New Methods to Evaluate Endothelial Function:  
Method for Assessing Endothelial Function in Humans Using  
a Strain-Gauge Plethysmography: Nitric Oxide-Dependent  
and -Independent Vasodilation

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Received September 1, 2003; Accepted October 15, 2003

Abstract. The vascular endothelium is involved in the release of various vasodilators, including nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factor as well as vasoconstrictors. NO plays an important role in the regulation of vascular tone, the inhibition of platelet aggregation, and the suppression of smooth muscle cell proliferation. Several diseases are associated with changes in endothelial function mediated through reduced NO bioavailability. In addition, endothelial dysfunction is an early feature of atherosclerosis and vascular diseases in humans. Therefore, it is clinically important to estimate the degree of endothelial dysfunction. Several methods have been used to assess endothelial function in humans. Recently, we have evaluated the effects of intra-arterial infusion of NO agonists, such as acetylcholine, methacholine, and bradykinin, and NO antagonists on forearm blood flow using mercury-filled Silastic strain-gauge plethysmography. The response to the intra-arterial infusion of vasoactive agents should be considered the gold standard in assessing endothelial function, because the use of agonists to stimulate NO release allow us to draw more specific conclusions concerning the role of basal and stimulated NO release. However, the invasive method is time-consuming and is a burden for patients. A noninvasive method of measuring forearm blood flow response to reactive hyperemia also is useful in assessing endothelial function. In this review we would like to explain in detail the methods of assessing endothelial function in humans using strain-gauge plethysmography.

Keywords: endothelial function, nitric oxide, forearm blood flow, plethysmography

Introduction

Until 1981, it has been thought that the vascular endothelium functions as a wall separating the blood vessel wall and the inside cavity. If, for example, the endothelium of the whole body can be collected, its total weight would be equal to that of the liver, and its total area would be equal to six tennis courts. In addition, endothelium secretes various vasoactive agents such as vasodilators and vasoconstrictors (1 – 3). Thus, it is concluded that vascular endothelium might be the biggest endocrine organ in the human body. Healthy endothelium maintains vascular tone and structure by regulating the balance between vasodilation and vasoconstriction, growth inhibition and growth promotion, antithrombosis and prothrombosis, anti-inflammation and proinflammation, and also antioxidation and prooxidation (1 – 3). Cardiovascular diseases are associated with endothelial dysfunction. It has been postulated that endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis, resulting in cardiovascular complications (4). Therefore, it is very important to assess endothelial function. Several methods are used to assess endothelial function in humans. In general, the measurement of the forearm blood flow (FBF) response to vasoactive agents using strain-gauge plethysmography.
plethysmography is the gold standard for assessing endothelial function in resistance arteries.

**Measurement of FBF using a strain-gauge plethysmography**

FBF is measured by using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R; D.E. Hokanson, Inc., Bellevue, WA, USA) as previously described (5, 6). Briefly, a strain gauge is attached to the upper part of the left arm and connected to a plethysmography device and is supported above the right atrium. A wrist cuff is inflated to a pressure of 50 mmHg above the systolic blood pressure to exclude the hand circulation from the measurements 1 min before each measurement and throughout the measurement of FBF. The upper arm congesting cuff is inflated to 40 mmHg for 7 s in each 15-s cycle to occlude venous outflow from the arm 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. Four plethysmographic measurements are averaged for analysis of FBF at baseline and during administration of drugs. Forearm vascular resistance is calculated as the mean arterial pressure divided by FBF.

**Assessment of endothelial function**

Subjects are fasted the previous night for at least 12 h. They are kept in the supine position in a quiet, dark, air-conditioned room (constant temperature 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter is inserted into the left brachial artery for the infusion of vasoactive agents for the recording of arterial pressure with a pressure transducer under local anesthesia (1% lidocaine). Another catheter is inserted into the left deep antecubital vein to obtain blood samples. After the subjects are placed for 30 min in the supine position, FBF and arterial blood pressure are measured. Then the effects of the vasoactive agents such as endothelium-dependent vasodilators, acetylcholine (ACh), methacholine, bradykinin, and histamine, endothelium-independent vasodilator, sodium nitroprusside and isosorbide dinitrate (ISDN) on forearm hemodynamics are measured. Vasoactive agents are infused intra-arterially for 5 min at each dose using a constant rate infusion pump. The FBF is measured during the last 2 min of the infusion. The infusions of vasoactive agents are carried out in a random order. Each study proceeds after the FBF returned to baseline.

