></td>
<td>0.75</td>
<td>&lt;0.001</td>
<td></td>
<td>0.67</td>
<td>&lt;0.001</td>
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<td>Body mass index, kg/m²</td>
<td>0.27</td>
<td>&lt;0.001</td>
<td></td>
<td>0.29</td>
<td>&lt;0.001</td>
<td></td>
<td>0.15</td>
<td>0.17</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<td>0.45</td>
<td>&lt;0.001</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>&lt;0.001</td>
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<td>0.31</td>
<td>0.002</td>
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<td>Heart rate, bpm</td>
<td>0.15</td>
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<td></td>
<td>0.18</td>
<td>0.002</td>
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<td>0.10</td>
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<td>Total cholesterol, mmol/L</td>
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<td>0.25</td>
<td></td>
<td>−0.13</td>
<td>0.12</td>
<td></td>
<td>0.14</td>
<td>0.29</td>
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<td>Triglycerides, mmol/L</td>
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<td>0.85</td>
<td></td>
<td>0.014</td>
<td>0.86</td>
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<td>−0.095</td>
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<td>HDL cholesterol, mmol/L</td>
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<td>−0.10</td>
<td>0.20</td>
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<td>−0.03</td>
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<td>LDL cholesterol, mmol/L</td>
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<td>−0.032</td>
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<td>0.21</td>
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<td>0.17</td>
<td>0.04</td>
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<td>Smoking, pack-year</td>
<td>0.24</td>
<td>&lt;0.001</td>
<td></td>
<td>0.45</td>
<td>&lt;0.001</td>
<td></td>
<td>0.15</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Framingham risk score</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td></td>
<td>0.56</td>
<td>&lt;0.001</td>
<td></td>
<td>0.51</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>FMD, %</td>
<td>−0.39</td>
<td>&lt;0.001</td>
<td></td>
<td>−0.41</td>
<td>&lt;0.001</td>
<td></td>
<td>−0.36</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Nitroglycerine-induced vasodilation, %</td>
<td>−0.32</td>
<td>&lt;0.001</td>
<td></td>
<td>−0.34</td>
<td>&lt;0.001</td>
<td></td>
<td>−0.36</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>0.41</td>
<td>&lt;0.001</td>
<td></td>
<td>0.42</td>
<td>&lt;0.001</td>
<td></td>
<td>0.39</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Carotid IMT, mm</td>
<td>0.58</td>
<td>&lt;0.001</td>
<td></td>
<td>0.59</td>
<td>&lt;0.001</td>
<td></td>
<td>0.85</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein cholesterol; FMD, flow-mediated vasodilation; IMT, intima-media thickness.

