ORIGINAL RESEARCH

Diagnostic Criteria of Flow-Mediated Vasodilation for Normal Endothelial Function and Nitroglycerin-Induced Vasodilation for Normal Vascular Smooth Muscle Function of the Brachial Artery

Tatsuya Maruhashi, MD, PhD; Masato Kajikawa, MD, PhD; Shinji Kishimoto, MD, PhD; Haruki Hashimoto, MD; Yuji Takaeko, MD; Takayuki Yamaji, MD; Takahiro Harada, MD; Yiming Han, MS; Yoshiki Aibara, MS; Farina Mohamad Yusoff, MD; Takayuki Hidaka, MD, PhD; Yasuki Kihara, MD, PhD; Kazuaki Chayama, MD, PhD; Ayumu Nakashima, MD, PhD; Chikara Goto, PhD; Hirofumi Tomiyama, MD, PhD, FAHA; Bonpei Takase, MD, PhD, FAHA; Takahide Kohro, MD, PhD; Toru Suzuki, MD, PhD; Tomoko Ishizu, MD, PhD; Shinichiro Ueda, MD, PhD; Tsutomu Yamazaki, MD, PhD; Tomoo Furumoto, MD, PhD; Kazuomi Kario, MD, PhD; Teruo Inoue, MD, PhD; Shinji Koba, MD, PhD; Kentaro Watanabe, MD, PhD; Yasuhiko Takemoto, MD, PhD; Takuzo Hano, MD, PhD; Masataka Sata, MD, PhD; Yutaka Ishibashi, MD, PhD; Koichi Node, MD, PhD; Koji Maemura, MD, PhD; Yusuke Ohya, MD, PhD; Taiji Furukawa, MD, PhD; Hiroshi Ito, MD, PhD; Hisao Ikeda, MD, PhD; Akira Yamashina, MD, PhD; Yukihito Higashi, MD, PhD, FAHA

***Background*—**Diagnostic criteria of flow-mediated vasodilation (FMD), an index of endothelial function, and nitroglycerin-induced vasodilation (NID), an index of vascular smooth muscle function, of the brachial artery have not been established. The purpose of this study was to propose diagnostic criteria of FMD and NID for normal endothelial function and normal vascular smooth muscle function.

***Methods and Results*—**We investigated the cutoff values of FMD and NID in subjects with (risk group) and those without cardiovascular risk factors or cardiovascular diseases (no-risk group) in 7277 Japanese subjects (mean age 51.4±10.8 years) from the Flow-Mediated Dilation Japan study and the Flow-Mediated Dilatation Japan Registry study for analysis of the cutoff value of FMD and in 1764 Japanese subjects (62.2±16.1 years) from the registry of Hiroshima University Hospital for analysis of the cutoff value of NID. Receiver-operator characteristic curve analysis of FMD to discriminate subjects in the no-risk group from patients in the risk group showed that the optimal cutoff value of FMD to diagnose subjects in the no-risk group was 7.1%. Receiver-operator characteristic curve analysis of NID to discriminate subjects in the no-risk group from patients in the risk group showed that the optimal cutoff value of NID to diagnose subjects in the no-risk group was 15.6%.

***Conclusions*—**We propose that the cutoff value for normal endothelial function assessed by FMD of the brachial artery is 7.1% and that the cutoff value for normal vascular smooth muscle function assessed by NID of the brachial artery is 15.6% in Japanese subjects.

***Clinical Trial Registration*—**www.umin.ac.jp Unique identifiers: UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409 **(*J Am Heart Assoc*. 2020;9:e013915. DOI: 10.1161/JAHA.119.013915.)**

Key Words: diagnostic criteria • endothelial function • flow-mediated vasodilation • nitroglycerin-induced vasodilation • vascular smooth muscle function

Vascular dysfunction is an important feature of atherosclerosis. Noninvasive vascular function tests such as flow-mediated vasodilation (FMD), pulse wave velocity, cardio-ankle vascular index, ankle brachial pressure index, and augmentation index have been performed for diagnosis of vascular dysfunction and identification of individuals at high risk for cardiovascular complications.[1-3] However, diagnostic criteria for vascular dysfunction have not been established, and the lack of diagnostic criteria is an issue for clinical application of these noninvasive vascular function tests. The lack of diagnostic criteria causes difficulties in appropriate interpretation and utilization of the results of vascular function

A list of affiliations has been placed at the end of this article.
Correspondence to: Yukihito Higashi, MD, PhD, FAHA, Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail: yhigashi@hiroshima-u.ac.jp

Received July 11, 2019; accepted December 6, 2019.

Clinical Perspective

What Is New?

- We investigated the cutoff values of flow-mediated vasodilation and nitroglycerin-induced vasodilation of the brachial artery for discrimination of subjects with and those without cardiovascular risk factors or cardiovascular disease.
- We propose that cutoff value for normal endothelial function assessed by flow-mediated vasodilation of the brachial artery is 7.1% and that the cutoff value for normal vascular smooth muscle function assessed by nitroglycerin-induced vasodilation of the brachial artery is 15.6%.

What Are the Clinical Implications?

- These diagnostic criteria of flow-mediated vasodilation and nitroglycerin-induced vasodilation of the brachial artery are useful for appropriate interpretations and utilization of the results of vascular function tests for the management of cardiovascular disorders.

tests for the management of cardiovascular disorders. Therefore, establishment of diagnostic criteria is needed for the application of these vascular function tests to daily clinical practice.

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays an important role in the development, progression, and maintenance of this condition, leading to cardiovascular complications.[4,5] Endothelial dysfunction precedes the development of overt atherosclerosis and clinical diseases. Therefore, assessment of endothelial function potentially enables early detection of atherosclerosis followed by appropriate interventions, leading to the prevention of cardiovascular complications. Noninvasive diagnostic tools for the assessment of endothelial function have been developed. For example, digital pulse volume amplitude tonometry is used for measuring microcirculatory reactive hyperemia. Changes of pulse wave due to β2 agonist treatment or reactive hyperemia can be detected by digital photoplethysmography. Skin laser Doppler is also used as a noninvasive technique for assessment of endothelial function. However, these methodologies have some limitations, such as an expensive modality, low reproducibility, and susceptibility to oxygen saturation. Several systemic circulating blood markers, such as nitric oxide (NO) product, oxidative stress markers, adhesion molecules, and inflammation markers, have also been measured as indices of endothelial function. However, circulating biochemical markers do not sufficiently reflect endothelial function. FMD of the brachial artery, an index of endothelium-dependent vasodilation, has been widely used for assessment of endothelial function in humans because of its noninvasive nature, reflecting NO production in the endothelium and correlating with coronary artery endothelial function.[6] Nitroglycerin-induced vasodilation (NID) of the brachial artery, an index of endothelium-independent vasodilation, has been used as a control test of FMD. Recent studies have shown that not only FMD but also NID per se is impaired in patients with cardiovascular risk factors or cardiovascular diseases, indicating that NID can be used as a vascular biomarker reflecting vascular smooth muscle function.[7-10] However, diagnostic criteria of FMD and NID have not been established. Because of the lack of diagnostic criteria, it is clinically difficult to provide an appropriate explanation of the results of FMD and NID measurements to the subject and to use the results in the management of cardiovascular disorders. For the establishment of diagnostic criteria, data on FMD and NID measured using the same standardized protocols are necessary. Recently, data on FMD and NID of the brachial artery measured using the same instrument and the same protocol have been obtained in Japan, making it possible to propose diagnostic criteria of vascular function tests. Therefore, we investigated the cutoff values of FMD and NID in subjects with and those without cardiovascular risk factors or cardiovascular diseases to propose diagnostic criteria for normal endothelial function and normal vascular smooth muscle function in Japanese subjects.

