Assessment of Endothelial Function
History, Methodological Aspects, and Clinical Perspectives

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SUMMARY

In 1986, endothelial function was measured for the first time in patients with atherosclerotic coronary arteries. Since then, several methods for assessment of endothelial function, such as endothelium-dependent vasodilation induced by intra-arterial infusion of vasoactive agents using coronary angiography, Doppler flow guide wire, mercury-filled Silastic strain-gauge plethysmography, flow-mediated vasodilation, reactive hyperemia-peripheral arterial tonometry, and vascular response using an oscillometric method have been performed in humans. This review focuses on the assessment of endothelial function, including measurement history, methodological issues, and clinical perspectives. (Int Heart J 2015; 56: 125-134)

Key words: Plethysmography, Flow-mediated vasodilation, Reactive hyperemia-peripheral arterial tonometry, Biochemical marker

Almost 30 years have passed since the first assessment of endothelial function in humans. Results of studies on endothelial function in humans have shown reliable methods for measurement of endothelial function, relationships between atherosclerosis and endothelial function, mechanisms of endothelial dysfunction, and clinical implication such as the effects of interventions and cardiovascular events. Endothelial dysfunction is the initial step of atherosclerosis and is involved in the development of atherosclerosis, and it might increase the risk of cardiovascular and cerebrovascular diseases, resulting in cardiovascular events. It is possible that measurement of endothelial function will reveal one of the earliest changes that can be seen in atherosclerosis. Therefore, it is clinically important to assess endothelial function using an established method. Several methods have been used for assessment of endothelial function in humans, including endothelium-dependent vasodilation induced by intra-arterial infusion of vasoactive agents using coronary angiography, a Doppler flow guide wire, and plethysmography, as well as flow-mediated vasodilation (FMD), reactive hyperemia-peripheral arterial tonometry (RH-PAT), and vascular response using an oscillometric method. Although vascular responses to intra-arterial infusion of vasoactive agents, such as agonists to stimulate nitric oxide (NO) release and antagonists of NO, should be considered as the gold standard for assessment of endothelial function, this technique is invasive and burdensome for the subjects. FMD elicited by shear stress-induced NO production from endothelial cells is now widely used worldwide. RH-PAT is also noninvasive, simple, and reproducible. Each method has advantages and disadvantages with respect to methodological aspects and clinical perspectives.

Clinical Perspectives of Assessment of Endothelial Function

Structure and physiology of the endothelium: Both vascular structure and function play an important role in the development and maintenance of atherosclerosis. If the endothelium of the whole body could be collected, its total weight would be equal to that of the liver, its total area would be equal to that of 6 tennis courts, and its total length is 100,000 km (2.5 times around the globe). Endothelial cells secrete various vasoactive agents such as vasodilators, including NO, prostanoylin and endothelin-derived hyperpolarizing factor, and vasoconstrictors, including endothelin-1, angiotensin II and thromboxane A2. Thus, the vascular endothelium might be the largest endocrine organ in the human body. A healthy endothelium maintains vascular tone and structure by regulating the balance between vasodilation and vasoconstriction, growth inhibition and growth promotion, antiinflammation and proinflammation, and also antioxidant and pro-oxidation.

Endothelial function and atherosclerosis: Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays an important role in the development of this condition (Figure 1). Endothelium-dependent vasodilation is impaired

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Key words: Plethysmography, Flow-mediated vasodilation, Reactive hyperemia-peripheral arterial tonometry, Biochemical marker
in various vascular beds, including the forearm, coronary, leg and renal arteries, in patients with cardiovascular diseases and subjects with coronary risk factors. It is clinically important to select an appropriate intervention that is effective in improving endothelial function in patients with cardiovascular diseases. Several interventions, including pharmacological therapy, administration of antihypertensive drugs such as renin-angiotensin system inhibitors, statins, and thiazolidinedione derivatives, supplementation therapy such as administration of L-arginine, estrogen replacement, administration of a cofactor of NO tetrahydrobiopterine, treatment with antioxidant vitamins, and lifestyle modifications such as aerobic exercise, body weight reduction, smoking cessation, and sodium restriction have been shown to improve endothelial function. These findings suggest that endothelial dysfunction in patients with cardiovascular diseases is reversible.