**Table 3. Clinical Characteristics of 3 Groups of IMT of the Brachial Artery**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low Group</th>
<th>Intermediate Group</th>
<th>High Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=130)</td>
<td>(n=129)</td>
<td>(n=129)</td>
</tr>
<tr>
<td>Brachial IMT, mm</td>
<td>0.19±0.03</td>
<td>0.28±0.02*</td>
<td>0.40±0.07†</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.5±13.1</td>
<td>44.6±19.3*</td>
<td>64.3±13.6†</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.1±3.0</td>
<td>22.3±3.8*</td>
<td>24.0±5.4†</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120.8±16.3</td>
<td>126.9±18.0*</td>
<td>141.6±23.6†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>68.9±12.5</td>
<td>73.8±12.9*</td>
<td>79.8±14.8*†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67.6±13.1</td>
<td>65.8±12.0</td>
<td>71.6±12.0†</td>
</tr>
<tr>
<td>Smoking, pack-year</td>
<td>2.6±8.5</td>
<td>14.0±23.2*</td>
<td>30.3±36.2†</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.02±0.88</td>
<td>4.91±0.98</td>
<td>4.86±1.05</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.29±0.71</td>
<td>1.73±1.50</td>
<td>1.67±1.03</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.57±0.56</td>
<td>1.49±0.51</td>
<td>1.41±0.41</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.66±0.96</td>
<td>2.74±0.85</td>
<td>2.82±0.87</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.9±1.5</td>
<td>6.3±2.3</td>
<td>7.3±3.0†</td>
</tr>
<tr>
<td>Framingham risk score</td>
<td>2.42±5.25</td>
<td>5.52±4.23*</td>
<td>8.37±3.35†</td>
</tr>
<tr>
<td>FMD, %</td>
<td>6.59±3.68</td>
<td>5.39±3.17*</td>
<td>3.30±2.58†</td>
</tr>
<tr>
<td>Nitroglycerine-induced vasodilation, %</td>
<td>15.5±6.5</td>
<td>14.9±5.6</td>
<td>11.2±5.1†</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>3.73±0.50</td>
<td>3.87±0.57</td>
<td>4.32±0.71†</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>13 (10)</td>
<td>42 (32.8)*</td>
<td>99 (77.3)*†</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>11 (8.5)</td>
<td>39 (30.7)*</td>
<td>64 (50.0)*†</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>6 (4.6)</td>
<td>23 (17.8)*</td>
<td>48 (37.2)*†</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>24 (18.5)</td>
<td>59 (46.8)*</td>
<td>85 (69.1)*†</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>0 (0)</td>
<td>8 (6.2)</td>
<td>20 (15.5)</td>
</tr>
<tr>
<td>Cerebrovascular disease, n (%)</td>
<td>1 (0.8)</td>
<td>5 (3.9)*</td>
<td>15 (11.6)*†</td>
</tr>
</tbody>
</table>

IMT indicates intima-media thickness; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FMD, flow-mediated vasodilation.

*P<0.05 vs low group; †P<0.05 vs intermediate group.
FMD, and nitroglycerine-induced vasodilation in the brachial artery offers the opportunity to investigate the interrelation between morphological and functional parameters within the same artery by a single examination.

In the present study, we found that there was a significant relationship between brachial IMT and nitroglycerine-induced vasodilation, suggesting that vascular smooth muscle function is also impaired in relation to increased brachial IMT. In addition, a significant inverse correlation was found between brachial IMT and FMD. These findings suggest that abnormalities in vascular smooth muscle function, and not endothelial function per se, are associated with brachial IMT.

It is well known that cardiovascular risk factors increase both carotid IMT and femoral IMT. In the present study, we confirmed that brachial IMT also significantly correlated with cardiovascular risk factors, including aging, systolic blood pressure, diastolic blood pressure, glucose level, and smoking status, and that prevalence of hypertension, dyslipidemia, diabetes mellitus, and smoking, as well as the incidence of CVD, increased in relation to increase in brachial IMT. In addition, brachial IMT was positively related to Framingham risk score, which is a risk calculator and an index of cumulative cardiovascular risk commonly used for assessing the probability of heart attack or death from heart disease within 10 years. These findings suggest that brachial IMT may be helpful for estimation of the extent of atherosclerosis and for risk stratification of patients with cardiovascular risk factors. As for the prognostic value of brachial IMT, Frick et al reported a lack of correlation between brachial IMT and brachial FMD. Although we selected a general population, including healthy subjects and patients with coronary heart diseases in the present study, it should be noted that patients in their study population were limited to male patients aged <70 years who underwent coronary angiography because of chest pain, as well as a positive exercise stress test. Further studies are needed to determine whether endothelial function per se has a direct impact on intima-media thickening in a clinical setting.

Table 4. Multivariate Analysis of the Relation Between Intima-Media Thickness of Brachial Artery and Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>t Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.52</td>
<td>8.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>−0.11</td>
<td>−2.57</td>
<td>0.01</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.034</td>
<td>0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.15</td>
<td>3.12</td>
<td>0.002</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.044</td>
<td>1.03</td>
<td>0.30</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.06</td>
<td>1.52</td>
<td>0.13</td>
</tr>
<tr>
<td>Smoking</td>
<td>−0.03</td>
<td>0.68</td>
<td>0.50</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>0.18</td>
<td>4.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Weidinger et al reported a lack of correlation between brachial IMT and brachial FMD. Although we selected a general population, including healthy subjects and patients with coronary heart diseases in the present study, it should be noted that patients in their study population were limited to male patients aged <70 years who underwent coronary angiography because of chest pain, as well as a positive exercise stress test. Further studies are needed to determine whether endothelial function per se has a direct impact on intima-media thickening in a clinical setting.