Methods

Study 1: Diagnostic Criteria of FMD for Normal Endothelial Function

We investigated the cutoff value of FMD of the brachial artery for discrimination of subjects with and those without cardiovascular risk factors or a history of cardiovascular diseases. Study 1 was conducted in subjects from the FMD-J (Flow-Mediated Dilation Japan) study[11] and the FDR (Flow-Mediated Dilation Japan Registry) study.[12] Detailed information on the subjects and protocol of the FMD-J and FDR studies is publicly available.[11,13] In brief, the FMD-J study was a prospective multicenter study conducted at 22 university hospitals and affiliated clinics in Japan to examine the usefulness of FMD assessment for the management of patients at risk for cardiovascular disease. The FMD-J study included 3 study arms: study A, study B, and study C. Patients aged 20 to 74 years who had been diagnosed as having coronary artery disease were enrolled in study A; patients aged 20 to 74 years with controlled hypertension or diabetes mellitus who had been receiving antihypertensive or antidiabetic treatment for at least 6 months were enrolled in study B, and subjects who underwent annual health checkups as mandated by the company were enrolled in study C. In the FDR study subjects who underwent annual health-screening

examinations with agreement for measurement of FMD were enrolled at the following 3 institutions participating in the FMD-J study: Tokyo Medical University (the core center of the FMD-J study), Hiroshima University, and National Defense Medical College. Some of the data from the FMD-J study and the FDR study have been previously reported elsewhere.[12-14] This study was performed in accordance with the Declaration of Helsinki. The ethical committees of the participating institutions approved the study protocol. Informed consent for participation in the study was obtained from all subjects. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, and UMIN000012952).

Subjects in Study 1

A total of 8652 Japanese subjects (4179 subjects from the FMD-J study and 4473 subjects from the FDR study) were enrolled in study 1. Participants with unclear images of the brachial artery interface (n=1263) and participants with missing information on cardiovascular risk factors (n=112) were excluded. Finally, 7277 subjects were enrolled in study 1.

Study 2: Diagnostic Criteria of NID for Normal Vascular Smooth Muscle Function

In study 2 we investigated the cutoff value of NID of the brachial artery for discrimination of subjects with and those without cardiovascular risk factors or a history of cardiovascular diseases. In the FMD-J study and the FDR study, measurement of NID was not performed. Therefore, study 2 was conducted in subjects who had undergone health-screening examinations or who had visited the outpatient clinic at Hiroshima University Hospital.

Subjects in Study 2

Between July 2007 and June 2019, a total of 2579 subjects underwent measurement of FMD and NID of the brachial artery at Hiroshima University Hospital. Subjects undergoing treatment with nitrate (n=128), patients with malignancy who were undergoing treatment with steroid, nonsteroidal anti-inflammatory drugs, or immunosuppressive drugs (n=155), patients with a diagnosis of secondary hypertension (n=370), and subjects with missing information on cardiovascular risk factors (n=162) were excluded. Finally, 1764 subjects were enrolled in study 2. Subjects enrolled in study 2 were also used as external samples to validate the determined FMD cutoff value in study 1. This study was performed in accordance with the Declaration of Helsinki. The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000003409).

Study Protocol

The same study protocol was used in study 1 and study 2. Subjects fasted the previous night and abstained from caffeine, alcohol, smoking, and antioxidant vitamins on the day of the vascular function test. Each of the subjects was kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 23 -26°C) throughout the study. The vascular function test was performed at least 20 minutes after maintaining the supine position. The observers were blind to the form of the examination.

Measurements of FMD and NID of the Brachial Artery

The same protocol for the measurement of FMD of the brachial artery was used in both the FMD-J study and the FDR study. In both studies FMD was measured using UNEXEF 18G (UNEX Co, Nagoya, Japan), an ultrasound instrument specialized for FMD measurement, except for 10 cases in the FMD-J study. 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, Nagoya, Japan) 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. 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 3 minutes after cuff deflation (Figure 1A). Changes in brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage

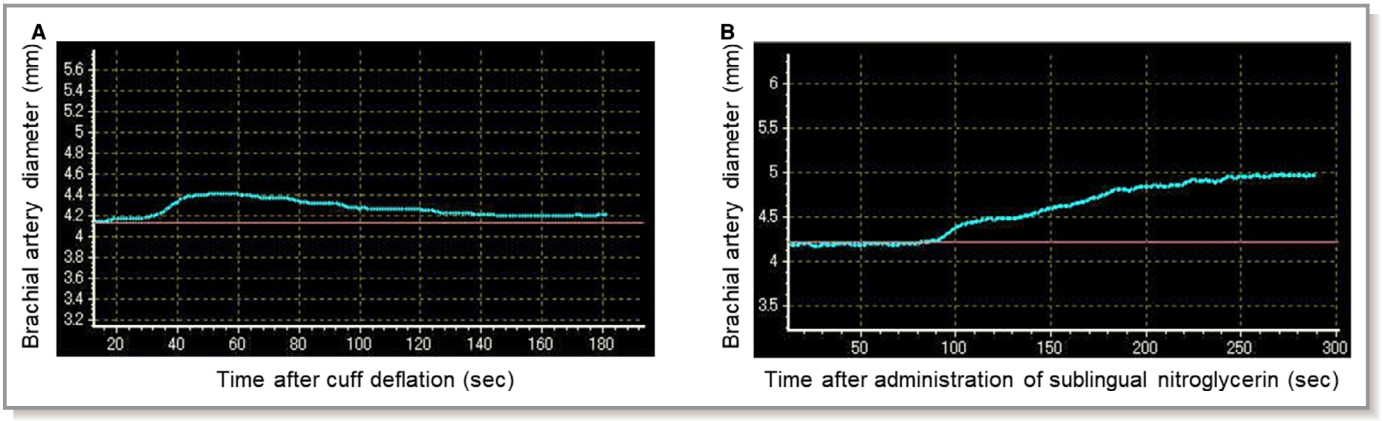

Figure 1. Representative images of change in brachial artery diameter after cuff deflation for measurement of flow-mediated vasodilation (**A**) and after administration of a sublingual nitroglycerin tablet for measurement of nitroglycerin-induced vasodilation (**B**).

change in peak vessel diameter from the baseline value. Percentage of FMD [(peak diameter−baseline diameter)/baseline diameter] was used for analysis.[15] All of the sonographers specialized in FMD measurement at the participating institutions received training for a standard protocol of FMD measurement and training for scanning and analysis of the record at the core laboratory located in Tokyo Medial University in the FMD-J study. All recordings of brachial artery scans obtained during the measurement of FMD were sent from the participant institutions to the core laboratory in Tokyo Medical University by universal serial bus flash drives and were individually analyzed by a well-experienced reader at the core laboratory without any information about the patients in the FMD-J study. The intraclass correlation coefficient between each participating institution and the core laboratory has been previously described.[13] The correlation coefficient between FMD analyzed at the core laboratory and at participant institutions was 0.84 (P<0.001). The intraobserver variability (coefficient of variation) was 10.1% to 11.2%.[16]

NID of the brachial artery was measured using UNEX EF 18G (UNEX Co). The response to nitroglycerin was used for the measurement of NID. After acquisition of baseline rest images for 30 seconds, a sublingual tablet (75 μg nitroglycerin) was given, and images of the brachial artery were recorded continuously until the dilation reached a plateau after administration of the nitroglycerin (Figure 1B). NID was automatically calculated as a percentage change in peak diameter from the baseline value. Percentage NID [(peak diameter−baseline diameter)/baseline diameter] was used for analysis.

