Flow-Mediated Vasodilation and Anatomical Variation of the Brachial Artery (Double Brachial Artery) in Healthy Subjects and Patients With Cardiovascular Disease

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**Background:** The purpose of this study was to evaluate the effect of anatomical variation of the brachial artery on flow-mediated vasodilation (FMD) in healthy subjects and patients with cardiovascular disease (CVD).

**Methods and Results:** There was no significant difference in the prevalence of double brachial artery between healthy subjects (6.1%) and patients with CVD (6.5%). In healthy subjects, FMD was larger in a single brachial artery than in large and small vessels of a double brachial artery (7.2±3.4% vs. 4.7±3.3% and 4.5±2.5%, P<0.01, respectively). In patients with CVD, there were no significant differences in FMD among a single brachial artery, large vessel of a double brachial artery and small vessel of a double brachial artery (3.3±1.4%, 3.1±2.3% and 3.6±2.1%). FMD in a single brachial artery was smaller in patients with CVD than in healthy subjects. There were no significant differences in FMD in the large vessel of a double brachial artery between the 2 groups or in the small vessel of a double brachial artery between the 2 groups. Nitroglycerine-induced vasodilation was similar in all arteries in healthy subjects and patients with CVD.

**Conclusions:** When measuring FMD, the existence of a double brachial artery should be checked. FMD measured in a double brachial artery may be underestimated in healthy subjects.  

**Key Words:** Atherosclerosis; Double brachial artery; Flow-mediated vasodilation

The vascular endothelium plays an important role in regulation of vascular tone, inhibition of platelet aggregation, and suppression of smooth muscle cell proliferation.1–4 Endothelial dysfunction is an early feature of atherosclerosis and vascular disease in humans.5 Therefore, it is clinically important to estimate the degree of endothelial function. Measurement of flow-mediated vasodilation (FMD) in the brachial artery using ultrasound is non-invasive and is an accurate indicator of nitric oxide (NO) production.6–13 Measurement of FMD is widely performed as an index of conduit artery endothelial function. Several studies have shown that FMD is an independent predictor for cardiovascular outcomes.14–17 It is thought that FMD is useful as a surrogate endpoint of cardiovascular outcome and an index of atherosclerosis treatment.

Guidelines and detailed methods for FMD measurement have recently been established,18–20 but there is no information on anatomical consideration of the brachial artery for assessing vascular function. It is well known that there are various anomalies in the brachial artery and it has been reported that
**Figure 1.** Representative smaller artery and larger artery on the double brachial artery side and artery on the single brachial artery side (arrows) in a healthy subject and a patient with cardiovascular disease.
3.0–15.1% of the population have 2 brachial arteries in the upper arm. The purpose of this study was to clarify the impact of anatomical variation of the brachial artery on measurement of FMD.

**Methods**

**Subjects**

Bilateral upper arms were investigated for brachial artery anatomical variation using duplex ultrasound sonography in 213 consecutive healthy subjects and in 169 consecutive patients with cardiovascular disease (CVD), including coronary artery disease and hypertension. To compare FMD in an artery with an anomaly and that in an artery without an anomaly, subjects who had 2 arteries in the upper arm on 1 side and a single artery on the other side were enrolled. Thirteen of the 213 healthy subjects (10 men, 3 women; age range, 20–52 years; mean age, 29±10 years) who had a double brachial artery; 11 of the 169 patients with CVD (9 men, 2 women; age range, 55–78 years; mean age, 68±7 years) who had a double brachial artery; 200 age- and sex-matched healthy subjects without a double brachial artery (154 men, 46 women; age range, 21–51 years; mean age, 29±8 years); and 200 patients with CVD who had no double brachial artery (164 men, 36 women; age range, 55–78 years; mean age, 68±9 years) were enrolled in this study. The prevalence of double brachial artery was calculated for all subjects. The study protocol was approved by the Ethics Committee of Hiroshima University Graduate School of Biomedical Sciences. Written informed consent for participation in the study was obtained from all subjects.

**Study Protocol**

We measured vascular response to reactive hyperemia and sublingual nitroglycerine in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 h. The study began at 08:30 hours. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22–25°C) throughout the study. A 23-G polyethylene catheter was inserted into the left deep anteceubital vein to obtain blood samples. Thirty minutes after maintaining the prone position, basal brachial artery diameter was measured. Then FMD and nitroglycerine-induced vasodilation were measured. These tests were carried out in a randomized fashion. Each study proceeded after brachial artery diameter had returned to baseline.