**Endothelial function and cardiovascular events:** From a clinical perspective, it is clinically important to confirm whether or not endothelial function is associated with an increased risk of cardiovascular events. Several lines of evidence have shown that measurement of endothelial function, including plethysmography using intra-arterial infusion of vasoactive agents, coronary flow or diameter response to vasoactive agents, FMD and RH-PAT, is an independent predictor of cardiovascular events (Table 1). Perticone, et al demonstrated cardiac outcome characterized by the three tertiles of acetylcholine-induced vasodilation in forearm circulation in patients with essential hypertension, and they found that patients with the lowest tertile of acetylcholine-induced vasodilation had a significantly higher event ratio than did patients with moderate and high tertiles of acetylcholine-induced vasodilation. Severe coronary endothelial dysfunction is associated with increased cardiovascular events in patients with coronary artery diseases. Schachinger, et al demonstrated that coronary endothelial dysfunction assessed by intra-arterial infusion of acetylcholine was an independent predictor of atherosclerotic disease progression and subsequent cardiovascular events in patients with coronary artery diseases. Both acetylcholine-induced vasodilation and FMD are also useful for predicting cardiovascular events in these patients. In patients with peripheral arterial disease also, conduit artery endothelial dysfunction assessed by brachial artery FMD independently predicts long-term cardiovascular outcome. In addition, peripheral endothelial dysfunction assessed by RH-PAT independently correlated with future cardiovascular events in symptomatic outpatients with unexplained chest pain and in patients with heart failure who had normal left ventricular ejection fraction. Lerman and Zeiher reported the results of a multivariate analysis of hazard ratios in studies showing an association between coronary or peripheral endothelial function and cardiovascular events. However, some studies have shown that endothelial function is not associated with future cardiovascular events in a relatively low grade of atherosclerosis and in a severe stage of atherosclerosis (Table 1), though the reasons why endothelial function is not a predictor of cardiovascular events in these patients remains unclear.

**Putative mechanisms of endothelial dysfunction:** Putative mechanisms of impairment of endothelial function in cardiovascular diseases are abnormality of shear stress, increase in the endogenous endothelial NO synthase (eNOS) inhibitor asymmetrical dimethylarginine, increases in vasoconstrictors such as angiotensin II, endothelin-1, and norepinephrine, proinflammation, and inactivation of NO by reactive oxygen species.

**History of the Assessment of Endothelial Function**

It is clinically important to estimate the degree of endothelial dysfunction. Several methods have been developed and used to assess endothelial function in humans (Figure 2). In 1986, Ludmer, et al demonstrated, for the first time, paradoxical coronary artery vasoconstriction induced by intracoronary infusion of acetylcholine in patients with atherosclerotic coronary arteries. Endothelial function in coronary circulation was directly measured using coronary angiography and a Doppler flow guide wire. In 1990, Panza, et al reported that endothelium-dependent vasodilation induced by intra-arterial infusion of acetylcholine in forearm circulation, but not endothelium-independent vasodilation induced by sodium nitroprusside using a mercury-filled Silastic strain-gauge plethysmography, was impaired in patients with essential hypertension. In 1992, measurement of FMD in the brachial artery using high-resolution ultrasound was used as a method for assessing endothelial function. Technological advances have enabled evaluation of continuous changes in brachial artery diameter automatically using ultrasonography with an edge detection and wall tracking system, providing the true peak diastolic diameter during reactive hyperemia. In renal circulation also, the renal blood flow response to intravenous infusion of the NO substrate L-arginine was assessed as an index of renal endothelial function. In the digit circulation, the peripheral vasodilator response to reactive hyperemia using RH-PAT has been assessed as an index of endothelial function since 2003. Recently, we developed a new device for fully automatic measurement of endothelial function using an oscillometric method, named enclosed zone FMD (ezFMD).