Assessment of Cardiovascular Risk Factors and Cardiovascular Diseases

Hypertension, dyslipidemia, diabetes mellitus, smoking, and chronic kidney disease were included in cardiovascular risk factors. Hypertension was defined as treatment with oral antihypertensive agents or systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg without medication. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program.[17] Diabetes mellitus was defined according to the recommendations of the American Diabetes Association.[18] We defined smokers as those who were current smokers. The estimated glomerular filtration rate was calculated using the Japanese equation.[19] Chronic kidney disease was defined as estimated glomerular filtration rate <60 mL/min per 1.73 m^2.[20] Coronary artery disease, cerebrovascular disease, and peripheral artery disease were included in cardiovascular diseases. Coronary artery disease included angina pectoris, myocardial infarction, and unstable angina. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. Peripheral artery disease was defined as current intermittent claudication with ankle-brachial index <0.9 or a history of intervention, including angioplasty and bypass graft.

Statistical Analyses

Results are presented as means±SD for continuous variables and as percentages for categorical variables. 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 the chi-squared test. Continuous variables were compared by using unpaired Student t test. Receiver-operator characteristic (ROC) curve analyses were performed to assess the sensitivity and specificity and to confirm the optimal cutoff values of FMD and NID to diagnose subjects without cardiovascular risk factors or a history of cardiovascular diseases. Cutoff values were determined according to the highest Youden index from the ROC curve analyses. The data were processed using JMP version 11 (SAS Institute, Cary, NC).

Results

Study 1: Diagnostic Criteria of FMD for Normal Endothelial Function

Cutoff Value of FMD of the Brachial Artery

The baseline clinical characteristics of the subjects are summarized in Table 1. The mean age of the subjects was 51.4±10.8 years (median 50 years; interquartile range 44-59 years; range 18-88 years). Of the 7277 subjects (5817 men and 1460 women), 3243 (44.6%) had hypertension, 3767 (51.8%) had dyslipidemia, 703 (9.7%) had diabetes mellitus, 2187 (30.1%) were current smokers, 693 (9.5%) had chronic kidney disease, and 703 (9.7%) had cardiovascular diseases. There were significant differences in FMD between subjects with and those without hypertension (5.4±2.8% versus 6.9±3.1%, $P<0.001$), between subjects with and those without dyslipidemia (5.8±2.9% versus 6.7±3.2%, $P<0.001$), and between subjects with and those without diabetes mellitus (5.0±2.6% versus 6.3±3.1%, $P<0.001$). The mean value of FMD in all subjects was 6.2±3.1% (median 6.0%; interquartile range 4.2% to 8.0%). We divided the subjects into 2 groups: subjects without cardiovascular risk factors or a history of cardiovascular diseases (no-risk group, n=1542) and subjects with cardiovascular risk factors or a history of cardiovascular diseases (risk group, n=5735). The mean values of FMD were 7.5±3.3% (median 7.3%; interquartile range 5.3% to 9.4%) in the no-risk group and 5.9±2.9% (median 5.7%; interquartile range 3.9% to 7.5%) in the risk group.

ROC curve analysis of FMD to discriminate subjects in the no-risk group from patients in the risk group showed that the optimal cutoff value of FMD to diagnose subjects in the no-risk group was 7.1% with an area under the curve (AUC) value of 0.65 (95% CI 0.64-0.67), sensitivity of 0.53, and specificity of

Table 1. Clinical Characteristics of Subjects

Variables	All Subjects (n=7277)	No-Risk Group (n=1542)	Risk Group (n=5735)
Age, y	51.4±10.8	44.5±9.8	53.3±10.3
Men, n (%)	5817 (80.0)	1000 (65.2)	4811 (83.9)
Body mass index, kg/m^2	23.5±3.3	21.8±2.8	24.0±3.3
Systolic blood pressure, mm Hg	127.3±16.5	117.1±11.6	130.0±16.5
Diastolic blood pressure, mm Hg	79.5±11.7	72.9±8.6	81.3±11.8
Heart rate, bpm	63.6±10.2	62.1±9.4	64.0±10.4
Total cholesterol, mmol/L	5.21±0.88	4.90±0.62	5.29±0.91
Triglycerides, mmol/L	1.44±1.03	0.89±0.33	1.59±1.10
HDL cholesterol, mmol/L	1.53±0.41	1.74±0.39	1.47±0.39
LDL cholesterol, mmol/L	3.03±0.77	2.75±0.51	3.11±0.81
Glucose, mmol/L	5.60±1.14	5.16±0.53	5.71±1.23
HbA_{1c}, %	5.7±0.7	5.4±0.3	5.7±0.7
eGFR, mL/min per 1.73 m^2	76.8±14.5	81.7±13.4	75.5±14.5
Current smokers, n (%)	2187 (30.1)	0 (0)	2187 (38.1)
Complications, n (%)			
Hypertension	3243 (44.6)	0 (0)	3243 (56.6)
Dyslipidemia	3767 (51.8)	0 (0)	3767 (65.7)
Diabetes mellitus	703 (9.7)	0 (0)	703 (12.3)
Chronic kidney disease	693 (9.5)	0 (0)	693 (12.1)
Cardiovascular disease	703 (9.7)	0 (0)	703 (9.7)
Medication use, n (%)			
Antihypertensive drugs	2053 (28.2)	0 (0)	2053 (35.8)
Lipid-lowering drugs	1147 (15.8)	0 (0)	1147 (20.0)
Antidiabetic drugs	498 (6.8)	0 (0)	498 (8.7)
FMD, %	6.2±3.1	7.5±3.3	5.9±2.9

All results are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

0.70 (Figure 2A). We validated the determined FMD cutoff value of 7.1% by using subjects enrolled in study 2 as external samples. When the FMD cutoff value of 7.1% to diagnose subjects in the no-risk group was applied to subjects enrolled in study 2, the sensitivity was 0.51, and the specificity was 0.90.

We divided the subjects into 2 groups by sex. Clinical characteristics of the male and female subjects are summarized in Table 2. In men ROC curve analysis showed that the optimal cutoff value of FMD to diagnose subjects in the no-risk group was 7.2% with an AUC value of 0.63 (95% CI 0.61-0.65), sensitivity of 0.48, and specificity of 0.71. In women ROC curve analysis showed that the optimal cutoff value of FMD to diagnose subjects in the no-risk group was 6.2% with an AUC value of 0.70 (95% CI 0.67-0.73), sensitivity of 0.60, and specificity of 0.73.