Figure 5. A, Bar graphs show the prevalences of cardiovascular risk factors in subjects with low, intermediate, and high intima-media values of thickness of the brachial artery. B, Bar graphs show the prevalence of cardiovascular disease of subjects with low, intermediate, and high values of intima-media thickness of the brachial artery.

Figure 6. Scatter plots show the relationship between brachial intima-media thickness (IMT) and carotid IMT.
reported that brachial IMT predicts late cardiovascular events in male patients admitted for evaluation of chest pain. Further studies are needed to determine the relationship between brachial IMT and their prognostic value for occurrence of cardiovascular events in a general population.

An autopsy study showed that atherosclerosis was a common finding in the brachial artery in 52 consecutive subjects, even in young subjects.22 Interestingly, the grade of atherosclerosis in the brachial artery is related to that in common carotid and coronary arteries.22 In the present study, we demonstrated that brachial IMT significantly correlated with carotid IMT. In addition, area under the curve values of ROC curves revealed that both measurements of brachial IMT and carotid IMT are equally effective for diagnosing patients without clinical evidence of CVDs and patients with CVDs.

In the present study, although there were significant relationships between brachial IMT and cardiovascular risk factors in men, there was no significant relationship between brachial IMT and body mass index or between brachial IMT and smoking status in women. Although the reason for no relationships of brachial IMT with body mass index and smoking status in women remains unclear, the following possibilities are postulated: (1) There is a difference in fat distribution and protective effects of estrogen on atherosclerosis in women.35 The difference in fat distribution may contribute to different relationships in men and women.35 It has been reported that men accumulate more visceral fat in the central region of the body, carrying a greater risk of metabolic complications than subcutaneous fat,36 whereas women accumulate more fat subcutaneously. The difference with regard to the distribution of fat between men and women and endogenous estrogens before menopause may, at least in part, explain the weak relationships between brachial IMT and cardiovascular risk factors in women. (2) It has been reported that smoking significantly increases carotid IMT in men but not in women without cardiovascular risk factors other than smoking, suggesting protective effects of female hormones against smoking-induced thickening of the arterial wall.37 (3) The relatively small number of female subjects in the present study may have contributed to the different relationships in men and women.

In conclusion, brachial IMT is associated with cardiovascular risk factors and may be a marker of atherosclerosis.
and a predictor of coronary heart disease. Further studies are needed to standardize the method for measurement of brachial IMT and determine whether brachial IMT can be used as a surrogate marker of future cardiovascular events.

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We thank Megumi Wakisaka, Kichiro Kawano, and Satoko Michiyama for their excellent secretarial assistance.

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Intima-Media Thickness of Brachial Artery, Vascular Function, and Cardiovascular Risk Factors

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Vascular hypoxic-preconditioning relies on TRPV4-dependent calcium influx and proper intercellular gap-junctions communication

Short title: TRPV4 and Connexins in vascular preconditioning

Géraldine Rath, Julie Saliez, Gaëtane Behets, Miguel Romero-Perez, Elvira Leon-Gomez, Caroline Bouzin, Joris Vriens, Bernd Nilius, Olivier Feron and Chantal Dessy.