**Methodology of Assessment of Endothelial Function**

**Plethysmography - measurement of forearm blood flow:** Preparation of subjects should be carefully considered prior to and during the measurement of endothelial function. Subjects
### Table 1. Endothelial Function and Cardiovascular Events

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects (number)</th>
<th>Follow-up period (year)</th>
<th>Target vessel</th>
<th>Study design</th>
<th>Measurement for endothelial function</th>
<th>Cardiovascular events</th>
<th>Predictor of cardiovascular events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penttinen, et al 10</td>
<td>Hypertension (225)</td>
<td>1.3</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>Pletysmography</td>
<td>Fatal and nonfatal myocardial infarction, fatal and nonfatal stroke, transient cerebral ischemic attack, unstable angina, coronary revascularization procedures (bypass surgery or angioplasty), and symptomatic aortoiliac occlusive disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Neunteufel, et al 18</td>
<td>Chest pain syndrome (73)</td>
<td>5</td>
<td>Brachial artery</td>
<td>Retrospective</td>
<td>FMD</td>
<td>Death, myocardial infarction, percutaneous coronary intervention, and coronary artery bypass surgery</td>
<td>Yes</td>
</tr>
<tr>
<td>Schachinger, et al 9</td>
<td>Coronary artery disease (147)</td>
<td>7.7</td>
<td>Coronary artery/brachial artery</td>
<td>Retrospective</td>
<td>Coronary artery response to acetylcholine/FMD</td>
<td>Cardiovascular death, unstable angina pectoris, myocardial infarction, coronary bypass surgery, coronary angioplasty, ischemic stroke, or revascularization of peripheral arteries</td>
<td>Yes</td>
</tr>
<tr>
<td>Suwaidi, et al 8</td>
<td>Coronary artery disease (157)</td>
<td>2.2</td>
<td>Coronary artery/brachial artery</td>
<td>Retrospective</td>
<td>Coronary artery response to acetylcholine</td>
<td>Myocardial infarction, heart failure, and surgical or percutaneous coronary revascularization</td>
<td>Yes</td>
</tr>
<tr>
<td>Modena, et al 11</td>
<td>Postmenopausal women with hypertension (400)</td>
<td>5.6</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Hospitalization for coronary heart disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Gokce, et al 17</td>
<td>Peripheral artery disease (199)</td>
<td>1.2</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiac death, myocardial infarction, unstable angina, or stroke</td>
<td>Yes</td>
</tr>
<tr>
<td>Targonski, et al 10</td>
<td>Patients without coronary artery disease (503)</td>
<td>6.6</td>
<td>Coronary artery</td>
<td>Prospective</td>
<td>Coronary artery response to acetylcholine</td>
<td>Ischemic or hemorrhagic stroke or transient ischemic attack</td>
<td>Yes</td>
</tr>
<tr>
<td>Schindler, et al 9</td>
<td>Patients without coronary artery disease (130)</td>
<td>1.9</td>
<td>Coronary artery</td>
<td>Prospective</td>
<td>Coronary artery response to acetylcholine</td>
<td>Cardiovascular death, acute coronary syndrome, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary bypass grafting, ischemic stroke, or peripheral revascularization</td>
<td>Yes</td>
</tr>
<tr>
<td>Heitzer, et al 10</td>
<td>Coronary artery disease (281)</td>
<td>4.5</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>Pletysmography</td>
<td>Death from cardiovascular causes, sudden cardiac death, myocardial infarction, ischemic stroke, percutaneous coronary intervention, coronary artery bypass surgery, and peripheral bypass revascularization</td>
<td>Yes</td>
</tr>
<tr>
<td>Halcox, et al 11</td>
<td>Patients undergoing cardiac catheterization (308)</td>
<td>1.9</td>
<td>Coronary artery</td>
<td>Retrospective</td>
<td>Coronary artery response to acetylcholine</td>
<td>Cardiovascular death, acute myocardial infarction, unstable angina pectoris, and acute ischemic stroke</td>
<td>Yes</td>
</tr>
<tr>
<td>Fathi, et al 10</td>
<td>Coronary artery disease (444)</td>
<td>2</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Death, myocardial infarction, hospitalization for acute coronary syndrome, stroke, and coronary revascularization</td>
<td>No</td>
</tr>
<tr>
<td>Frick, et al 10</td>
<td>Chest pain syndrome (398)</td>
<td>3.3</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiac death, myocardial infarction, percutaneous coronary intervention as well as bypass surgery, repeat coronary angiography with documented progression of coronary atherosclerosis, or hospitalization for worsening angina and exclusion of instability</td>
<td>No</td>
</tr>
<tr>
<td>Fischer, et al 11</td>
<td>Chronic heart failure (67)</td>
<td>3.9</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiovascular death, hospitalization due to worsening of heart failure (NYHA class IV, pulmonary oedema), or necessity for heart transplantation or chronic inotropic support or implantation of a ventricular assist device or death</td>
<td>Yes</td>
</tr>
<tr>
<td>Muyer, et al 10</td>
<td>Chronic heart failure (75)</td>
<td>3</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiovascular death, myocardial infarction, unstable angina, decompensated heart failure, and nonhemorrhagic stroke</td>
<td>No</td>
</tr>
<tr>
<td>Huang, et al 7</td>
<td>Peripheral artery disease (267)</td>
<td>0.8</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiovascular death, myocardial infarction, unstable angina, coronary revascularization procedure (catheter-based or surgically), transient ischemic attack, and stroke</td>
<td>Yes</td>
</tr>
<tr>
<td>Suzuki, et al 10</td>
<td>Metabolic syndrome (819)</td>
<td>6.8</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Stroke, myocardial infarction, or vascular death</td>
<td>Yes</td>
</tr>
<tr>
<td>Morimoto, et al 9</td>
<td>End-stage renal disease (199)</td>
<td>3.5</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Any cause of death</td>
<td>No</td>
</tr>
<tr>
<td>Rossi, et al 10</td>
<td>Postmenopausal women (2264)</td>
<td>3.8</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Cardiovascular death, myocardial infarction, revascularization procedure (catheter-based or surgically), transient ischemic attack, and stroke</td>
<td>Yes</td>
</tr>
<tr>
<td>Yehoch, et al 10</td>
<td>Subjects without coronary heart disease (3026)</td>
<td>5</td>
<td>Brachial artery</td>
<td>Prospective</td>
<td>FMD</td>
<td>Myocardial infarction, definite angina, coronary revascularization (coronary artery bypass grafting and percutaneous coronary intervention), resuscitated cardiac arrest, stroke, or cardiovascular disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Rubinstein, et al 10</td>
<td>Symptomatic outpatients with unexplained chest pain (270)</td>
<td>5.8</td>
<td>Digit artery</td>
<td>Prospective</td>
<td>RH-PAT</td>
<td>All-cause death, cardiovascular death, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, ischemic or haemorrhagic stroke or transient ischaemic attack, hospitalization for any cardiac cause</td>
<td>Yes</td>
</tr>
<tr>
<td>Akiyama, et al 10</td>
<td>Heart failure with normal left ventricular ejection fraction (321)</td>
<td>2.4</td>
<td>Digit artery</td>
<td>Prospective</td>
<td>RH-PAT</td>
<td>Cardiovascular death, nonfatal myocardial infarction, unstable angina pectoris, nonfatal ischemic stroke, hospitalization for heart failure, decompenstation, or coronary revascularization</td>
<td>Yes</td>
</tr>
</tbody>
</table>