Next, we divided the subjects into 4 groups according to age decades from the 30s through the 60s. Clinical characteristics according to the age decades are summarized in Table 3. ROC curve analyses showed that the optimal cutoff values of FMD to diagnose subjects in the no-risk group were 8.9% with an AUC value of 0.58 (95% CI 0.54-0.61) in subjects younger than 40 years, 7.1% with an AUC value of 0.61 (95% CI 0.58-0.64) in subjects aged 40 to 49 years, 5.9% with an AUC value of 0.59 (95% CI 0.56-0.63) in subjects aged 50 to 59 years, and 4.5% with an AUC value of 0.63 (95% CI 0.57-0.68) in subjects aged 60 years or older.

Study 2: Diagnostic Criteria of NID for Normal Vascular Smooth Muscle Function

Cutoff Value of NID of the Brachial Artery

The baseline clinical characteristics of the subjects are summarized in Tables 4 and 5. The mean age of the subjects was 62.2±16.1 years (median 67 years; interquartile range, 54-73 years; range 18-92 years). Of the 1764 subjects (1109 men and 655 women), 1449 (82.1%) had hypertension, 1291 (73.2%) had dyslipidemia, 552 (31.3%) had diabetes mellitus, 334 (18.9%) were current smokers, 453 (26.2%) had chronic kidney disease, and 424 (24.0%) had cardiovascular diseases. The mean value of NID in all subjects was 11.9±6.0% (median 11.7%; interquartile range 7.6% to 15.6%). There were significant differences in NID between subjects with and those without hypertension (11.1±5.7% versus 15.8±5.8%, $P<0.001$), between subjects with and those without dyslipidemia (11.5±5.9% versus 13.2±6.0%, $P<0.001$), and between subjects with and those without diabetes mellitus (10.5±5.8% versus 12.6±6.0%, $P<0.001$). We divided the subjects into 2 groups: subjects without cardiovascular risk factors or a history of cardiovascular diseases (no-risk group, n=72) and subjects with cardiovascular risk factors or a history of cardiovascular diseases (risk group, n=1692). The mean values of NID were 17.7±5.7% (median 17.4%; interquartile range 13.2% to 21.7%) in the no-risk group and 11.7±5.9% (median 11.4%; interquartile range 7.4% to 15.3%) in the risk group.

ROC curve analysis of NID to discriminate subjects in the no-risk group from patients in the risk group showed that the optimal cutoff value of NID to diagnose subjects in the no-risk group was 15.6% with an AUC value of 0.77 (95% CI 0.72-0.82), sensitivity of 0.64, and specificity of 0.77 (Figure 2B).

Discussion

In the present study we investigated the cutoff values of FMD and NID of the brachial artery for discrimination of subjects with and those without cardiovascular risk factors or cardiovascular diseases to propose diagnostic criteria for normal endothelial function and normal vascular smooth muscle

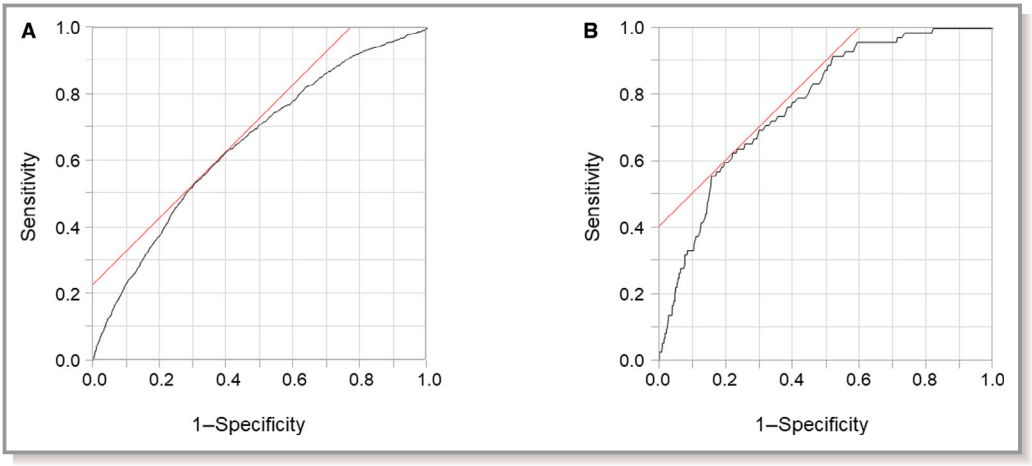

Figure 2. Receiver-operating characteristic curves of flow-mediated vasodilation (**A**) and nitroglycerin-induced vasodilation (**B**) of the brachial artery to discriminate subjects in the no-risk group from patients in the risk group.

Table 2. Clinical Characteristics of Subjects According to Sex

Variables	Men (n=5817)	Women (n=1460)
Age, y	51.0±10.2	52.9±12.8
Body mass index, kg/m^2	23.8±3.2	22.6±3.7
Systolic blood pressure, mm Hg	128.5±15.6	122.6±18.8
Diastolic blood pressure, mm Hg	80.6±11.5	75.3±11.6
Heart rate, bpm	63.4±10.2	64.7±10.2
Total cholesterol, mmol/L	5.19±0.87	5.27±0.87
Triglycerides, mmol/L	1.54±1.09	1.07±0.61
HDL cholesterol, mmol/L	1.47±0.39	1.75±0.41
LDL cholesterol, mmol/L	3.03±0.77	3.03±0.78
Glucose, mmol/L	5.65±1.14	5.38±1.13
HbA_{1c}, %	5.7±0.7	5.7±0.7
eGFR, mL/min per 1.73 m^2	76.4±14.0	78.6±16.0
Current smokers, n (%)	2062 (35.5)	125 (8.6)
Complications, n (%)		
Hypertension	2617 (45.0)	626 (42.9)
Dyslipidemia	3115 (53.6)	652 (44.7)
Diabetes mellitus	567 (9.8)	136 (9.3)
Chronic kidney disease	554 (9.5)	139 (9.5)
Cardiovascular disease	600 (10.3)	103 (7.1)
Medication use, n (%)		
Antihypertensive drugs	1554 (26.7)	499 (34.2)
Lipid-lowering drugs	876 (15.1)	271 (18.6)
Antidiabetic drugs	413 (7.1)	85 (5.8)
FMD, %	6.1±3.0	6.6±3.4
No-risk group, n (%)	1006 (17.3)	536 (36.7)

All results are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

function in Japanese subjects. The cutoff value of FMD for discrimination of subjects with and those without cardiovascular risk factors or cardiovascular disease was 7.1%, and that of NID was 15.6%. Therefore, we propose that the cutoff value for normal endothelial function assessed by FMD of the brachial artery is 7.1% and that the cutoff value for normal vascular smooth muscle function assessed by NID of the brachial artery is 15.6% in Japanese subjects.