Materials and Methods

Materials

Modular incubator chamber was from Billups Rothenberg and the hypoxic chamber used for cell culture hypoxia was an InVivo2 Hypoxia Workstation 500 from Ruskin. HUVECs were from Clonetics. Carbenoxolone, PKH26 red fluorescent cell linker and antibodies directed to Cx43 clone CXN-6 and β-actin were all provided by Sigma. Cx40-A antibody was from Gentaur, TRPV4 channel and phospho-ser1177-eNOS antibodies were from Abcam and Upstate respectively. eNOS and caveolin-1 antibodies were both from BD. All secondary antibodies (peroxidase-conjugated affinipure goat anti-mouse IgG or goat anti-rabbit IgG, TRITC-donkey anti-goat IgG, FITC-mouse anti-rabbit IgG) were from Jackson ImmunoResearch. Calcein-AM was provided by Fluka and the FACScan used for flow cytometry was from Becton Dickinson.

Measurement of $[Ca^{2+}]_i$ in HUVECs

Endothelial cells were plated on coverslips and submitted to the described N, H or PC protocols. They were then incubated with 2 μM Fura-2/AM at 37°C for 30 min and extensively
washed and placed in a temperature-controlled perfusion chamber for 15 min. Cells were observed with a Zeiss Axiovert 100 microscope in epifluorescence mode using a long pass filter cut-off at 510 nm and a dichroic at 405 nm. Alternating wavelength excitation of 340 and 380 nm was provided by a motorized filter wheel. The image pairs monitored by IonOptix Myocam camera were processed by the associated software (IonOptix, Milton, MA) and are presented as the fluorescence ratio (340/380 nm), a direct index to the [Ca2+]i. After rinsing coverslips, endothelial cells were left to recover for 5 min before 4alphaPDD stimulation. Each curve was calibrated using saturated and zero [Ca2+] condition (EGTA-Ca2+-buffered solutions containing ionomycin 10µM and Mn2+ quenching1).

**Western blotting**

For eNOS monomer/dimer assay, non-denatured cell lysates in ice-cold buffer (composition in 50mM Tris-HCl, pH8; 180mM NaCl; 0.5mM EDTA, 0.2% NP40; 100mM phenylmethylsulfonyl fluoride; 1mM DTT; PIC) were separated with 2xSDS sample buffer by low temperature SDS-PAGE at 30mA.

For membrane expression of caveolin-1, total cell membranes were isolated by the method of Nagamatsu et al. with slight modifications2. Briefly, cells were homogenized in 1ml of homogenization buffer (10mM Tris-HCl, pH7.4, 1mM EDTA, 200mM sucrose, 1mM phenylmethylsulfonyl fluoride). The nuclei and cell debris were removed from the homogenate by centrifugation at 900g for 10min at 4°C. The resulting supernatant was centrifuged at 110000g for 75min at 4°C (SW40 rotor, Beckman ultracentrifuge). The membrane pellet was solubilized in buffer (10mM Tris-HCl, pH7.4, 1mM EDTA, 0.5% Triton X-100, 1mM phenylmethylsulfonyl fluoride) for a minimum of 1h at 4°C. Insoluble material was removed by centrifugation at 14000g for 10min at 4°C and 1µg/ml aprotinin added to solubilized membrane samples prior to use.
Results

Supplemental figure I

A.

Total endothelial relaxation in mesenteric arteries isolated from mice exposed to “in vivo” hypoxia-reoxygenation or hypoxic-preconditioning. Acetylcholine (Ach) evoked relaxation in second branch superior mesenteric arteries isolated from mice exposed to either prolonged hypoxia-reoxygenation (H+R), preconditioning followed by prolonged hypoxia-reoxygenation (PC+H+R), or normoxia (N). A, endothelial relaxation was evaluated in presence of indomethacin (10µM) after contraction with phenylephrin (1µM) (n=7). B, Table including EC50 and Emax values.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (M)</th>
<th>Emax (%response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5.10.10^{-8}</td>
<td>78.01 ± 4.90</td>
</tr>
<tr>
<td>H+R</td>
<td>1.37.10^{-7}</td>
<td>61.91 ± 10.00</td>
</tr>
<tr>
<td>PC+H+R</td>
<td>3.50.10^{-8}</td>
<td>97.20 ± 0.74</td>
</tr>
</tbody>
</table>

*, P< 0,05 ; **, P < 0,01 ; ***, P< 0,001 vs normoxic conditions (N)
References