The use of a nitric oxide (NO) synthase inhibitor N⁶-monomethyl-L-arginine (L-NMMA) is useful to confirm the role of basal and stimulated NO release. We intra-arterially administer L-NMMA at a dose of 8 μmol/min for 5 min while the basal FBF and arterial blood pressure are recorded, and then vasoactive agents are administered. After the administration of L-NMMA, the effect of endothelium-dependent vasodilation is inhibited for at least 4 h in extremities circulation in humans.

**NO-dependent and -independent vasodilation**

We evaluated the role of NO in basal FBF (basal vascular tone) in 42 healthy men (mean age, 47 ± 15 [S.D.] years). Intra-arterial infusion of L-NMMA (8 μmol/min for 5 min) decreased basal FBF from 4.6 ± 1.2 [S.D.] to 2.6 ± 0.7 [S.D. mL/min per 100 mL tissue (P<0.001), indicating that NO contributes to about 50% of the regulation of basal FBF (Fig. 1). The rest of the regulation of basal FBF may be based on factors other than NO, such as endothelium-derived hyperpolarizing factor (EDHF) and prostaglandins.

ACh is the most frequently prescribed class of endothelium-dependent vasodilators in experimental models and humans. It is thought that the vasodilatory effect of ACh is to bind the muscarine receptor and activate endothelial NO synthase, and NO, which is produced by L-arginine in the presence of endothelial NO synthase in the endothelium, stimulates cytosolic guanylate cyclase and increases cGMP content in vascular smooth muscle cells, resulting in relaxation of vascular tone. The vasodilator response to ACh is also mediated by a variety of factors, although the main contributor to this response is NO. However, there is little information about whether other endothelium-dependent vasodilators, such as EDHF and prostaglandins other than NO, contribute to ACh-induced vasodilation in vasculature.

![Fig. 1. The role of nitric oxide in basal forearm blood flow (FBF) in humans. Basal FBF was decreased by the infusion of N⁶-monomethyl-L-arginine (L-NMMA) (left). Bar graphs show the effect of L-NMMA infusion on basal FBF (right).](image-url)
in humans. We evaluated the FBF response to intra-arterial infusion of ACh (7.5, 15, and 30 µg/min; for 5 min) alone and in the presence of L-NMMA (8 µmol/min); glibenclamide (0.125 mg/min), a potassium-ATP channel inhibitor; indomethacin (50 µg/min); a cyclooxygenase inhibitor; or miconazol (0.25 mg/min), a cytochrome P-450 inhibitor, in 7 healthy men (mean age, 27 ± 5 [S.D.] years). Infusion of ACh significantly increased the FBF response in a dose-dependent manner. Intra-arterial infusion of L-NMMA attenuated ACh-induced vasodilation, glibenclamide attenuated ACh-induced vasodilation (Fig. 2), and miconazol also attenuated the FBF response to ACh (Fig. 3). On the other hand, infusion of indomethacin did not alter the FBF response to ACh (Figs. 2 and 3). These findings suggest that although ACh-induced vasodilation is mainly caused by NO in forearm circulation in humans, potassium-ATP channels, and cytochrome P-450, but not prostaglandins, plays, at least in part, an important role in ACh-induced vasodilation. EDHF also may contribute to ACh-induced vasodilation in forearm resistance arteries in humans. The response to the intra-arterial infusion of vasoactive agents should be considered the gold standard in assessing endothelial function. However, the invasive method used for the intra-arterial drug infusion is time-consuming and is a burden for the subjects. The noninvasive method of measuring reactive hyperemia is simple, reproducible, and does not cause adverse effects. To obtain reactive hyperemia, FBF is occluded by inflating of the cuff on the left upper arm at a pressure of 280 mmHg for 5 min. After the release of ischemic cuff occlusion, FBF is measured for 3 min. Nitroglycerine (NTG), an endothelium-independent vasodilator, is sublingually administered at the dose of 0.3 mg by one tablet (Nihonkayaku Co., Tokyo). After the administration of NTG, FBF is measured for 5 min. These studies are carried out in a randomized fashion. Each study proceeded after FBF had returned to the baseline. In the preliminary study, after the release of cuff occlusion or the sublingual NTG, FBF returned to baseline within 10 min. Thus, the end of the response to reactive hyperemia or sublingual NTG is followed by a 15-min recovery period. Figure 4 shows the FBF response to reactive hyperemia in 57 healthy men (mean age, 44 ± 10 [S.D.] years). Interestingly, we have recently shown that the peak FBF response to reactive hyperemia strongly correlates with the FBF response to ACh (30 µg/min for 5 min) (7). It is well known that vasodilation during reactive hyperemia is caused by multiple factors. NO plays a vital role in the vasodilation evoked by reactive hyperemia. Tagawa et al. (8) have recently reported that NO plays a minor role in the peak FBF response to reactive hyperemia and mainly contributes to the mid-to-late phase of the FBF response after peak vasodilation. Although L-NMMA does not completely
inhibit the forearm vascular relaxation caused by reactive hyperemia, NO is intimately involved in the FBF response to reactive hyperemia. However, the role of factors other than NO in reactive hyperemia-induced vasodilation is not established at present.