Recently, FMD of the brachial artery has been widely used for assessment of endothelial function in humans. FMD of the brachial artery has been shown to be associated with cardiovascular risk factors, structural arterial disease, and cardiovascular outcome, providing incremental prognostic information. Therefore, measurement of FMD of the brachial artery is recommended for the assessment of cardiovascular risk.[21] Although the precise mechanisms by which vasodilation occurs during ischemic reactive hyperemia in FMD measurement have not been fully elucidated, shear stress–induced NO production in the endothelium has been proposed as a principal mediator of FMD. Endothelial dysfunction may be caused by oxidative stress, which can decrease NO production and increase NO inactivation, leading to decreased NO bioavailability and consequent FMD impairment. Meta-analyses have shown that an increase in the FMD value of 1% is associated with a 12% to 13% reduction in the odds of cardiovascular events independent of conventional cardiovascular risk factors, indicating that FMD may be a useful vascular biomarker for cardiovascular risk stratification.[22,23] Clinical studies demonstrated relatively close correlations between brachial endothelial function assessed by FMD and coronary artery endothelial function assessed by diameter change in the epicardial coronary artery in response to shear stress or intracoronary acetylcholine infusion.[24-26] This relationship may explain, in part, the ability of FMD of the brachial artery to predict future cardiovascular events. However, at present, as a vascular biomarker, measurement of FMD in the brachial artery is given a Class III recommendation (no benefit) with evidence level of B in the guidelines from the American College of Cardiology Foundation/American Heart Association for assessment of cardiovascular risk in asymptomatic adults and in the position paper from the European Society of Cardiology Working Group for the role of vascular biomarkers for primary and secondary prevention.[2,27] Important issues in practical application of FMD include technical difficulties, low reproducibility, the lack of standardized protocols, and the lack of diagnostic criteria. FMD measurement requires a trained sonographer with a comprehensive understanding of a standard protocol, measurement conditions, scanning, and analysis of the recordings. In addition, there is a large variation in the protocols for FMD measurement due to differences in testing modality, position of occlusion cuff placement (forearm or upper arm), cuff occlusion pressure (50 mm Hg above systolic blood pressure, 44 mm Hg above systolic blood pressure, 200, 250, 260, or 300 mm Hg), cuff occlusion duration (3, 4, 4.5, or 5 minutes), and time frame for measurement of diameter changes during reactive hyperemia (measurement at 1 arbitrary time point, 45 or 60 seconds; measurements at multiple arbitrary time points, 45-60-90-180-300 seconds, 20-40-60-90-120 seconds, or 60-90 seconds; or measurement within a fixed time window during reactive hyperemia, 0 to 60, 0 to 90, 0 to 120, 45 to 60, 60 to 75, 60 to 90, or 45 to 90 seconds). These limitations make it difficult to establish diagnostic criteria for appropriate interpretation of results of FMD in clinical practice. However, testing modalities and protocols for FMD measurement have recently been standardized in Japan. The FMD-J study, a prospective multicenter study, was conducted at 22 university hospitals and affiliated clinics in Japan from May 2010 to August 2015 to assess the

Table 3. Clinical Characteristics of Subjects According to Age Decades

Variables	<40 y (n=941)	40 to 49 y (n=2260)	50 to 59 y (n=2434)	≥60 y (n=1642)
Age, y	34.5±4.4	44.8±3.2	54.2±3.2	66.1±5.2
Men, n (%)	711 (75.6)	1927 (85.3)	2000 (82.2)	1179 (71.8)
Body mass index, kg/m^2	22.7±3.7	23.6±3.5	23.6±3.1	23.9±3.1
Systolic blood pressure, mm Hg	120.6±14.6	125.1±14.8	128.3±16.4	132.6±17.8
Diastolic blood pressure, mm Hg	74.4±11.3	79.8±11.6	81.5±11.8	79.2±11.2
Heart rate, bpm	63.5±10.0	63.3±10.0	63.7±10.3	64.0±10.5
Total cholesterol, mmol/L	4.91±0.82	5.26±0.84	5.37±0.85	5.05±0.91
Triglycerides, mmol/L	1.23±1.06	1.47±1.05	1.49±0.97	1.46±1.05
HDL cholesterol, mmol/L	1.54±0.40	1.52±0.39	1.56±0.42	1.48±0.40
LDL cholesterol, mmol/L	2.81±0.73	3.08±0.75	3.14±0.76	2.92±0.81
Glucose, mmol/L	5.08±0.58	5.45±1.03	5.67±1.10	5.99±1.41
HbA_{1c}, %	5.4±0.3	5.5±0.6	5.7±0.7	6.0±0.8
eGFR, mL/min per 1.73 m^2	86.2±13.4	79.2±13.2	75.2±13.4	70.6±15.0
Current smokers, n (%)	276 (29.3)	816 (36.1)	829 (34.1)	266 (16.2)
Complications, n (%)				
Hypertension	143 (15.2)	683 (30.2)	1096 (45.0)	1321 (80.5)
Dyslipidemia	274 (29.1)	1059 (46.9)	1295 (53.2)	1139 (69.4)
Diabetes mellitus	10 (1.1)	110 (4.9)	224 (9.2)	359 (21.9)
Chronic kidney disease	12 (1.3)	102 (4.5)	227 (9.3)	352 (21.4)
Cardiovascular disease	9 (1.0)	50 (2.2)	163 (6.7)	481 (29.3)
Medication use, n (%)				
Antihypertensive drugs	37 (3.9)	302 (13.4)	581 (23.9)	1133 (69.0)
Lipid-lowering drugs	16 (1.7)	155 (6.9)	303 (12.5)	673 (41.0)
Antidiabetic drugs	8 (0.9)	75 (3.3)	163 (14.0)	252 (15.4)
FMD, %	7.8±3.1	6.9±3.1	5.8±2.8	5.0±2.7
No-risk group, n (%)	457 (48.6)	601 (26.6)	401 (16.5)	83 (5.1)

All results are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

clinical usefulness of semiautomatic measurement of FMD of the brachial artery in the management of patients with cardiovascular risk factors or cardiovascular diseases.[11,13] In the FMD-J study most of the FMD measurements were performed using the UNEX EF 18G (UNEX Co, Nagoya, Japan), an ultrasound instrument specialized for FMD measurement equipped with an automated edge-tracking system. Furthermore, FMD measurements were performed using a standardized protocol in which an occlusion cuff was placed around the forearm and inflated to 50 mm Hg above systolic blood pressure for 5 minutes to induce reactive hyperemia with continuous assessment of diameter change during reactive hyperemia. Therefore, the protocols for FMD measurement have been standardized, and data on FMD measured using the same instrument and the same standardized protocol have been obtained, making it possible to propose diagnostic criteria of FMD for normal endothelial function in Japan. The cutoff value of FMD for discrimination of subjects with and those without cardiovascular risk factors or cardiovascular diseases was 7.1% in a total of 7277 Japanese subjects. Therefore, we propose that the cutoff value of FMD of the brachial artery for normal endothelial function is 7.1% in Japanese subjects.