**Measurement of Vascular Function**

Subjects who had double brachial artery had FMD measured at 3 sites: the smaller artery and larger artery on the double brachial artery side, and the artery on the single brachial artery side. Figure 1 shows a representative smaller artery and larger artery on the double brachial artery side and artery on the single brachial artery side. Every measurement of FMD was randomly performed on separate days. A high-resolution linear array transducer was coupled to computer-assisted analysis software (prosound-a7; Hitachi Aloka Medical, Tokyo, Japan) that used an automated edge detection system for measurement of artery diameter. A blood pressure cuff was placed around the forearm. The target artery was scanned longitudinally. When the clearest B-mode image of the 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 (MP-PH0001; Hitachi Aloka Medical) to ensure consistency of the image. A baseline image was acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a sample volume. The blood pressure cuff was then inflated to 50mmHg above systolic pressure for 5 min. 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. A Doppler signal is recorded from the center of the vessel with an automated set of range gate. We adjusted sample volume to be as wide as possible in the vessel wall in every artery. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 s and then the blood pressure cuff was inflated to 50mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as percent change relative to the vessel diameter before cuff inflation.

FMD was calculated as the percent change in peak vessel diameter from the baseline value. %FMD (peak diameter−baseline diameter/baseline diameter) was used for analysis. Blood flow was calculated as 3 time-averaged velocity integrals (cm/s)×cross-sectional area of artery (r²)×heart rate (per min). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow. Artery shear stress was calculated as 8μV/diameter, where μ is blood velocity and V is artery velocity at peak hyperemia. After a 10-min period to allow artery baseline conditions to be re-established, another baseline scan was performed. Representative baseline and peak vasodilation of the smaller artery and larger artery on the double brachial artery side and artery on the single brachial artery side are shown in a healthy subject (Figure 2) and in a patient with CVD (Figure 3).

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation. After storing of baseline resting images, a sublingual nitroglycerine tablet (75μg) was given, and images of the artery were recorded for 5 min. The response of the artery diameter to nitroglycerine was expressed as percent change relative to vessel diameter. Nitroglycerine-induced vasodilation was automatically calculated as the percent change in peak vessel diameter from the baseline value. %Nitroglycerine (peak diameter−baseline diameter/baseline diameter) was used for analysis.

**Statistical Analysis**

Results are presented as mean±SD. P<0.05 was considered to indicate statistical significance. Mann-Whitney U-test was used to evaluate differences between groups. Multi-group comparisons of variables were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni correction. The data were processed using StatView IV (SAS Institute, Cary, NC, USA) or Super analysis of variance (Abacus Concepts, Berkeley, CA, USA).
Results

Clinical Characteristics

The prevalence of double brachial artery was 6.1% (13/213) in healthy subjects and 6.5% (11/169) in patients with CVD, and was not significantly different between the 2 groups.

Baseline clinical characteristics of the 13 healthy subjects with a double brachial artery, the 11 CVD patients with a double brachial artery, the 200 healthy subjects without a double brachial artery and the 200 CVD patients without a double brachial artery are summarized in Table 1. Patients with CVD who had a double brachial artery also had diabetes mellitus (n=2), hypertension (n=9), dyslipidemia (n=5), and coronary artery disease (n=9). Patients with CVD who had no double
brachial artery included 41 with diabetes mellitus, 181 with hypertension, 98 with dyslipidemia, and 176 with coronary artery disease. Systolic blood pressure and diastolic blood pressure were higher in patients with CVD than in healthy subjects. Patients with CVD included smokers, whereas there were no smokers among the healthy subjects. There were no significant differences in other parameters between the 4 groups.

**Vascular Function**

Vascular function of the 13 healthy subjects with a double brachial artery, the 11 CVD patients with a double brachial artery, the 200 healthy subjects without a double brachial artery and the 200 CVD patients without a double brachial artery is summarized in Table 2. Figure 4 shows FMD in the smaller artery and larger artery on the double brachial artery side and artery on the single brachial artery side in healthy subjects and patients with CVD and in the brachial artery in healthy subjects without a double brachial artery and in CVD patients without a double brachial artery.

