Correlation Between Flow-Mediated Vasodilatation of the Brachial Artery and Intima-Media Thickness in the Carotid Artery in Men

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Abstract—Endothelial dysfunction has been reported to be the initial step in atherosclerosis. A noninvasive technique that uses ultrasound to measure the intima-media thickness of the carotid artery has been applied to evaluate localized atherosclerosis. This study was undertaken to elucidate whether endothelial dysfunction in the brachial artery is related to the intima-media thickness of the carotid artery. Thirty-four men with atherosclerosis (mean±SE age 61±2 years) and 33 age-matched men without clinical atherosclerosis were examined. The intima-media thickness and plaque formation of the common carotid artery were assessed by B-mode ultrasonography. We also noninvasively measured brachial artery diameter by the same ultrasound machine when the subjects were at rest, during reactive hyperemia, which causes endothelium-dependent vasodilatation, and after sublingual administration of nitroglycerin, which causes endothelium-independent vasodilatation. The atherosclerosis group had a significantly greater intima-media thickness of the common carotid artery than did the control group (1.02±0.04 versus 0.91±0.03 mm, P<0.05). The flow-mediated diameter (FMD) increase (percent FMD=ΔD/D×100) in the atherosclerosis group was significantly smaller than that in the control group (2.8±0.4% versus 5.1±0.6%, P<0.01). A significant negative correlation between the intima-media thickness of the carotid artery and percent FMD was found in all of the subjects. On multiple regression analysis, percent FMD showed a significant negative correlation with the intima-media thickness of the common carotid artery. These findings support the concept that endothelial dysfunction is significantly related to atherogenesis. (Arterioscler Thromb Vasc Biol. 1999;19:2795-2800.)

Key Words: vasodilatation ■ endothelium ■ carotid artery ■ atherosclerosis

Evidence has accumulated that impairment of vascular endothelial function is the initial step in the development of atherosclerosis.1 One important finding is the impaired release of endothelium-dependent relaxing factor, which is now thought to be NO or its related substances, from endothelial cells.1 Flow-mediated dilatation (FMD) induced by reactive hyperemia has been known to be endothelium dependent,2 and this phenomenon can be detected during reactive hyperemia by high-resolution ultrasound in superficial arteries.3,4 Several coronary risk factors such as hypercholesterolemia,5 smoking,6 and hyperhomocysteinemia7 have been reported to be significantly related to decreased FMD. However, no study has been reported to demonstrate an association between increased intima-media thickness (IMT) of the carotid artery and decreased FMD.

A noninvasive technique that uses B-mode ultrasonography can visualize and assess the lumen and vessel wall of the carotid artery. We analyzed IMT of the right common carotid artery by using this method.7 IMT thickening consists of both an intimal atherosclerotic process and medial hypertrophy. Because IMT is increased in subjects with familial hypercholesterolemia8 and shows a progressive reduction with cholesterol-lowering treatment,9,10 IMT seems to be significantly related to the early phase of atherosclerosis.

This study was undertaken to elucidate whether impaired endothelial function in the brachial artery is related to IMT thickening in the common carotid artery. Because of the significant correlation between IMT and coronary or cerebrovascular disease,11-16 we examined the clinical significance of increased IMT in relation to impaired endothelial function in the study subjects.

Methods

Subjects
Thirty-four men with atherosclerosis aged 61.1±2.0 years (mean±SE) and 33 age-matched men without clinical atherosclerosis (controls) were enrolled in this study. These subjects were recruited from outpatient clinics, inpatient wards, and community volunteers. A history and physical examination were obtained, and laboratory tests were performed in all subjects to exclude diseases other than hypertension, hyperlipidemia, and diabetes mellitus. Exclusion criteria for this study included clinical manifestations of cerebrovascular disease, venous thromboembolism, or liver disor-
ders and a personal history of cancer(s). The inclusion criteria for the atherosclerosis group were as follows: (1) coronary artery disease, confirmed by coronary arteriography and/or a documented history of myocardial infarction within 5 years; (2) a clinical diagnosis of arteriosclerosis obliterans. All subjects were interviewed about their drinking and smoking habits. Each subject gave written, informed consent before enrollment in this study, after receiving a thorough explanation of the study design and protocol. This study was in agreement with the guidelines approved by the ethics committee at our institution.