Previous studies have shown that FMD in women is significantly higher than that in men and that there is a negative correlation between age and FMD.[12,28,29] In the present study, when the subjects were divided into 2 groups by sex, the optimal cutoff values of FMD were 7.2% in men and 6.2% in women. Although we do not know the precise reasons for the substantial difference in the optimal cutoff value of FMD between men and women in the present study, there are some possible explanations, including differences in

Table 4. Clinical Characteristics of the Subjects

Variables	All Subjects (n=1764)	No-Risk Group (n=72)	Risk Group (n=1692)
Age, y	62.2±16.1	31.8±15.3	63.5±14.8
Men, n (%)	1109 (62.9)	53 (73.6)	1056 (62.5)
Body mass index, kg/m^2	23.8±3.8	21.1±2.7	23.9±3.9
Systolic blood pressure, mm Hg	130.1±18.4	115.1±9.4	130.8±18.5
Diastolic blood pressure, mm Hg	76.7±12.1	66.5±9.3	77.1±12.0
Heart rate, bpm	69.5±12.0	64.7±11.5	69.7±12.0
Total cholesterol, mmol/L	4.94±0.97	4.48±0.62	4.96±0.98
Triglycerides, mmol/L	1.59±1.27	0.83±0.33	1.62±1.29
HDL cholesterol, mmol/L	1.53±0.43	1.68±0.38	1.52±0.43
LDL cholesterol, mmol/L	2.82±0.85	2.45±0.53	2.84±0.86
Glucose, mmol/L	6.31±2.19	4.89±0.92	6.37±2.21
HbA_{1c}, %	5.6±1.0	5.1±0.3	5.6±1.0
eGFR, mL/min per 1.73 m^2	70.4±20.3	88.8±12.8	114.8±39.8
Current smokers, n (%)	334 (18.9)	0 (0)	334 (19.7)
Complications, n (%)			
Hypertension	1449 (82.1)	0 (0)	334 (19.7)
Dyslipidemia	1291 (73.2)	0 (0)	1291 (76.3)
Diabetes mellitus	552 (31.3)	0 (0)	552 (32.6)
Chronic kidney disease	453 (26.2)	0 (0)	453 (27.4)
Cardiovascular disease	424 (24.0)	0 (0)	424 (25.1)
Medication use, n (%)			
Antihypertensive drugs	1223 (70.1)	0 (0)	1223 (73.2)
Lipid-lowering drugs	737 (42.2)	0 (0)	737 (44.1)
Antidiabetic drugs	224 (12.8)	0 (0)	224 (13.4)
NID, %	11.9±6.0	17.7±5.7	11.7±5.9

All results are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NID, nitroglycerin-induced vasodilation.

the numbers of subjects (5817 men versus 1460 women) and the percentages of subjects in the no-risk group (17.3% versus 36.7%) between men and women. When subjects were divided into 4 groups according to age decades, the optimal cutoff values of FMD were 8.9% in subjects younger than 40 years, 7.1% in subjects aged 40 to 49 years, 5.9% in subjects aged 50 to 59 years, and 4.5% in subjects aged 60 years or older, indicating that the optimal cutoff value of FMD declines with age. Because a negative correlation between age and FMD has been consistently shown in previous studies, the decline in the optimal cutoff value of FMD with age may be relevant.

We previously reported that FMD above 7.1% is significantly associated with a lower risk of cardiovascular events in patients with coronary artery disease. Given that the cutoff value of FMD for normal endothelial function was 7.1% in this study, FMD may be useful for identifying subjects not at high risk but at low risk for cardiovascular events. Although further improvement in methodology is required,[30] FMD may be an important diagnostic methodology to identify patients at low risk for cardiovascular events. Not all of the subjects with FMD ≤7.1% are at high risk for future cardiovascular events. We previously found that FMD <2.9% was associated with a higher risk of first major cardiovascular event in a retrospective study in which measurements of FMD were performed using the same protocol as that used in the present study.[31] Further prospective studies are needed to investigate the optimal cutoff for predicting future cardiovascular events.

Measurement of NID, an index of endothelium-independent vasodilation, has been performed as a control test of FMD to confirm that an impaired vasodilatory response to reactive hyperemia is not due to decreased reactivity of vascular smooth muscle cells to NO released from the endothelium but truly is a consequence of endothelial dysfunction. However,

Table 5. Clinical Characteristics of the Subjects According to Sex

Variables	Men (n=1109)	Women (n=653)
Age, y	59.7±17.6	66.5±11.9
Body mass index, kg/m^2	24.0±3.7	23.4±4.1
Systolic blood pressure, mm Hg	129.4±18.2	131.3±18.8
Diastolic blood pressure, mm Hg	77.3±12.1	75.7±12.0
Heart rate, bpm	68.7±12.1	70.9±11.9
Total cholesterol, mmol/L	4.82±0.95	5.15±0.96
Triglycerides, mmol/L	1.70±1.47	1.40±0.81
HDL cholesterol, mmol/L	1.47±0.41	1.63±0.43
LDL cholesterol, mmol/L	2.74±0.83	2.97±0.86
Glucose, mmol/L	6.35±2.30	6.26±2.00
HbA_{1c}, %	5.6±1.1	5.6±0.7
eGFR, mL/min per 1.73 m^2	71.6±20.8	68.5±19.1
Current smokers, n (%)	289 (26.1)	44 (6.7)
Complications, n (%)		
Hypertension	877 (79.1)	570 (87.3)
Dyslipidemia	764 (68.9)	525 (80.4)
Diabetes mellitus	321 (28.9)	230 (35.2)
Chronic kidney disease	261 (24.1)	190 (29.6)
Cardiovascular disease	325 (29.3)	98 (15.0)
Medication use, n (%)		
Antihypertensive drugs	737 (67.4)	484 (74.6)
Lipid-lowering drugs	413 (37.8)	323 (49.6)
Antidiabetic drugs	137 (12.5)	86 (13.2)
NID, %	12.4±5.9	11.2±6.1
No-risk group, n (%)	53 (4.8)	19 (2.9)

All results are presented as mean±SD. eGFR indicates estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NID, nitroglycerin-induced vasodilation.

recent studies have revealed that NID per se is impaired in patients with cardiovascular risk factors or cardiovascular diseases, indicating an association between vascular smooth muscle dysfunction and cardiovascular disorders and raising the possibility that NID of the brachial artery can be used as a vascular biomarker.[7-10,32] A recent clinical study demonstrated significant correlations of NID in the brachial artery with NID in the left anterior descending coronary artery and NID in the left circumflex coronary artery assessed by intracoronary isosorbide dinitrate infusion.[33] Although the mechanisms underlying the impairment of NID in patients with cardiovascular risk factors have not been fully elucidated, involvement of increased oxidative stress in vascular smooth muscle cells has been postulated. Oxidative stress has been shown to inhibit NO-mediated intracellular signaling pathways in vascular smooth muscle cells, leading to impaired relaxation of vascular smooth muscle cells and consequent NID impairment.[10] We previously reported that vascular smooth muscle function assessed by NID is maintained in patients at low risk for cardiovascular disease in whom endothelial function assessed by FMD was already impaired, whereas both FMD and NID were impaired in patients at high risk for cardiovascular disease.[9] These findings suggest that FMD is an appropriate marker of an early stage of atherosclerosis, whereas NID can be used as a vascular biomarker of an advanced stage of atherosclerosis. However, the cutoff value of NID for normal vascular smooth muscle function is unclear. Measurement of NID was not performed in the FMD-J study or in the FDR study. Therefore, we investigated the cutoff value of NID using data from subjects who underwent health-screening examinations or who visited the outpatient clinic at Hiroshima University Hospital. The cutoff value of NID for discrimination of subjects with and those without cardiovascular risk factors or cardiovascular diseases was 15.6%. Therefore, we propose that the cutoff value of NID of the brachial artery for normal vascular smooth muscle function is 15.6% in Japanese patients. Because of the small number of subjects in the no-risk group (n=72), we did not perform analysis of cutoff values of NID by sex or age decades.