In healthy subjects with a double brachial artery, baseline artery diameter increased in the order of small and large arteries in a double artery, and single brachial artery (2.21±0.52 mm vs. 2.93±0.60 mm vs. 3.27±0.55 mm, P<0.001, respectively). FMD was lower in small and large arteries in a double brachial artery than in a single brachial artery (4.5±2.5% and 4.7±3.3% vs. 7.2±3.4%, P<0.01, respectively), whereas FMD was similar in small and large arteries in a double brachial artery. There was no significant differences in baseline diameter or FMD in a single brachial artery between healthy subjects with and without a double brachial artery (3.27±0.55 mm vs. 3.27±0.55 mm, P<0.001, respectively). These was no significant difference in other parameters between the 4 groups.

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<th>Table 1. Clinical Subject Characteristics</th>
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<td><strong>Healthy subjects without a DBA (n=200)</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
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Data given as mean±SD or n (%). *P<0.05 vs. healthy subjects. BMI, body mass index; CVD, cardiovascular disease; DBA, double brachial artery; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol.

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<th>Table 2. Vascular Function</th>
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<td><strong>Healthy subjects without a DBA (n=200)</strong></td>
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<td><strong>Baseline artery diameter (mm)</strong></td>
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<td><strong>Brachial artery</strong></td>
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<td><strong>Shear stress (dyne/cm²)</strong></td>
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<td><strong>Increase in hyperemic flow (%)</strong></td>
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<td><strong>FMD (%)</strong></td>
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<td><strong>Nitroglycerine-induced vasodilation (%)</strong></td>
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<td><strong>Nitroglycerine-induced vasodilation (%)</strong></td>
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Data given as mean±SD. *P<0.05 vs. single brachial artery in the same group; †P<0.05 vs. large vessel in DBA in the same group; ‡P<0.05 vs. single brachial artery in healthy subjects.

FMD, flow-mediated vasodilation. Other abbreviations as in Table 1.
in baseline diameter in a single brachial artery between healthy subjects with and without a double brachial artery (3.73±0.62 mm vs. 3.71±0.53 mm). FMD and nitroglycerine-induced vasodilation were similar in small and large arteries in a double brachial artery and single brachial artery in CVD patients with a double brachial artery and in a brachial artery in CVD patients without a double brachial artery (FMD: 3.6±2.1% vs. 3.1±2.3% vs. 3.3±1.4% vs. 3.5±1.2%; nitroglycerine-induced vasodilation: 14.1±7.1% vs. 13.2±3.9% vs. 12.4±4.2% vs. 12.8±2.3%).

FMD in a single brachial artery was smaller in CVD patients with and without a double brachial artery than in healthy subjects with and without a double brachial artery (3.3±1.4% and 3.5±1.2% vs. 7.2±3.4% and 7.0±1.3%, P<0.01, respectively). There were no significant differences in FMD in large and small arteries of a double brachial artery between healthy subjects and patients with CVD. Nitroglycerine-induced vasodilation was similar in all arteries in healthy subjects and patients with CVD.

**Discussion**

FMD was significantly smaller in a double brachial artery than in a single brachial artery in healthy subjects. Anatomical variation of the brachial artery did not alter FMD in patients with CVD. In a double brachial artery, there was no significant difference in FMD between healthy subjects and patients with CVD, while FMD in a single brachial artery was significantly greater in healthy subjects than in patients with CVD. Nitroglycerine-induced vasodilation was similar in a single brachial artery and double brachial artery in healthy subjects and patients with CVD. These findings suggest that endothelial function, but not smooth muscle function, is selectively altered in a double brachial artery compared with that in a single brachial artery in healthy subjects and that both endothelial function and vascular smooth muscle function are similar in a double brachial artery and single brachial artery in patients with CVD.

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis, leading to cardiovascular complications. FMD in the brachial artery is an appropriate method of assessing endothelial function in humans, because it is non-invasive and reflects NO production. Several clinical studies have shown that endothelial function assessed by FMD can serve as an independent predictor of cardiovascular events. Therefore, from a clinical perspective, it is important to estimate the basal condition of endothelial function. Unfortunately, brachial artery anatomical variation is neglected in measurement of FMD. It has been reported that 3.0–15.1% of the population have 2 arteries in an upper arm because of variations of the arterial pattern in the upper limb such as a double brachial artery, high origin of the radial artery, and high origin of the ulnar artery. In the present study, we found that the prevalence of double brachial artery was 6.1% in healthy subjects and 6.5% in patients with CVD. The frequency of brachial artery anatomical variation is not low. Therefore, attention must be given to brachial artery anatomical variation when endothelial function is measured.

