Flow-Mediated Vasodilation*

A Diagnostic Instrument, or an Experimental Tool?

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Brachial arterial flow-mediated dilation (FMD), assessed by high-resolution ultrasonography, reflects endothelium-dependent vasodilator function. FMD is diminished in patients with atherosclerosis and with coronary risk factors, and improves with risk-reduction therapy. Therefore, the measurement of FMD can be a good prognostic instrument in preventive cardiology, is useful to predict short-term postoperative cardiovascular events in a high-risk population and to assess long-term cardiovascular risk in a lower risk population, and is an excellent experimental tool to detect changes in endothelial function after new therapeutic interventions. In this review article, the pathophysiology of FMD, based on reactive hyperemia, is extensively discussed. Furthermore, an overview is given of the actual clinical indications of FMD measurement. ***(CHEST 2005; 127:2254–2263)***

Key words: coronary artery disease; endothelial dysfunction; flow-mediated vasodilation; review

Abbreviations: CAD = coronary artery disease; eNOS = endothelial nitric oxide synthase; FMD = flow-mediated dilation; IMT = intima-media thickness; NO = nitric oxide; PWV = pulsewave velocity

Coronary angiography has been the cornerstone in the diagnosis of coronary artery disease (CAD) for decades and is used to quantify significant prognostic information about epicardial coronary arteries.[1,2] However, this technique is restricted to analyzing the lumen and does not assess the functional reactivity of the coronary arteries (*ie*, endothelial function). Furthermore, angiography is invasive and may miss significant atherosclerosis that is present in the vessel wall before eventual encroachment on the lumen occurs.[3] Therefore, the assessment of vessel function may yield additive prognostic information to that derived from standard coronary angiography only.

Normal vascular endothelial cells support cardiovascular function by promoting vasodilatation, and by inhibiting platelet aggregation, WBC adhesion, and smooth muscle cell proliferation. In contrast, a dysfunctional endothelium is characterized by an impaired endothelium-dependent vasodilation response, which favors platelet aggregation and WBC adhesion, and promotes smooth muscle cell proliferation. Endothelial dysfunction is characterized by a decreased production and/or local bioavailability of nitric oxide (NO). It plays a pivotal role in the development, progression, and clinical manifestations of atherosclerosis, as well as in the development of ischemia and thrombosis in the late stages of the disease, by promoting coronary vasoconstriction and thrombosis.

Impaired endothelium-dependent dilation in the coronary circulation is associated with coronary atherosclerosis[4] and coronary risk factors,[5] and improves with risk-reduction therapy.[6] Consequently, endothelial function has been defined as an "excellent barometer" of vascular health[7] and can be used to gauge cardiovascular risk. A pathogenic link between coronary endothelial dysfunction and cardiovascular events was almost simultaneously demonstrated by Suwaidi et al[8] and Schächinger et al.[9]

Endothelial function can be evaluated by the following different approaches: (1) measurement of morphologic and mechanical characteristics of the vascular wall (*eg*, intima media thickness, compliance, distensibility, and remodeling indexes); (2) determination of soluble endothelial markers (*eg*, von Willebrandt factor, plasminogen activator, inhibitor complex thrombomodulin adhesion molecules, and N-oxides); and (3) measurement of the endothelium-dependent regulation of vascular tone at focal sites of the circulation.[10]

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Invasive vasomotor techniques (*eg*, quantitative coronary angiography and strain gauge plethysmography) are considered to be the diagnostic standard for the evaluation of endothelium-dependent vasodilation. However, patients cannot be frequently subjected to these invasive techniques as is needed in clinical applications and population-based studies. Therefore, brachial artery flow-mediated dilation (FMD) measurement by high-resolution ultrasonography is a broadly applicable method that is used for the examination of endothelial function.[11–14]

Both peripheral and coronary endothelial dysfunction may be induced by several risk factors, as in familial hypercholesterolemia,[15,16] smoking,[17,18] diabetes mellitus,[19,20] and hyperhomocysteinemia.[21,22] Coronary endothelial function has been found to correlate with endothelial function in accessible peripheral arteries, such as the brachial artery.[23] A close relation has been demonstrated between FMD in the brachial artery and the coronary circulation, both in terms of morphologic lesions[24] and the functional responses to acetylcholine.[23,25]

Physiology of FMD

FMD is designated as an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased flow and, thereby, increased shear stress. When blood flow increases through a vessel, the vessel dilates. This physiologic response was first described by Schretzenmayer[26] and was subsequently demonstrated in a number of conduit arteries. An impaired (*ie*, diminished) FMD response reflects endothelial dysfunction.