In subjects with impaired NID, it is theoretically impossible to discern whether FMD impairment is a result of endothelial dysfunction reflecting a balance between NO production in the endothelium and NO destruction by reactive oxygen species or concomitant abnormalities of endothelial function and vascular smooth muscle function. In these patients FMD should be interpreted not as an index of endothelial function but as flow-mediated vascular reactivity reflecting both endothelium-dependent and endothelium-independent vasodilation.

Although it is possible to measure FMD or NID in other conduit arteries, such as the popliteal artery,[34] measurements of vascular functions in other peripheral arteries are technically challenging and are not regarded as a standard method for the assessment of vascular functions. Measurements of FMD and NID of the brachial artery have been performed for almost 40 years and have been regarded as standard noninvasive methods for the assessments of endothelial function and vascular smooth muscle function. We therefore investigated the diagnostic criteria for normal FMD and NID of the brachial artery in this study.

There are some limitations in this study. First, FMD and NID measurements were performed only in Japanese subjects. Therefore, it would be difficult to apply the current diagnostic criteria of FMD and NID to other races/ethnicities. Second, FMD measurements were performed using a protocol in which an occlusion cuff was placed around the forearm and inflated to 50 mm Hg above systolic blood pressure for 5 minutes to induce reactive hyperemia. Thus, it would be difficult to apply

the current diagnostic criterion of FMD to subjects in whom FMD measurement is performed using different protocols. Notably, brachial artery dilatation has been shown to be greater after upper arm occlusion than after forearm occlusion.[35] For example, the mean value of FMD of the brachial artery measured using upper arm occlusion was reported to be 6.0±3.5% in patients with stable coronary artery disease, whereas the mean value of FMD measured using forearm occlusion was 4.8±2.6% in patients with coronary artery disease.[3,36] Third, the response of the brachial artery to 75 μg of sublingually administered nitroglycerin was used for the measurement of NID. Therefore, it is difficult to apply the current diagnostic criterion of NID to subjects in whom different doses of nitroglycerin are used for NID measurement. The mean value of NID of the brachial artery measured using 800 μg sublingual nitroglycerin was reported to be 12.5±5.4% in patients with stable coronary artery disease, whereas the mean value of NID measured using 75 μg sublingual nitroglycerin was 10.5±5.6% in patients with cardiovascular disease.[9,36] Fourth, the number of subjects without cardiovascular risk factors or cardiovascular diseases included in analysis of the cutoff value of NID was small because the study subjects were recruited mainly from an outpatient clinic. In addition, subjects in the no-risk group were younger than those in the risk group. If a sufficient number of middle-aged and older subjects for statistical analysis had been included in the no-risk group, the cutoff value of NID for normal vascular smooth muscle function would have been lower. Further study is needed to investigate the cutoff value for normal NID in a population with a sufficiently large number of subjects who do not have cardiovascular risk factors or cardiovascular diseases. Fifth, baseline brachial artery diameter is a significant confounding factor in the measurements of FMD and NID calculated as a relative percentage change in baseline brachial artery diameter in response to reactive hyperemia or nitroglycerin.[37] A previous clinical study showed that the absolute percentage change in brachial artery diameter does not correctly scale for interindividual differences in baseline brachial artery diameter but overestimates vascular response for small baseline brachial artery diameters and vice versa. Allometric scaling, a statistical approach to account for the association between baseline and peak brachial artery diameters, may be a useful method for comparing different settings and/or populations whenever differences in baseline diameter are present between or within individuals.[37]

Conclusions

We investigated the cutoff values of FMD and NID of the brachial artery to establish diagnostic criteria for normal vascular function in Japanese subjects. We propose that the cutoff value of FMD for normal endothelial function is 7.1% and that the cutoff value of NID for normal vascular smooth muscle function is 15.6% in Japanese subjects. Further studies are needed to determine whether the current diagnostic criteria of FMD and NID for normal vascular function are appropriate for identifying subjects at low risk for cardiovascular events in a prospective large clinical study.

Acknowledgments

We thank Megumi Wakisaka, Miki Kumiji, Ki-ichiro Kawano, and Satoko Michiyama for their excellent secretarial assistance.

Sources of Funding

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (18590815 and 21590898 to Y.H.) and a Grant in Aid of the Japanese Arteriosclerosis Prevention Fund (to Y.H.).

Disclosures

None.

Authors' Affiliations

From the Department of Cardiovascular Medicine, Graduate School of Biomedical and Health Sciences (T.M., H.H., Y. Takaeko, T. Yamaji, T. Harada, Y. Han, T. Hidaka, Y.K.), Division of Regeneration and Medicine (M.K.), Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine (S.K., Y.A., F.M.Y., Y. Higashi), Department of Gastroenterology and Metabolism, Biomedical Sciences, Graduate School of Biomedical and Health Sciences (K.C., Y. Higashi), and Department of Stem Cell Biology and Medicine, Graduate School of Biomedical and Sciences (A.N.), Hiroshima University, Hiroshima, Japan; Hiroshima International University, Hiroshima, Japan (C.G., A.Y.); Department of Cardiology, Tokyo Medical University, Tokyo, Japan (H.T.); Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan (B.T.); Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Tochigi, Japan (T.K., K.K.); Cardiovascular Medicine, University of Leicester, United Kingdom (T.S.); Cardiovascular Division, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan (T.I.); Department of Clinical Pharmacology and Therapeutics, University of the Ryukyu School of Medicine, Okinawa, Japan (S.U.); Clinical Research Support Center, Faculty of Medicine, The University of Tokyo, Japan (T. Yamazaki); Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine,

Hokkaido, Japan (T.F.); Department of Cardiovascular Medicine, Dokkyo Medical University, Tochigi, Japan (T.I.); Division of Cardiology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan (S.K.); Department of Neurology, Hematology, Metaboism, Endocrinology and Diabetology (DNHMED), Yamagata University School of Medicine, Yamagata, Japan (K.W.); Department of Internal Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka, Japan (Y. Takemoto); Department of Medical Education and Population-Based Medicine, Postgraduate School of Medicine, Wakayama Medical University, Wakayama, Japan (T.H.); Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan (M.S.); Department of General Medicine, Shimane University Faculty of Medicine, Izumo, Japan (Y.I.); Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan (K.N.); Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan (K.M.); The Third Department of Internal Medicine, University of the Ryukyus, Okinawa, Japan (Y.O.); Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan (T.F.); Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan (H. Ito); Faculty of Fukuoka Medical Technology, Teikyo University, Omuta, Japan (H. Ikeda).