FMD indicates flow-mediated vasodilation; and RH-PAT, reactive hyperemia-peripheral arterial tonometry.
should fast the previous night for at least 12 hours prior to the study. The subjects are kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22°C to 25°C) throughout the study. Thirty minutes after maintaining the supine position, basal brachial forearm blood flow (FBF) is measured. FBF is measured with a mercury-filled Silastic strain-gauge plethysmography (EC-5R, D.E. Hokanson, Inc., Issaquah, WA, USA) as previously described (Figure 3A). Briefly, a strain gauge is attached to the upper part of the left arm and is connected to a plethysmography device, and supports above the right atrium. A wrist cuff is inflated to 50 mmHg above systolic blood pressure to exclude hand circulation from measurements taken 1 minute before measurement of FBF. The upper arm-congesting cuff is inflated to 40 mmHg for 7 seconds in 15-second cycles to occlude venous outflow from the arm by using a rapid cuff inflator (EC-20, D.E. Hokanson, Inc.). The FBF output signal is transmitted to a recorder. FBF is expressed as mL per minute per 100 mL of forearm tissue volume. Forearm vascular resistance is calculated as mean blood pressure divided by FBF and is expressed as mmHg per mL per minute per 100 mL of forearm tissue volume. Four plethysmographic measurements are averaged to obtain FBF at baseline, and infusion of an endothelium-dependent vasodilator such as acetylcholine, methacholine, serotonin, substance P or bradykinin, and an endothelium-independent vasodilator such as nitroglycerine, sodium nitroprusside.