Reactive Hyperemia

In peripheral arteries, a temporary increase in shear stress can be induced by increasing the local blood flow, the so-called *reactive hyperemia*. Reactive hyperemia can be achieved by inflating a pneumatic sleeve around the forearm to 220 mm Hg for 4 min and then deflating it. This results in an abrupt decrease in vascular resistance. After deflation, the arterial diameter is measured at 60 and 90 s to determine the maximum posthyperemia diameter. FMD is expressed as the absolute or relative change in diameter from the baseline measurements.

Shear Stress

Shear stress is mainly determined by blood flow and its attractive force exerted at a vector perpendicular to the long axis of the vessel (Fig 1). The endothelium acts as a mechanotransducer that senses changes in shear stress and subsequently

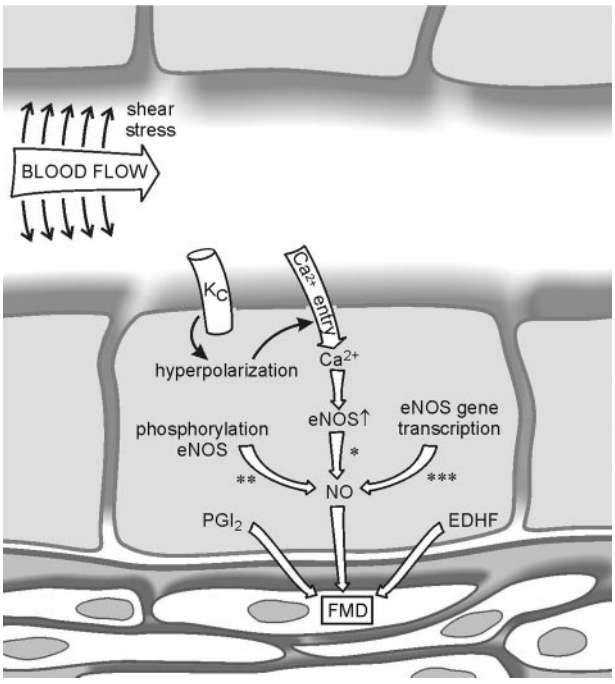

FIGURE 1. The genesis of FMD, in response to different changes in shear stress. * = very short-term changes; ** = changes taking place over slightly longer periods (minutes); *** = changes taking place over a longer time (many minutes or hours). PGI_2 = prostacycline; EDHF = endothelium-derived hyperpolarizing factor; Kc = calcium-activated potassium channel.

modifies the output of dilator factors. The endothelial signaling cascade, which is responsible for converting mechanical stimuli into the release of vasodilatory molecules, has not been fully clarified.

FMD has been shown to be predominantly dependent on endothelium-derived NO.[27,28] The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress.[29–31] The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry. Calcium activates endothelial NO synthase (eNOS), and the subsequent generation of NO appears to account for FMD.[28,32]

Several mechanisms are responsible for the increase in NO in response to changes in shear stress. Very short-term changes may be mediated by the increase in intracellular calcium that occurs when ion channels open. Slightly longer time periods of shear stress are responsible for the phosphorylation of eNOS via serine/threonine protein kinase and Akt/protein kinase B, even at low calcium concentrations. In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function. Longer time periods (many minutes or hours) of

shear stress activate eNOS gene transcription, which may result in continued increases in NO generation if shear stress is maintained at high levels.[33]

FMD depends not only on NO formation, but also on NO inactivation and the sensitivity of the underlying vascular smooth muscle for NO. Regular exercise simultaneously induces the up-regulation not only of eNOS but also of superoxide dismutase expression.[34] This feed-forward mechanism could prevent superoxide-mediated inactivation of NO and thus increase shear stress-dependent FMD.

Very recently, Mitchell et al[35] demonstrated that impaired FMD of the brachial artery may not be due to the impaired release of NO from the vascular endothelium, but from a lesser stimulus to NO release as a consequence of decreased flow velocity and shear stress during reactive hyperemia caused by impaired microvascular response.

The reduced NO bioavailability that characterizes endothelial dysfunction may induce important steps in the appearance and progression of the atherosclerotic lesions, including monocyte and leukocyte adhesion and platelet-vessel wall interaction. NO also reduces vascular tone, decreases endothelial permeability, and inhibits vascular smooth muscle cell migration and proliferation.

The principal mediator, but not the only mediator, of FMD is NO. In the eNOS knockout mice, FMD appears to be mediated by endothelium-derived prostanoids since it is blocked by indomethacin.[36] It is unknown whether mediators, such as the unidentified endothelium-derived hyperpolarizing factor, can cause FMD if both the NO and prostanoid pathways are blocked.