Some possible reasons for the difference in FMD between a single brachial artery and double brachial artery in healthy subjects are postulated. It is well known that increase in hyperemic flow, that is, increase in shear stress, mediates the production of NO from endothelial cells. Although increases in hyperemic flow and shear stress were similar in a double brachial artery and a single brachial artery in the present study, the percent and raw values of baseline blood flow and reactive hyperemic flow were significantly lower on the double brachial artery side than on the single brachial artery side (baseline flow: 30.1±20.2 ml/min and 47.5±24.3 ml/min vs. 74.6±22.5 ml/min, P<0.05, respectively; increase in hyperemic flow: 122.4±69.7 ml/min and 192.1±76.3 ml/min vs. 320.2±74.5 ml/min, P<0.05, respectively). The differences in baseline blood flow and reactive hyperemic flow may contribute to the differences in FMD between a double brachial artery and single brachial artery in healthy subjects.
artery. In patients with CVD also, raw values of baseline blood flow and reactive hyperemic flow were significantly lower on the double brachial artery side than on the single brachial artery side. Endothelial function assessed by FMD was impaired in patients with CVD, suggesting a decrease in shear stress-induced NO production in these patients. Under the condition of endothelial dysfunction, differences in baseline blood flow or hyperemic flow using an FMD measurement protocol may not directly affect FMD. The precise reason for the discrepancy in FMD in a double brachial artery and a single brachial artery between healthy subjects and patients with CVD, however, remains unclear.

Previous studies have clearly shown that CVD, including hypertension, dyslipidemia, diabetes mellitus and coronary artery disease, are associated with endothelial dysfunction in the forearm vasculature. We confirmed that endothelial function was impaired in a single brachial artery in patients with CVD compared with that in healthy subjects. FMD in a double brachial artery, however, was similar in healthy subjects and patients with CVD. We cannot deny the possibility that measurement of FMD in a double brachial artery is underestimated, especially in healthy subjects. When healthy subjects have double brachial arteries, the other side should be checked for the existence of a single brachial artery. If a single brachial artery is observed on the other side, FMD should be measured on the single brachial artery side.

It is well known that brachial artery diameter is an independent predictor of FMD. In the present study, there was a significant relationship between brachial artery diameter and FMD in a single brachial artery in 200 healthy subjects and in 200 patients with CVD (r=-0.41, P<0.001; r=-0.39, P<0.001, respectively). In addition, multiple regression analysis showed that brachial artery diameter was an independent predictor of FMD in healthy subjects and patients with CVD (β=0.18, t=4.23, P<0.001; β=0.17, t=3.87, P<0.001, respectively). We cannot find, however, a significant relationship between brachial artery diameter and FMD in a double brachial artery in healthy subjects and patients with CVD. Vessel diameter did not affect FMD in patients with CVD, while FMD was significantly smaller in a double brachial artery than in a single brachial artery in healthy subjects. In addition, in healthy subjects, there was no significant difference in FMD between the small double brachial artery and large brachial artery. Although the number of subjects in whom the effect of double brachial artery on FMD was evaluated is small, the present findings suggest that vessel diameter per se is not always a predictor of FMD, especially in a double brachial artery. In the present study, we would like to emphasize the impact of anatomical variation of the brachial artery on measurement of FMD, because the proportion of subjects who had a double brachial artery was relatively high.

Specialists in peripheral artery ultrasonography carefully checked for the existence of an anomaly of the brachial artery. We cannot deny the possibility, however, that anatomical variation of the brachial artery was overlooked, because we did not perform brachial artery angiography in all subjects.

Although we could not find a double brachial artery in bilateral arms in the present study, it is thought that FMD cannot be adequately measured in healthy subjects with a double brachial artery in bilateral arms.

The number of study subjects was relatively small. We cannot exclude the possibility that there was selection bias in the results. We confirmed, however, that endothelial function in a single brachial artery was impaired in patients with CVD compared with that in healthy subjects. A larger number of subjects is necessary to determine conclusively the effects of anatomical variation of the brachial artery on FMD.

In conclusion, in healthy subjects, we should confirm the existence of anatomical variation of the brachial artery when measuring FMD. It is possible that FMD measured in a double brachial artery is underestimated in healthy subjects. Measurement of FMD in a single brachial artery is recommended in healthy subjects. The existence of anatomical variation of the brachial artery is a pitfall in the assessment of endothelial function.

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