References

1. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. *Circulation*. 2010;122:e584–e636.
2. Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cifkova R, Cosentino F, De Carlo M, Gallino A, Landmesser U, Laurent S, Lekakis J, Mikhailidis DP, Naka KK, Protogerou AD, Rizzoni D, Schmidt-Trucksass A, Van Bortel L, Weber T, Yamashina A, Zimlichman R, Boutouyrie P, Cockcroft J, O'Rourke M, Park JB, Schillaci G, Sillesen H, Townsend RR. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. *Atherosclerosis*. 2015;241:507–532.
3. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Iwamoto A, Kajikawa M, Matsumoto T, Oda N, Kishimoto S, Matsui S, Hashimoto H, Aibara Y, Mohamad Yusoff F, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Tomiyama H, Takase B, Kohro T, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Ikeda H, Yamashina A, Higashi Y. Endothelial dysfunction, increased arterial stiffness, and cardiovascular risk prediction in patients with coronary artery disease: FMD-J (Flow-Mediated Dilation Japan) Study A. *J Am Heart Assoc*. 2018;7:e008588 DOI: 10.1161/JAHA.118.008588.
4. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
5. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. *Circ J*. 2009;73:411–418.
6. Higashi Y. Assessment of endothelial function. History, methodological aspects, and clinical perspectives. *Int Heart J*. 2015;56:125–134.
7. Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, Celermajer DS. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol*. 1998;32:123–127.
8. Raitakari OT, Seale JP, Celermajer DS. Impaired vascular responses to nitroglycerin in subjects with coronary atherosclerosis. *Am J Cardiol*. 2001;87:217–219, A218.
9. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Kajikawa M, Matsumoto T, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Higashi Y. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. *Arterioscler Thromb Vasc Biol*. 2013;33:1401–1408.
10. Maruhashi T, Kihara Y, Higashi Y. Assessment of endothelium-independent vasodilation: from methodology to clinical perspectives. *J Hypertens*. 2018;36:1460–1467.
11. Tomiyama H, Kohro T, Higashi Y, Takase B, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Yamashina A. A multicenter study design to assess the clinical usefulness of semi-automatic measurement of flow-mediated vasodilatation of the brachial artery. *Int Heart J*. 2012;53:170–175.
12. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Kajikawa M, Matsumoto T, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Tomiyama H, Takase B, Yamashina A, Higashi Y. Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart*. 2013;99:1837–1842.
13. Tomiyama H, Kohro T, Higashi Y, Takase B, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Ikeda H, Yamashina A. Reliability of measurement of endothelial function across multiple institutions and establishment of reference values in Japanese. *Atherosclerosis*. 2015;242:433–442.
14. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Kajikawa M, Matsumoto T, Kihara Y, Chayama K, Noma K, Nakashima A, Tomiyama H, Takase B, Yamashina A, Higashi Y. Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. *Circulation*. 2012;126:598–603.
15. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.
16. Tomiyama H, Higashi Y, Takase B, Node K, Sata M, Inoue T, Ishibashi Y, Ueda S, Shimada K, Yamashina A. Relationships among hyperuricemia, metabolic syndrome, and endothelial function. *Am J Hypertens*. 2011;24:770–774.
17. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA*. 2001;285:2486–2497.
18. American Diabetes Association. Clinical practice recommendations 1999. *Diabetes Care*. 1999;22(suppl 1):S1–S114.
19. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida ACollaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–992.
20. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL, Eckardt KU. The definition, classification, and prognosis of chronic kidney disease: a KDIGO controversies conference report. *Kidney Int*. 2011;80:17–28.
21. Charakida M, Masi S, Luscher TF, Kastelein JJ, Deanfield JE. Assessment of atherosclerosis: the role of flow-mediated dilatation. *Eur Heart J*. 2010;31:2854–2861.
22. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging*. 2010;26:631–640.
23. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. *J Am Heart Assoc*. 2015;4:e002270. DOI: 10.1161/JAHA.115.002270.
24. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.
25. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, Kurita A. Endothelium-dependent flow-mediated vasodilation in

coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol*. 1998;82:1535–1539, A1537–1538.

26. Teragawa H, Ueda K, Matsuda K, Kimura M, Higashi Y, Oshima T, Yoshizumi M, Chayama K. Relationship between endothelial function in the coronary and brachial arteries. *Clin Cardiol*. 2005;28:460–466.
27. Akamatsu D, Sato A, Goto H, Watanabe T, Hashimoto M, Shimizu T, Sugawara H, Sato H, Nakano Y, Miura T, Zukeran T, Serizawa F, Hamada Y, Tsuchida K, Tsuji I, Satomi S. Nitroglycerin-mediated vasodilatation of the brachial artery may predict long-term cardiovascular events irrespective of the presence of atherosclerotic disease. *J Atheroscler Thromb*. 2010;17:1266–1274.
28. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24:1468–1474.
29. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, Lehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham heart study. *Circulation*. 2004;109:613–619.
30. Matsui S, Kajikawa M, Maruhashi T, Hashimoto H, Kihara Y, Chayama K, Goto C, Aibara Y, Yusoff FM, Kishimoto S, Nakashima A, Noma K, Kawaguchi T, Matsumoto T, Higashi Y. New assessment of endothelial function measured by short time flow-mediated vasodilation: comparison with conventional flow-mediated vasodilation measurement. *Int J Cardiol*. 2018;265:24–29.
31. Kajikawa M, Maruhashi T, Hida E, Iwamoto Y, Matsumoto T, Iwamoto A, Oda N, Kishimoto S, Matsui S, Hidaka T, Kihara Y, Chayama K, Goto C, Aibara Y, Nakashima A, Noma K, Higashi Y. Combination of flow-mediated vasodilation and nitroglycerine-induced vasodilation is more effective for prediction of cardiovascular events. *Hypertension*. 2016;67:1045–1052.
32. Maruhashi T, Nakashima A, Matsumoto T, Oda N, Iwamoto Y, Iwamoto A, Kajikawa M, Kihara Y, Chayama K, Goto C, Noma K, Higashi Y. Relationship between nitroglycerine-induced vasodilation and clinical severity of peripheral artery disease. *Atherosclerosis*. 2014;235:65–70.
33. Maruhashi T, Kajikawa M, Nakashima A, Iwamoto Y, Iwamoto A, Oda N, Kishimoto S, Matsui S, Higaki T, Shimonaga T, Watanabe N, Ikenaga H, Hidaka T, Kihara Y, Chayama K, Goto C, Aibara Y, Noma K, Higashi Y. Nitroglycerine-induced vasodilation in coronary and brachial arteries in patients with suspected coronary artery disease. *Int J Cardiol*. 2016;219:312–316.
34. Iwamoto A, Kajikawa M, Maruhashi T, Iwamoto Y, Oda N, Kishimoto S, Matsui S, Kihara Y, Chayama K, Goto C, Noma K, Aibara Y, Nakashima A, Higashi Y. Vascular function and intima-media thickness of a leg artery in peripheral artery disease: a comparison of Buerger disease and atherosclerotic peripheral artery disease. *J Atherodcler Thromb*. 2016;23:1261–1269.
35. Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, Goodfellow J. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci (Lond)*. 2001;101:629–635.
36. Ostad MA, Wild PS, Schnorbus B, Schulz A, Munzel T, Warnholtz A. Predictive value of brachial reactive hyperemia and flow-mediated dilation in stable coronary artery disease. *Clin Hemorheol Microcirc*. 2014;56:247–257.
37. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens*. 2013;31:287–291.