In human forearm circulation, endothelial responses to blood flow depend on the characteristics of the flow stimulus. FMD after brief episodes of hyperemia is often almost exclusively mediated by NO, whereas dilatation during sustained hyperemia is unaffected by the inhibition of NO synthesis.[37] Additionally, FMD is diminished by reductions in extracellular calcium and sodium,[38] and is improved by magnesium.[39]

Endothelium-Independent Vasodilation With Nitroglycerin

An exogenous NO donor (*eg*, a single high dose of nitroglycerin [0.4 mg]) is used to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation, reflecting vascular smooth muscle function.[40] As cardiovascular risk factors increase in number, smooth muscle dysfunction becomes apparent, and thus the nitroglycerin response is progressively impaired independently of endothelial dysfunction.[41] This should be taken into account when studying patients with either coronary or systemic atherosclerosis. In hypertensive subjects, for example, nitroglycerin-mediated vasodilation is also impaired, suggesting an impairment of overall vascular function but not necessarily a single impairment of endothelial function.[42]

Rather than increasing doses, a single bolus of nitrates is preferable, because nitrates may further bring changes in smooth muscle function or arterial compliance. Peak vasodilation occurs 3 to 4 min after nitrate administration. This measurement can only take place at least 10 min after reactive hyperemia, since baseline conditions are not yet reestablished prior to this moment.

Indication for FMD Measurement

Risk Stratification for Cardiovascular Events

Two major invasive studies[8,9] have disclosed the relation between coronary endothelial dysfunction and cardiovascular events. Several studies have addressed the question of whether endothelial function in a peripheral artery as such also provides prognostic information about cardiac events. Heitzer et al[43] demonstrated that patients with CAD with impaired forearm microvessel responses to acetylcholine developed more cardiovascular events over a 4.5-year follow-up period. Perticone et al[44] examined endothelial function in response to intraarterial acetylcholine infusion in 225 patients with newly diagnosed hypertension. Patients with lower vasodilator responses had a significantly higher risk of experiencing a cardiac or vascular event (relative risk, 2.084). Very recently, Fathi et al[45] examined the value of the measurement of brachial artery reactivity in predicting cardiovascular events in a group of 444 patients with a significant risk of cardiovascular events. They demonstrated that patients with FMD of $< 2\%$ had significantly more cardiovascular events than those with normal FMD ($> 6.3\%$) or mildly abnormal FMD (2.1 to 6.3%). However, in this study, the mean intima-media thickness (IMT), rather than FMD, was the vascular factor independently associated with mortality.

Risk Stratification in Patients With Chest Pain

Neunteufl et al[24] demonstrated in patients with angina pectoris ($n = 74$) that those with lesions who were undergoing standard coronary angiography had reduced FMD. Angiography revealed CAD ($\geq 30\%$ diameter stenosis) in 44 patients (age range, 32 to 67 years) and smooth coronary arteries in 30 patients

(age range, 22 to 73 years). Patients with CAD showed markedly impaired mean (± SD) FMD compared to the group without CAD (5.7 ± 4.8% vs 12.6 ± 6.7%, respectively; $p < 0.0001$). The authors further demonstrated a relation between FMD and the extent of CAD (*ie*, one-vessel, two-vessel, or three-vessel disease).

In another study, Neunteufl et al[46] described the 5-year prognostic value of FMD in patients with chest pain. The evaluated end points in this study were death, myocardial infarction, percutaneous transluminal angioplasty, and coronary artery bypass surgery. These cardiovascular events occurred more often in patients with impaired FMD compared with patients with preserved FMD (*ie*, FMD < 10% vs FMD > 10%, and FMD 50% vs FMD 15%, respectively; $p = 0.002$). Events after the first month were observed only in patients with FMD of < 10%, and in this group hospitalization also was necessary more often.

Shroeder et al[47] examined 122 consecutive patients with a clinical suspicion of CAD. Of these patients, 101 were found to have any angiographically detectable disease of any severity. FMD of the brachial artery was significantly better in the group with no CAD (mean FMD, 7.0 ± 3.5%) as opposed to the group with CAD (mean FMD, 3.8 ± 4.1%). FMD showed a 71% sensitivity and a 81% specificity in predicting any CAD. These researchers found that the optimal cutoff point for FMD in terms of sensitivity and specificity in predicting CAD was ≤ 4.5%. These studies suggest that FMD is a parameter worth measuring in patients with clinical suspicion of CAD before coronary angiography, especially if the results of one of the screening tests is negative.

Risk Stratification for Postoperative Cardiovascular Events

Gokce et al[48] demonstrated that impaired FMD of the brachial artery independently predicts short-term cardiovascular events in patients undergoing vascular surgery. No relation was observed between nitroglycerin-mediated dilation and events, and between the extent of reactive hyperemia and events. The predictive value of FMD was independent of vessel size. These observations imply that the results cannot be explained by the altered function of vascular smooth muscle variation in the stimulus for vasodilation, or by larger vessel size in patients who have experienced cardiovascular events. Therefore, FMD measurement can be used as an independent factor in risk stratification in patients undergoing vascular surgery, with high short-term postoperative cardiovascular risk. A potential approach would be to combine the results of endothelial function testing with other test or clinical scoring systems of surgical risk.