Study Design
Each subject made 1 visit to the University of Tokyo Hospital. Blood sampling was performed in the morning of the examination, after a 14-hour overnight fast, to measure the serum lipid profile and other biochemical parameters. Serum total cholesterol and triglyceride concentrations were measured enzymatically, and the serum HDL cholesterol concentration was measured by the heparin–Ca$^{2+}$ precipitation method. Plasma glucose concentration was assayed by the glucose oxidase method, and the hemoglobin A1c level was measured by high-performance liquid chromatography.

Measurement of IMT of the Carotid Artery
Ultrasound measurements of IMT of the common carotid artery were performed by an examiner who was unaware of the subjects’ clinical backgrounds. IMT of the carotid artery was measured from high-resolution, 2-dimensional ultrasound images obtained by an SSA-270A ultrasound machine (Toshiba) with a 7.5-MHz linear-array transducer.

The subject reclined on the examination table for 15 minutes before the initial carotid ultrasound scanning. IMT measurement of the carotid artery was performed according to the method of Salonen and Salonen as described previously in a quiet, temperature-controlled (22°C to 24°C) room. This measurement was applied to the far wall of the right carotid artery. While subjects were in the supine position, a linear-array ultrasound probe (7.5 MHz), which was part of the same ultrasound machine, was applied longitudinally to the surface of the skin on the right side of the neck. Longitudinal scanning was performed from the common carotid artery to the bifurcation of the common carotid artery. Scanning was performed in the optimal position. Blood pressure was monitored in the left arm every 2 minutes during the study by an automated blood pressure recorder. An ECG monitor integrated with the ultrasound machine was also applied. The ultrasound images were recorded on S-VHS videotape with an SLV-R75 videocassette recorder (Sony). After the bifurcation of the common carotid artery was confirmed, IMT was measured from the B-mode screen with electronic calipers to within 10 mm proximal to the bifurcation. Four points were measured in the scan, which was synchronized with the R-wave peaks on the ECG to avoid possible errors resulting from variable arterial compliance. Two scans were made for each subject. The mean IMT was calculated from 8 points. The variability of the ultrasound measurements of IMT was studied by performing 5 measurements over 1 month in 12 volunteers. The coefficient of variation for measurement of IMT was 4.20±0.67%.

Measurement of Flow-Mediated and Nitroglycerin-Induced Dilatation of the Brachial Artery
After the carotid ultrasound scanning, flow-mediated dilatation (FMD) and nitroglycerin (NTG)-induced dilatation of the brachial artery were measured by the examiner who performed the carotid ultrasound study. Studies of FMD and NTG-induced dilatation were performed according to the method described previously. The examinations were conducted by the same examiner throughout the study. The diameter of the artery was measured from high-resolution, 2-dimensional ultrasound images obtained by an ultrasound machine with a 7.5-MHz linear-array transducer. Machine operating parameters were kept constant during each study.

The right brachial artery was scanned over a longitudinal section 3 to 5 cm above the right elbow. Depth and gain settings were optimized to identify the lumen-to-wall interface. Automated blood pressure recording in the left arm was continued every 2 minutes during the study. An ECG monitor integrated with the ultrasound machine was also applied. When an adequate image was obtained, the surface of the skin was marked, and the arm was kept in the same position throughout the study. A pneumatic tourniquet placed around the forearm distal to the target artery was inflated to a pressure of 250 mm Hg, and inflation was held for 5 minutes. Increased flow was then induced by sudden cuff deflation. A second scan was performed continuously for 60 seconds before and for 120 seconds after cuff deflation. Then, 15 minutes later, another resting scan was recorded to confirm vessel recovery. Sublingual NTG spray (300 μg, Myocol spray, Toa Eiyo Co) was then administered, and 3 to 5 minutes later the last scan was performed.