**Figure 2.** History of the assessment of endothelial function.

**Figure 3.** A: Measurement of forearm blood flow (FBF) in response to vasoactive agents using a mercury-filled Silastic strain-gauge plethysmography (Hokanson system). Role of nitric oxide in basal forearm blood flow (FBF) in healthy subjects. B: Basal FBF was decreased by infusion of N^\text{G}-monomethyl-L-arginine (L-NMMA). Bar graphs show the effect of L-NMMA infusion on basal FBF. Modified from Higashi Y, et al. J Pharma Sci 2003; 93: 399-404.
side or isosorbide dinitrate, is performed. Thirty minutes after maintaining the supine position, basal FBF is measured. The effects of infusion of an endothelium-dependent vasodilator and an endothelium-independent vasodilator on FBF are then determined. After administration of an endothelium-dependent vasodilator and an endothelium-independent vasodilator, FBF is measured during the last 2 minutes of infusion. These studies are carried out in a randomized fashion. Each study proceeded after FBF had returned to baseline. After infusion of an endothelium-dependent vasodilator and an endothelium-independent vasodilator, FBF returns to baseline within 30 minutes. Thus, the end of infusion of an endothelium-dependent vasodilator or an endothelium-independent vasodilator is followed by a 30-minute recovery period. To examine the effect of exercise on release of NO, FBF during infusion of an endothelium-dependent vasodilator and an endothelium-independent vasodilator in the presence of the NO synthase inhibitor N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) should be measured. FBF is calculated by observers who do not know the study protocol and results from the linear portions of the plethysmographic recordings.

Measurement of vascular responses to intra-arterial infusion of vasoactive agents should be considered as the gold standard for assessing endothelial function, because the use of agonists to stimulate NO release and the use of antagonists of NO allow us to draw more specific conclusions concerning the role of basal and stimulated NO release. However, this technique is invasive and burdensome for the subjects. A noninvasive method for measuring forearm blood flow response to reactive hyperemia is also useful for assessing endothelial function. Interestingly, basal FBF was reduced by 50% after intra-arterial infusion of L-NMMA in healthy subjects, suggesting that approximately 50% of the basal blood flow in peripheral circulation is, at least in part, regulated by NO (Figure 3B). Measurement of FMD: Subjects are prepared by the same protocol as that for measurement of forearm blood flow using plethysmography. Thirty minutes after maintaining the supine position, basal brachial artery diameter is measured. The vascular response to reactive hyperemia in the brachial artery is used for assessment of endothelium-dependent FMD. After completion, nitroglycerine-induced vasodilation with confirmation that the brachial artery diameter had recovered to the baseline value is measured. A manual technique is acceptable for measuring vascular diameter, but, if possible, an automated edgetracking system for measurement of vascular diameter and blood flow is required. As an example of measurement of FMD, an automated edgetracking system should be used. A high-resolution linear artery transducer is coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that uses an automated edge detection system for measurement of brachial artery diameter (Figure 4A). A blood pressure cuff is placed around the forearm. The brachial artery is 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 is obtained, the transducer is 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 are set to optimize the images of the arterial lumen wall interface. When the tracking gate is placed on the intima, the artery diameter is automatically tracked, and the waveform of diameter changes over the cardi-

![Figure 4](image-url)
ac cycle is displayed in real time using the FMD mode of the tracking system. This allows the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow is assessed at baseline and during peak hyperemic flow, which is confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity is calculated from the color Doppler data and is displayed as a waveform in real time (Figure 4B). The baseline longitudinal image of the artery is acquired for 30 seconds, and then the blood pressure cuff is inflated to 50 mmHg above systolic pressure for 5 minutes. The longitudinal image of the artery is recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals are obtained for 20 seconds at baseline and for 10 seconds immediately after cuff deflation. Changes in brachial artery diameter are immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD is automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD ([Peak diameter - Baseline diameter]/Baseline diameter) is used for analysis. Blood flow volume is calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr²). Reactive hyperemia is calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

It is accepted that measurement of FMD is an index of conduit artery endothelial function. Measurement of FMD is noninvasive and reflects NO production. However, this technique has relatively low reproducibility and requires a skilled operator. Although the precise mechanism by which vasodilation occurs during reactive hyperemia in FMD measurement has not been fully elucidated, shear stress-induced NO has been proposed as a principal mediator of FMD (Figure 4C). Measurement of nitroglycerine-induced vasodilation: The response to nitroglycerine is used for assessment of endothelium-independent vasodilation. Nitroglycerine-induced vasodilation is measured as described previously. Briefly, in general, after acquiring baseline rest images for 30 seconds, a sublingual tablet or spray (75 μg to 300 μg nitroglycerine) is given, and images of the artery are recorded continuously until the dilation has reached a plateau after administration of nitroglycerine. Subjects who have received nitrate treatment and subjects in whom the sublingually administered nitroglycerine tablet is not dissolved during the measurement are excluded from the study. Nitroglycerine-induced vasodilation is calculated as a percent change in peak vessel diameter from the baseline value. Percentage of nitroglycerine-induced vasodilation ([Peak diameter - Baseline diameter]/Baseline diameter) is used for analysis.