Evaluation of New Therapies on Endothelial Function

The assessment of FMD of the brachial artery in clinical trials is increasingly used because of its user-friendly, efficient, and noninvasive nature. Both parallel-group and crossover designs for short-term and longer term intervention trials have successfully used FMD measurement as a reliable technique. A significant improvement in FMD can be seen with 20 to 30 patients in a crossover design study, and with 40 to 60 patients in a parallel-group design study. In studies of this size, the minimal statistically significant improvement that can be detected with intervention is an absolute change in FMD of 1.5 to 2%. An important parameter in such trials is the time-dependent reproducibility of FMD. An acceptable reproducibility is a mean difference of 2 to 3% in FMD over time (on a baseline vasodilation of about 10%). An important factor for the interpretation of the results is the change in baseline diameter. For example, a decrease in baseline diameter will result in an increase in FMD. This increase in FMD is a result of the change in resting tone and not of the beneficial effect of the intervention on endothelial function.[33] Follow-up studies assessing the effects of therapeutic interventions on endothelial function need to have highly standardized study protocols regarding all variable factors.

Investigation of Neurally Mediated Syncope

Takase et al[49] demonstrated that patients with neurally mediated syncope have significantly higher endothelium-dependent and endothelium-independent vasodilation of the brachial artery compared with control subjects. Paradoxical peripheral vasodilation is one of the suspected mechanisms of neurally mediated syncope. Parasympathetic stimulation following sympathetic activation contributes to this vasodilation. Due to increased vagal activity, there is an augmented presence of the postsynaptic transmitter acetylcholine. The increased level of acetylcholine can cause vasodilation through NO.

In the past, head-up tilt table testing was the diagnostic technique of choice for clinically assessing a patient's susceptibility to neurally mediated syncope, particularly of the vasovagal type.[50] However, tilt testing is a sensitive, but nonspecific, method for confirming neurocardiogenic syncope. Most studies have suggested that such testing discriminates relatively well between symptomatic patients and asymptomatic control subjects, of whom 10 to 15% have

false-positive test results. The sensitivity of tilt table testing is more difficult to evaluate because there is no accepted diagnostic "gold standard." However, sensitivity (measured against a classic presentation) has been estimated to range from 32 to 85%, with most reports favoring the higher end of this range.[51] Therefore, the measurement of FMD might contribute to the diagnostic accuracy of neurally mediated syncope.

Variable Factors in FMD Measurement

Changes in FMD have been described not only in the presence of classic risk factors, but also in a variety of other factors such as endogenous, exogenous, environmental, and familiar factors (Table 1). This observation is very interesting but makes a correct serial evaluation of FMD in the same subject or a comparison of FMD between subjects very difficult because of the special attention that should be paid to all factors that can influence FMD.

Age and gender are well-known classic risk factors. Diminished endothelial function with advancing age already has been described.[52,53] The mechanisms remain uncertain but may relate to age-associated increases in reactive oxygen species production.[54] In women, there is even an age-related decline in FMD.

Sustained arterial hypertension blunts FMD in conduit arteries in the peripheral[55] and coronary circulation.[56–58] The degree of coronary endothelial dysfunction depends on the severity and duration of arterial hypertension as indexed by the degree of left ventricular hypertrophy.[59,60] The pathogenesis of the association between endothelial dysfunction and hypertension is not fully understood. There is evidence that endothelial dysfunction may antedate and possibly contribute to the development of essential hypertension.[61] However, the larger recent Framingham Heart study[62] failed to determine whether endothelial dysfunction is a cause or a consequence of hypertension, or, alternatively, whether FMD and systolic BP are associated with a third factor, such as arterial stiffness.

Diabetic patients demonstrate a glucose-mediated endothelial dysfunction. Potential mechanisms for this include reduced NO bioavailability caused by the increased formation of reactive oxygen intermediates,[63,64] glucose autooxidation,[65] activation of protein kinase C,[66,67] formation of advanced glycosylation end products,[68] decreased NO synthase expression,[69] and direct chemical inactivation of NO by glucose.[70] Short-term antioxidant stress possibly plays a role in glucose-mediated endothelial dysfunction, because pretreatment with antioxidant vitamins may reverse hyperglycemia-induced impairment in endothelial function.[71,72]

FMD is also impaired by elevated levels of cholesterol, whereas the plasma level of triglycerides does not affect FMD.[73,74] In hypercholesterolemia, the impaired vasodilator response may result from increased NO inactivation by oxygen free radicals generated by the presence of oxidized low-density lipoprotein cholesterol and/or lipoprotein (a) particles in the subendothelial space,[75] or, in some circumstances, by substrate-limited NO production.