The ultrasound images were recorded on S-VHS videotape with an SLV-R75 videocassette recorder (Sony). The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia ("m line") at a fixed distance. The mean diameter was calculated from 5 cardiac cycles synchronized with the R-wave peaks on the ECG. All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance. Maximal vasodilatation was observed 45 to 60 seconds after cuff release. The diameter change caused by FMD was expressed as the percent change relative to that at the initial resting scan (percent FMD). The diameter change caused by NTG was expressed in the same way, as the percent change relative to that at the recovery scan (percent NTG-induced dilatation). The pulse-wave velocity profile of blood flow was simultaneously recorded. Mean flow velocity was calculated by measuring the area under this velocity profile curve. Blood flow (in milliliters per minute) was then calculated by multiplying the cross-sectional area of the brachial artery, which was based on the diameter and the mean flow velocity. Changes in diameter of 0.1 to 0.2 mm can be detected accurately with this method. The coefficient of variation for measurements of percent FMD was 5.84±0.25% and that for percent NTG-induced dilatation was 3.97±0.24%, as we reported before.

Statistical Analysis
All data in the text, tables, and figures are expressed as mean±SEM. Differences between the 2 groups were analyzed by Student’s unpaired t test. Simple correlation of percent FMD with IMT and of percent NTG-induced dilatation with IMT was determined. Standardized regression coefficients from multiple regression analysis of IMT in relation to various factors were analyzed. A value of P<0.05 was considered statistically significant.

Results
Clinical Characteristics
Thirty of the 34 subjects with atherosclerosis regularly took NTG products for clinical reasons. On the other hand, 1 of the 33 subjects without clinical atherosclerosis had been prescribed an NTG product by his family doctor for chest discomfort. The clinical and metabolic characteristics of the 34 men with atherosclerosis and of the 33 men without clinical atherosclerosis (controls) are presented in Table 1. Age, body mass index, and mean blood pressure of the men with atherosclerosis were not statistically different from those of the men without clinical atherosclerosis. Serum total cholesterol, HDL cholesterol, triglyceride, fasting plasma glucose, and hemoglobin A1c levels were similar between the 2 groups. The percent prevalences of hypertension, hyperlipidemia, diabetes mellitus, and current smoking are presented in Table 1, and these were not statistically different between the 2 groups.

Two men without clinical atherosclerosis and 6 men with atherosclerosis were taking 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Six men without clinical atherosclerosis and 18 men with atherosclerosis were taking calcium channel antagonists (P<0.01). Five men without
Subjects Without Clinical Atherosclerosis (n=33) | Subjects With Clinical Atherosclerosis (n=34)
---|---
Age, y | 57.0±1.6 | 61.1±2.0
Body mass index, kg/m² | 24.0±0.7 | 24.0±0.6
Mean blood pressure, mm Hg | 96±2 | 95±2
Total cholesterol, mmol/L | 5.25±0.13 | 5.20±0.16
HDL cholesterol, mmol/L | 1.37±0.08 | 1.19±0.05
Triglyceride, mmol/L | 2.29±0.52 | 1.96±0.24
Fasting plasma glucose, mmol/L | 6.00±0.28 | 6.27±0.33
Hemoglobin A₁c, % | 5.8±0.2 | 6.0±0.3
Regular NTG user, % | 3 | 88*  
Hypertension, % | 46 | 56
Hyperlipidemia, % | 39 | 35
Diabetes mellitus, % | 18 | 35
Current smoker, % | 42 | 56

*Significantly different from subjects without clinical atherosclerosis.

**Significantly different from subjects without clinical atherosclerosis.

FMD and NTG-Induced Dilatation of the Brachial Artery
All subjects tolerated the study well. There were no significant differences in the brachial artery diameter at rest (5.05±0.09 mm in the subjects without atherosclerosis versus 5.27±0.10 mm in the subjects with atherosclerosis) and the magnitude of reactive hyperemia produced by cuff inflation and release in the 2 groups (data not shown). As shown in Figure 2, the percent FMD in the subjects with atherosclerosis was significantly smaller than that of subjects without clinical atherosclerosis (2.78±0.43% versus 5.10±0.59%, P<0.01).