Nitroglycerine-induced vasodilation, an index of endothelium-independent vasodilation, has been assessed as a control test to ensure that impaired FMD is not due to underlying vascular smooth muscle dysfunction or alterations in vascular structure but truly a consequence of endothelial dysfunction. Indeed, both endogenous NO and exogenous NO act on vascular smooth muscle cells. Therefore, endothelial function should be defined after both measurements of endothelium-dependent and endothelium-independent vasodilation. If there is a significant difference in nitroglycerine-induced vasodilation, as an index of endothelium-independent vasodilation, the ratio of endothelium-dependent vasodilation to endothelium-independent vasodilation should be calculated to confirm real endothelial function. Indeed, some investigators have used the data on the ratio as an index of endothelial function even under the condition of no differences in nitroglycerine-induced vasodilation in the subjects studied. It is well known that nitroglycerine-induced vasodilation per se is impaired in subjects with cardiovascular risk factors or with coronary atherosclerosis. Recently, we have shown associations of nitroglycerine-induced vasodilation with cardiovascular risk factors and prevalence of cardiovascular diseases and a relationship between nitroglycerine-induced vasodilation and FMD in a general population including healthy subjects and patients with cardiovascular disease. In coronary circulation also, coronary artery dilation in response to nitroglycerine was impaired in patients with coronary heart disease and is an independent predictor of long-term cardiovascular events in these patients. These findings suggest that nitroglycerine-induced vasodilation per se may be a marker of the grade of atherosclerosis. FMD should be interpreted as an index of vascular function reflecting both endothelium-dependent vasodilation and endothelium-independent vasodilation in subjects with impaired nitroglycerine-induced vasodilation. In addition, it is expected that nitroglycerine-induced vasodilation may be a predictor of cardiovascular events. However, there is no evidence regarding the relationship between nitroglycerine-induced vasodilation and future cardiovascular events.

Measurement of RH-PAT: Digital pulse volume amplitude is measured using a peripheral arterial tonometry (PAT) device (Endo-PAT2000; Itamar Medical, Caesarea, Israel) (Figure 5), as previously described. PAT in response to reactive hyperemia is assessed. Briefly, digital probes are placed on the tip of each index finger and a blood pressure cuff is placed around one upper arm as the study arm, and the other arm serves as a control arm. PAT probes are placed on the index or middle finger of each hand for continuous recording of the PAT signal. After a 10-minute equilibration period, the blood pressure cuff is inflated to 50 mmHg above systolic pressure for 5 minutes. The PAT signal is then recorded for 10 minutes after the cuff is deflated. RH-PAT recordings are automatically analyzed by computer-assisted analysis software. The ratio of average amplitude of the PAT signal over a period of 1 minute starting 1 minute after cuff deflation to average amplitude of the PAT signal for 3 minutes at baseline (RH-PAT index) is calculated and normalized to the control arm.

RH-PAT is a noninvasive technique to assess microvascu-
lar endothelial function. RH-PAT reflects changes in finger pulse volume amplitude during reactive hyperemia. This technique is equivalent to finger plethysmography. It has been shown that a change in RH-PAT is, at least in part, NO-dependent. RH-PAT index is automatically measured by a special device and is operator-independent. At present, measurement of RH-PAT is the most simple and reproducible method. Measurement of RH-PAT is based on a technique similar to that of FMD. However, the Framingham Heart Study has shown that there is no significant correlation between RH-PAT and FMD and that there are different contributors to cardiovascular risk factors between RH-PAT and FMD. These findings suggest that RH-PAT and FMD assess different endothelial functions as the microvasculature and conduit artery and that assessment of different vascular beds have different contributors to cardiovascular disease and different prediction for cardiovascular outcomes.