FMD has been shown to be blunted in obese individuals. FMD is significantly lower in healthy obese subjects, compared with healthy nonobese volunteers.[76] The mechanisms of obesity-induced endothelial dysfunction are multifactorial, including dyslipidemia, elevated BP, increased inflammation, oxidative stress, and changes in glucose metabolism.

Additionally, weight reduction with a very-low-calorie diet improves FMD in obese individuals. This improvement is related to the reduction in plasma glucose concentration, suggesting that changes in glucose metabolism may determine endothelial vasodilatory function in obese persons.[77]

Patients with even mild hyperhomocysteinemia have an impaired endothelium-dependent vasodilation. The underlying pathobiological mechanisms are multiple. Hyperhomocysteinemia leads to the increased oxidative inactivation of NO by oxygen-derived free radicals that are formed during the autooxidation of homocysteine, and/or accumulate as a consequence of the homocysteine-mediated inhibition of antioxidant enzymes or of increased endothelial synthesis of the endogenous NO synthase inhibitor asymmetric dimethylarginine. Additionally, elevated homocysteine levels induce the expression of several chemokines and adhesion molecules. Folic acid is well-accepted as the therapeutic strategy for hyperhomocysteinemia. In healthy adults without conventional atherosclerotic risk factors but with mildly elevated homocysteine levels (*ie*, > 13 μmol/

Table 1—*Factors That May Impede FMD*

Age
Gender
Arterial hypertension
Diabetes mellitus
Cholesterol
Obesitas
Hyperhomocysteinemia
CAD
Hormonal (*eg*, catecholamines, estrogen)
Diurnal variation
Low birth weight
Familial history (diabetes and CAD)
Smoking (active and passive)

L), folic acid not only lowers the plasma level of homocysteinemia, it also improves FMD.

It has been shown that a high percentage of patients with unstable angina pectoris have concurrent diminished FMD. Interestingly, the disturbance of endothelial function was reversible after the treatment of acute coronary syndromes.[78] In patients with stable CAD, plasma levels of C-reactive protein were associated with FMD, confirming the relationship between inflammation and the integrity of the endothelium.[79]

FMD is also influenced by hormonal factors, such as circulating levels of estrogen, progesterone, and catecholamines. The investigator should be cognizant of professional sleep deprivation,[80] mental stress (catecholamines),[81] and the phase of the subject's menstrual cycle (estrogen),[82] as these factors may also affect FMD.

Etsuda et al[83] and Otto et al[84] showed a significant diurnal variation in FMD within healthy young men (FMD: 8:00 AM, 4.0%; 12:00 PM, 5.3%; 5:00 PM, 9.7%; 9:00 PM, 6.9%), demonstrating the importance of standardizing the time of day for assessment. Additionally, Title et al[71] and Plotnick et al[85] demonstrated an attenuated response in FMD after a glucose or fat load. These transient reductions lasted up to 3 and 4 h, respectively. Clearly, this indicates the importance of the timing of meals before testing. Therefore, it is recommended that the time of day for testing (between 7:00 and 10:00 AM) be standardized and that a fast of at least 8 h be required.

To limit the factors that can affect FMD vascular reactivity (Table 2), the patients should fast, including caffeine, for at least 8 h before the study and should not have exercised before the test. Preferably FMD measuring should take place in the morning, in a quiet, temperature-controlled room. In addition, patients should not smoke, and all vasoactive medications should be withheld for at least four half-lives, if possible. There are no racial differences in conduit vessel FMD.[42]

Low birth weight is associated with impaired endothelial function in childhood, which is a key early event in atherogenesis. Growth *in utero* may be associated with long-term changes in vascular function that are manifested by the first decade of life and that may influence the long-term risk of cardiovascular disease. FMD correlates positively and significantly with birth weight.[86]

Healthy young adults with a family history of premature CAD have impaired FMD, even in the absence of other risk factors.[87] This impaired brachial artery FMD not only coincides but also correlates with a greater IMT of the common carotid artery, indicating early functional and structural changes in the vascular endothelium in the offspring of patients with premature CAD.[88] Similar results were obtained in first-degree relatives of patients with type 2 diabetes.[89] Endogenous factors as well as environmental factors, like passive smoking,[90] may impair brachial artery FMD.