Among the subjects without clinical atherosclerosis, percent FMD was not significantly different between nonsmokers and smokers (5.62±0.69% versus 4.39±1.02%). Among the subjects with clinical atherosclerosis, the percent FMD in smokers was lower than that in nonsmokers (1.99±0.50% versus 3.79±0.68%, P<0.05). The percent NTG-induced dilatation in the subjects with atherosclerosis was also significantly smaller than that of subjects without clinical atherosclerosis (7.83±0.68% versus 12.28±0.83%, P<0.01). Among the subjects without clinical atherosclerosis, the percent NTG-induced dilatation was not significantly different between nonsmokers and smokers (11.91±1.00% versus 12.78±0.43%). Among the subjects with clinical atherosclerosis, the percent NTG-induced dilatation was not significantly different between nonsmokers and smokers (8.58±1.04% versus 7.24±0.91%). The percent FMD was significantly related to the percent NTG-induced dilatation by simple regression analysis in the subjects without atherosclerosis, in the subjects with atherosclerosis, and in all subjects combined (r = -0.56, P=0.0004; r = -0.77, P<0.0001; and r = -0.71, P<0.0001, respectively).

IMT of the Common Carotid Artery and Percent FMD of the Brachial Artery
To investigate the relationship between IMT and percent FMD, the following analysis was performed in the 67 subjects. As shown in Figure 3, IMT was inversely related to percent FMD by simple regression analysis (r = -0.36, P<0.01). When the subjects with atherosclerosis were excluded, IMT was still inversely related to percent FMD (r = -0.38, P<0.05). Multiple regression analysis was performed to investigate whether percent FMD was an independent variable related to IMT. Table 2 shows standardized regression coefficients of IMT in relation to age, body mass...
index, mean blood pressure, percent FMD, serum total cholesterol, serum HDL cholesterol, serum triglyceride, fasting plasma glucose, and plasma hemoglobin A1c levels. This analysis showed that IMT was inversely related to percent FMD only \( (P<0.05) \).

**IMT of the Common Carotid Artery and Percent NTG-Induced Dilatation of the Brachial Artery**

IMT was also inversely related to percent NTG-induced dilatation by simple regression analysis in the 67 subjects \( (r=0.31, P<0.05) \). However, when the subjects were divided into 2 groups (ie, those with and without atherosclerosis) as shown in Figure 3, IMT was not inversely related to percent NTG-induced dilatation in either group. On multiple regression analysis, IMT was not significantly related to age, body mass index, mean blood pressure, percent NTG-induced dilatation, serum total cholesterol, serum HDL cholesterol, serum triglyceride, fasting plasma glucose, and plasma hemoglobin A1c levels.

**Discussion**

IMT of the common carotid artery as measured by B-mode ultrasonography is thought to be an initial pathological change secondary to the atherosclerotic process. IMT of the common carotid artery has been used as a noninvasive end point in epidemiological studies and clinical trials to assess the progression and regression of atherosclerosis in the body.\(^\text{12-16}\) Carotid arterial IMT, expressed as a single measurement or a rate of change, is used as a surrogate end point for atherosclerosis of the coronary arteries.\(^\text{12-16}\) On the other hand, FMD in the brachial artery has recently been used to detect endothelial function and is widely accepted as a noninvasive technique.\(^\text{3}\) There have been few reports, however, demonstrating the relationship between FMD in the brachial artery and coronary artery endothelial function.\(^\text{23}\) Furthermore, the relationship between FMD in the brachial artery and the progression of coronary artery disease and/or

**TABLE 2. Standardized Regression Coefficients From Multiple Linear Regression Analysis of IMT in Relation to Independent Variables in 67 Subjects**

<table>
<thead>
<tr>
<th>Independent Variable(^*)</th>
<th>Standardized Coefficient</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>% FMD</td>
<td>-0.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Age</td>
<td>0.14</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>0.05</td>
<td>0.68</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.05</td>
<td>0.73</td>
</tr>
<tr>
<td>Hemoglobin A(_1c)</td>
<td>-0.04</td>
<td>0.84</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.08</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^*\)In all cases, IMT was the dependent variable.
its related events has not been demonstrated. Therefore, before FMD in the brachial artery becomes a useful surrogate for assessing the predisposition to atherosclerosis in patients, the relationship between IMT of the common carotid artery and FMD in the brachial artery will need to be determined.24