New methods for assessment of endothelial function - measurement of ezFMD: Oscillometric noninvasive blood pressure measurement is widely performed in a clinical setting. The theory is that the arterial wall contains zero stress and the vessel is minimally distended when external or cuff pressure is equal to arterial pressure. Cuff wave pressure is a signal of variation of internal cuff pressure and arises from volumetric change in the cuff, which, in turn, originates from volumetric pulse change in the artery. When arterial vessel volume increases, cuff volume decreases and cuff internal pressure increases. Repetition of changes in arterial vessel volume and cuff volume shows the oscillation signal. The magnitude of oscillation and volumetric change in the artery shows a close proportional relation. Cuff pressure associated with the largest oscillation amplitude can be considered as mean blood pressure. The vascular response to reactive hyperemia in the brachial artery is assessed for oscillation amplitude measurement of ezFMD (Figure 6A). The pulse wave is measured with an OPV 1500 (Nihon Koden, Co., Tokyo). Oscillometry is a commonly used noninvasive method for measuring blood pressure with a sphygmomanometer cuff tied around the upper arm. After the cuff is inflated to a level higher than systolic blood pressure, it is deflated slowly, and blood pressure is estimated on the basis of oscillation signals recorded from the internal cuff pressure. At first, blood pressure is measured 5 times on the proximal forearm in a seated position at rest with this device, and after interrupting blood flow for 5 minutes with the cuff, blood pressure for 5 minutes is consecutively measured automatically. The cuff is inflated up to systolic pressure plus 50 mmHg. For every cuff pressure deflation of 5 mmHg, two oscillation signal pulses are detected. In each step, detection of two oscillation signal pulses and deflation of the cuff are repeated, and cuff pressure is released at the point when diastolic pressure is reached. The average of the oscillation amplitude of two pulses is considered as the typical value of the oscillation amplitude at each cuff pressure step. Maximum typical value of one measurement sequence to calculate the compliance change is used. ezFMD is calculated by the following equation: %ezFMD = [(peak oscillation amplitude - baseline oscillation amplitude)/baseline oscillation amplitude] × 100. In the after-occlusion period, the average of the third to fifth of 5

measurements is used for analysis of peak oscillation amplitude.

ezFMD was significantly lower in patients with cardiovascular diseases than in age- and gender-matched healthy subjects, whereas there was no significant difference between baseline oscillation amplitudes in the two groups (Figure 6B). In addition, cardiovascular risk factors were independent predictors of ezFMD. ezFMD significantly correlated with conventional FMD (Figure 6C). Figure 6D shows a Bland-Altman plot of the differences between ezFMD and FMD values versus the corresponding averages of the two indices. There was good agreement between ezFMD and FMD. Conventional measurement of FMD using ultrasonography is measured by change in vascular diameter, whereas ezFMD is based on change in vascular volume. Although both ezFMD and FMD are equally useful for assessing endothelial function, measurement of ezFMD is easier and less biased than measurement of FMD.

Biochemical markers for endothelial function: Measurement of biochemical markers in blood and urine as an index of endothelial function is easy and noninvasive. Several biochemical markers, such as NO product, second messenger of NO production, adhesion molecules, oxidative stress markers, and inflammation markers, have been measured as indices of endothelial function (Table II). However, measurement of a biochemical marker does not sufficiently reflect endothelial function. At present, a biochemical marker would only be a surrogate of the assessment of endothelial function by physiological and pharmacological stimuli methods. It is expected that a specific biochemical marker for endothelial function will be developed in the near future.

In conclusion, this review focused on the assessment of endothelial function, including measurement history, methodological aspects, and clinical perspectives. Although assessment of endothelial function is widely performed at present, there are a number of methodological issues. In addition, the absence of diagnostic criteria makes it difficult to interpret the results of vascular response to vasoactive agents using plethysmography, FMD, and RH-PAT in clinical practice. Some studies have shown that improvement of endothelial function by intervention reduces cardiovascular events. However, it is unclear whether augmentation or improvement of endothelial function is directly related to reduction in cardiovascular events in patients with cardiovascular disease. Further studies are needed to determine whether improvement in endothelial function is a predictor of cardiovascular outcomes in a large clinical trial. In addition, the development of a new device for measurement of endothelial function that is easy to use, convenient, and accurate is needed.

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Disclosures
None.

References
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