Table 2—*Practical Setup*

Quiet, temperature-controlled examination room
Examination between 7:00 AM
Starvation (including caffeine) ≥ 8 h
No smoking (active and passive)
No exercise, no night work
No mental stress
Stop vasoactive medication ≥ 4 × half-life
In case of long-term follow-up: cave changes in BP, cholesterol level, and weight; be aware of stage of menstrual cycle

Technique Validity, Reliability, and Reproducibility in Clinical Trials

In healthy people, FMD is 7 to 10%[14,20,25,87,90] of the baseline diameter, but in patients with cardiovascular disease FMD is impaired or absent, with FMD values of 0 to 5%. Recently, in the Framingham Heart Study[62] (n = 2,883; age range, 33 to 88 years), the mean FMD in women was 3.3% (interquartile range, 1.1 to 4.9%), and 2.4% in men (interquartile range, 0.7 to 3.7%).

Numerous factors may contribute to the variability of FMD, mainly equipment-related, surgery-related, and physiologic influences. Hijmering et al[91] analyzed the reproducibility in healthy volunteers (n = 112). The intrasession variability was 1.1% (extremes, 0.06 to 2.0%), and the intersession variability was 3.6% for observer 1 and 3.8% for observer 2.

Sorensen et al[14] calculated that in clinical trials a mean improvement in FMD of ≥ 2% is necessary to detect a treatment benefit and that for any individual a difference of 4 to 8% is necessary to account for natural variability. Welsch et al[92] studied the clinical trial design and calculated that in a study with a cross-sectional design (*ie*, smokers vs nonsmokers) 23 subjects would be required to detect an FMD difference of 60% (two-tailed) (*eg*, 5% vs 8%, respectively, at 90% power). To achieve an FMD difference of 40% (*eg*, 5% vs 7%, respectively, at 90% power), 46 subjects have to be included.

To achieve a power of 90% in a study with an interventional design (*ie*, pre-posttreatment), 10 and 19 subjects are required to detect FMD differences of 60% and 40%, respectively (two-tailed). To achieve a power of 80%, 46 subjects are needed to detect an FMD difference of 20% (two-tailed).

FMD measurement can be considered to be a

stable and reproducible technique[92] when performed under strictly controlled conditions. Importantly, to ensure high reproducibility, it is recommended that study designs incorporate a single tester to ensure that subjects are scanned by the same individual each visit.

Interrelationship Among FMD, IMT, and PWV

FMD of the brachial artery, pulsewave velocity (PWV), and carotid IMT are all noninvasive techniques that are used for the assessment of cardiovascular risks, and are all used as surrogate markers for atherosclerosis. IMT detects morphologic changes of the carotid artery, consisting of both an intimal atherosclerotic process and medial hypertrophy.[93] IMT is measured by B-mode ultrasonography. PWV reflects arterial distensibility and is measured by pressure or volume pulsewave analysis using a transducer.[94]

There is a significant correlation between aortic PWV and carotid IMT,[95,96] and there is a negative correlation between FMD and IMT.[97] Recently, Kobayashi et al[98] demonstrated that decreased FMD in the brachial artery was related to increased brachial-ankle and heart-carotid PWV, as well as to increased carotid IMT. The combination of these three methods is very useful in predicting the burden of atherosclerosis, since it was found that subjects with the worst tertiles of all three measurements had a markedly higher prevalence of atherosclerotic disease and carotid plaques. The combination of FMD, IMT, and PWV serves as a more accurate indicator of clinical atherosclerosis than any single measurements.

Caveats in FMD Measurement

First, it should be noted that the FMD value is influenced by changes in baseline diameter. Baseline diameter is an important determinant of measures of the percent of change and must be considered when comparing vasodilator responses between different groups of subjects. Aging appears to result in progressive dilation of the brachial arteries at rest and in progressive diminution of the vasodilator response to a flow stimulus.

Most studies published so far have found a negative relationship between baseline diameter and FMD. Many studies have recognized this possible confounder and have matched for baseline diameters. However, drugs that produce increases in baseline diameter, such as angiotensin-converting enzyme inhibitors, angiotensin 1-receptor inhibitors, or calcium antagonists, will lead to smaller estimates of FMD without correction.

Second, there is no consensus about upper vs lower cuff placement. In addition, FMD can be studied in the radial, axillary, and superficial femoral arteries. However, arteries < 2.5 mm in diameter are difficult to measure, and vasodilation is generally less difficult to perceive in vessels > 5.0 mm in diameter.[14,99,100]

Third, and perhaps most important, is the specification of the ultrasound system used. Most authors use regular B-mode imaging, which is capable of discerning two adjacent points at least 0.2 to 0.3 mm apart.[14] To show a 5% increase in vasodilation in a brachial artery with 5 mm diameter, a diameter of 0.25 mm must be visualized. Clearly, without the use of modified B-mode systems or M-mode systems with radiofrequency signal processing, it is impossible to reliably detect small changes in FMD. Additionally, there are no long-term prognostic data that link FMD to really hard end points, such as overt atherosclerosis, myocardial infarction, stroke, and death.