**Endothelial function in cardiovascular diseases**

Several investigators have shown the impaired endothelium-dependent vasodilation in cardiovascular diseases, including hypertension (5, 6, 9–11), diabetes mellitus (12, 13), lipids disorders (14, 15), heart failure (16, 17), and smokers (18, 19), by using a strain-gauge plethysmography. Figure 5 shows that the ACh-induced vasodilation, but not ISDN-induced vasodilation, in forearm resistance arteries was significantly blunted in 20 patients with essential hypertension (10 men and 10 women; mean age, 47 ± 15 [S.D.] years) compared with 20 age- and sex-matched normotensive subjects (10 men and 10 women; mean age, 46 ± 15 [S.D.] years). There was no significant difference in change in the FBF response to ACh after L-NMMA infusion between hypertensive and normotensive subjects. These findings suggest that endothelium-dependent vasodilation is selectively impaired in patients with essential hypertension through a decrease in NO production. Endothelial dysfunction is an initial step in atherosclerosis, leading to increase in the incidences of cardiovascular outcome (Fig. 6). From a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial dysfunction in cardiovascular diseases. We can compare the effect of the most frequently prescribed interventions on endothelial function using this technique. Some interventions such as antihypertensive agents, angiotensin-converting enzyme inhibitors (20, 21), and angiotensin type I receptor blockers (22); various supplementation therapies such as antioxidant vitamins (6, 23), estrogen replacement therapy (24–26), NO substrate L-arginine (27), NO synthase cofactor tetrahydrobiopterine (28, 29); and several non-pharmacologic interventions such as aerobic exercise (5), body weight reduction (30), sodium restriction (31), and smoking cessation (32) improve endothelial dysfunction. In addition, the use of various vasoactive agents, including NO antagonists, allows us to draw more specific conclusions about the mechanisms of vasodilation and the mechanisms of endothelial dysfunction in cardiovascular diseases.

In conclusion, in this review we described in detail the methods of assessing endothelial function in humans by strain-gauge plethysmography. We consider that the measurement of the FBF response to vasoactive agents

![Fig. 4](image_url)  
**Fig. 4.** Line graph shows the time course of forearm blood flow (FBF) after the release of ischemic occlusion (reactive hyperemia).

![Fig. 5](image_url)  
**Fig. 5.** Line graphs show the effects of acetylcholine (ACh) and isosorbide dinitrate on forearm blood flow (FBF) in the normotensive and hypertensive subjects. Modified from Ref. 5.
by strain-gauge plethysmography is the gold standard for assessing endothelial function in resistance arteries. The vasodilatory response to reactive hyperemia is also useful in assessing resistance vessel endothelial function.

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