This study showed that IMT of the common carotid artery of subjects with atherosclerosis was significantly greater than that in subjects without clinical atherosclerosis. At the same time, endothelium-dependent vasodilatation in subjects with atherosclerosis was significantly smaller than that in subjects without clinical atherosclerosis. Endothelium-independent vasodilatation induced by NTG in subjects with atherosclerosis was also significantly smaller than that in subjects without clinical atherosclerosis. Atherosclerotic vessels, which are characterized by smooth muscle proliferation, may have an impaired smooth muscle response to NO, which is produced either by the endothelium or by sublingual nitrate administration. Furthermore, almost 90% of the subjects with atherosclerosis in this study had been prescribed nitrates. Regular use of nitrates may itself induce an impaired smooth muscle response to NO, manifested as “tolerance.” However, percent FMD was also significantly related to percent NTG-induced dilatation by simple regression analysis in the subjects without atherosclerosis. This suggests that early in the process of atherosclerosis, arterial wall changes are not only present as impaired endothelial function but also as functional changes in smooth muscle cell responses to endothelium-derived and/or exogenous nitro compounds. The mechanism underlying this remains unclear. It is possible that microscopic arterial structural changes may exist before the development of macroscopic anatomic changes.25,26

IMT was inversely related to percent FMD and percent NTG-induced dilatation by simple regression analysis for all subjects. When the subjects with atherosclerosis were excluded, IMT was inversely related to percent FMD only. Among the subjects with atherosclerosis, IMT was not related to either percent FMD or percent NTG-induced dilatation. This finding suggests that before the development of macroscopic anatomic atherosclerosis, IMT is well correlated with endothelial dysfunction. Multiple regression analysis also confirmed that percent FMD was an independent variable related to IMT. Furthermore, several subjects with atherosclerosis had a relatively thin IMT and a decreased percent FMD. This result may suggest that some subjects have impaired endothelial function before developing an early stage of atherosclerosis that can be measured as IMT. Therefore, it is important to know not only IMT but also endothelial function. We are not completely sure at this point that endothelial dysfunction precedes development of the early stages of atherosclerosis.

Active and passive smoking has been reported to be significantly involved in endothelial function2–27 and the progression of IMT.28,29 Smoking may have influenced the results of percent FMD and IMT in this study also. Actually, among the subjects with clinical atherosclerosis, even though IMT was not different with respect to smoking status, the percent FMD in smokers was lower than that of nonsmokers (1.99±0.50% versus 3.79±0.68%, P<0.05). However, the number of current smokers in both groups was not statistically different, and thus, it is likely that smoking habit did not influence our conclusions.

IMT of the common carotid artery can be reduced by cholesterol-lowering treatment.10,30–32 Pravastatin treatment in hyperlipidemic patients has resulted in a statistically significant reduction in IMT progression in the common carotid artery, and pravastatin treatment has also been associated with a reduction in fatal and nonfatal coronary events.32 The degree of carotid artery atherosclerosis as measured by B-mode ultrasound has been shown to be strongly and independently correlated with the presence of coronary atherosclerotic disease.11,23 Increased IMT of the common carotid artery has been shown to be an indicator of generalized atherosclerosis and to be associated with future cerebrovascular and cardiovascular events.12–16 These reports support the hypothesis that carotid arteriosclerosis may reflect generalized atherosclerosis.

Increased IMT of the common carotid artery and impaired FMD in the brachial artery are thought to be the initial steps in atherosclerosis, and they may even be reversible. Identifying patients without overt atherosclerosis but who have cardiovascular risk factor(s) is of clinical importance. Further studies are needed to evaluate the healthy population with or without risk factor(s).

In conclusion, the present study demonstrated a significant relationship between carotid artery IMT and endothelium-dependent vasodilatation in the brachial artery. These findings support the idea that endothelial dysfunction is significantly related to atherogenesis.

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References

lation of endothelial function in the human coronary and peripheral circulations. 


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