Conclusion

It has been shown that peripherally measured endothelial function in the brachial artery predicts future cardiovascular disease[23,47,101] and the postoperative occurrence of cardiovascular events.[48] It is also an excellent tool in the risk stratification of patients with chest pain, especially before they undergo angiography and when the first test results are negative. Additionally, FMD measurement can be useful in the difficult diagnosis of neurally mediated syncope and in judging the potential utility of new interventions for cardiovascular disease.[102]

The noninvasive evaluation of FMD is very attractive, and in this way it provides a minimal burden to the study population. Therefore, it is the preferred tool with which to study endothelial function in children and pregnant women. The following equipment needed is generally present in each clinical setting: a pneumatic cuff to create reactive hyperemia; and high-resolution ultrasonography to quantify diameter changes. The whole procedure is finished in < 30 min. However, because of the large variation in intersession FMD responses, often exceeding half of the baseline FMD value, the use of FMD as a "therapeutic target" for an individual patient is limited, particularly since the variation seems to be largely due to physiologic fluctuations.[91] However, the measurement of FMD is an attractive tool for serial measurements and long-term studies.

References

1 Coronary artery surgery study (CASS): a randomized trial of coronary artery bypass surgery: survival data. Circulation 1983; 68:939–950

2 Califf RM, Phillips HR III, Hindman MC, et al. Prognostic value of a coronary artery jeopardy score. J Am Coll Cardiol 1985; 5:1055–1063

3 Topol EJ, Nissen SE. Our preoccupation with coronary luminology: the dissociation between clinical and angiographic findings in ischemic heart disease. Circulation 1995; 92:2333–2342

4 Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990; 323:27–36

5 Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990; 81:491–497

6 Vita JA, Keaney JF Jr. Exercise: toning up the endothelium? N Engl J Med 2000; 342:503–505

7 Vita JA, Keaney JF Jr. Endothelial function: a barometer for cardiovascular risk? Circulation 2002; 106:640–642

8 Suwaidi JA, Hamasaki S, Higano ST, et al. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000; 101:948–954

9 Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000; 101: 1899–1906

10 Kelm M. Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects. Am J Physiol Heart Circ Physiol 2002; 282:H1–H5

11 Laurent S, Lacolley P, Brunel P, et al. Flow-dependent vasodilation of brachial artery in essential hypertension. Am J Physiol 1990; 258:H1004–H1011

12 Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. Circulation 1989; 79:93–100

13 Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340:1111–1115

14 Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. Br Heart J 1995; 74:247–253

15 Drexler H, Zeiher AM. Endothelial function in human coronary arteries *in vivo*: focus on hypercholesterolemia. Hypertension 1991; 18(suppl):II90–II99

16 Adams MR, McCredie R, Jessup W, et al. Oral L-arginine improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease. Atherosclerosis 1997; 129:261–269

17 Nitenberg A, Antony I, Foult JM. Coronary vasoconstriction induced by acetylcholine in young smokers with normal angiographic coronary vessels. Arch Mal Coeur Vaiss 1993; 86:1133–1136

18 Lekakis J, Papamichael C, Vemmos C, et al. Effect of acute cigarette smoking on endothelium-dependent brachial artery dilatation in healthy individuals. Am J Cardiol 1997; 79:529–531

19 Nitenberg A, Valensi P, Sachs R, et al. Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. Diabetes 1993; 42:1017–1025

20 Clarkson P, Celermajer DS, Donald AE, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. J Am Coll Cardiol 1996; 28:573–579

21 Montalescot G, Ankri A, Chadefaux-Vekemans B, et al. Plasma homocysteine and the extent of atherosclerosis in patients with coronary artery disease. Int J Cardiol 1997; 60:295–300

22 Woo KS, Chook P, Lolin YI, et al. Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 1997; 96:2542–2544

23 Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26:1235–1241

24 Neunteufl T, Katzenschlager R, Hassan A, et al. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. Atherosclerosis 1997; 129:111–118

25 Motoyama T, Kawano H, Kugiyama K, et al. Flow-mediated, endothelium-dependent dilatation of the brachial arteries is impaired in patients with coronary spastic angina. Am Heart J 1997; 133:263–267

26 Schretzenmayer A. Uber kreislaufregulatoische vörgänge an den grossen arterien bei der muskelarbeit. Pflugers Arch 1933; 232:S743–S748

27 Meredith IT, Currie KE, Anderson TJ, et al. Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. Am J Physiol 1996; 270:H1435–H1440

28 Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries *in vivo*. Circulation 1995; 91:1314–1319

29 Cooke JP, Rossitch E Jr, Andon NA, et al. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest 1991; 88:1663–1671

30 Miura H, Wachtel RE, Liu Y, et al. Flow-induced dilation of human coronary arterioles: important role of Ca(2+)-activated K(+) channels. Circulation 2001; 103:1992–1998

31 Olesen SP, Clapham DE, Davies PF. Haemodynamic shear stress activates a K+ current in vascular endothelial cells. Nature 1988; 331:168–170

32 Pohl U, Holtz J, Busse R, et al. Crucial role of endothelium in the vasodilator response to increased flow *in vivo*. Hypertension 1986; 8:37–44

33 Corretti MC, Anderson TJ, Benjamin EJ, et al. 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

34 Fukai T, Siegfried MR, Ushio-Fukai M, et al. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000; 105:1631–1639

35 Mitchell GF, Parise H, Vita JA, et al. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. Hypertension 2004; 44:134–139

36 Sun D, Huang A, Smith CJ, et al. Enhanced release of prostaglandins contributes to flow-induced arteriolar dilation in eNOS knockout mice. Circ Res 1999; 85:288–293

37 Mullen MJ, Kharbanda RK, Cross J, et al. Heterogenous nature of flow-mediated dilatation in human conduit arteries *in vivo*: relevance to endothelial dysfunction in hypercholesterolemia. Circ Res 2001; 88:145–151

38 Bevan JA. Flow regulation of vascular tone: its sensitivity to changes in sodium and calcium. Hypertension 1993; 22:273–281

39 Shechter M, Sharir M, Labrador MJ, et al. Oral magnesium therapy improves endothelial function in patients with coronary artery disease. Circulation 2000; 102:2353–2358

40 Ducharme A, Dupuis J, McNicoll S, et al. Comparison of nitroglycerin lingual spray and sublingual tablet on time of onset and duration of brachial artery vasodilation in normal subjects. Am J Cardiol 1999; 84:952–954
41 Adams MR, Robinson J, McCredie R, et al. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. J Am Coll Cardiol 1998; 32:123–127
42 Gokce N, Holbrook M, Duffy SJ, et al. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. Hypertension 2001; 38:1349–1354
43 Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001; 104:2673–2678
44 Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001; 104:191–196
45 Fathi R, Haluska B, Isbel N, et al. The relative importance of vascular structure and function in predicting cardiovascular events. J Am Coll Cardiol 2004; 43:616–623
46 Neunteufl T, Heher S, Katzenschlager R, et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. Am J Cardiol 2000; 86:207–210
47 Schroeder S, Enderle MD, Ossen R, et al. Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. Am Heart J 1999; 138:731–739
48 Gokce N, Keaney JF Jr, Hunter LM, et al. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 2002; 105:1567–1572
49 Takase B, Akima T, Uehata A, et al. Endothelial function and peripheral vasomotion in the brachial artery in neurally mediated syncope. Clin Cardiol 2000; 23:820–824
50 Oh JH, Kim JS, Kwon HC, et al. Predictors of positive head-up tilt test in patients with suspected neurocardiogenic syncope or presyncope. Pacing Clin Electrophysiol 2003; 26:593–598
51 Benditt DG. Neurally mediated syncopal syndromes: pathophysiological concepts and clinical evaluation. Pacing Clin Electrophysiol 1997; 20:572–584
52 Herrington DM, Fan L, Drum M, et al. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. J Cardiovasc Risk 2001; 8:319–328
53 Celermajer DS, Sorensen KE, Spiegelhalter DJ, et al. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 1994; 24:471–476
54 Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993; 90:7915–7922
55 Duffy SJ, Gokce N, Holbrook M, et al. Effect of ascorbic acid treatment on conduit vessel endothelial dysfunction in patients with hypertension. Am J Physiol Heart Circ Physiol 2001; 280:H528–H534
56 Antony I, Lerebours G, Nitenberg A. Loss of flow-dependent coronary artery dilatation in patients with hypertension. Circulation 1995; 91:1624–1628
57 Frielingsdorf J, Kaufmann P, Seiler C, et al. Abnormal coronary vasomotion in hypertension: role of coronary artery disease. J Am Coll Cardiol 1996; 28:935–941
58 Solzbach U, Hornig B, Jeserich M, et al. Vitamin C improves endothelial dysfunction of epicardial coronary arteries in hypertensive patients. Circulation 1997; 96:1513–1519
59 Hamasaki S, Al Suwaidi J, Higano ST, et al. Attenuated coronary flow reserve and vascular remodeling in patients with hypertension and left ventricular hypertrophy. J Am Coll Cardiol 2000; 35:1654–1660
60 Houghton JL, Davison CA, Kuhner PA, et al. Heterogeneous vasomotor responses of coronary conduit and resistance vessels in hypertension. J Am Coll Cardiol 1998; 31:374–382
61 Taddei S, Virdis A, Mattei P, et al. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. Circulation 1996; 94:1298–1303
62 Benjamin EJ, Larson MG, Keyes MJ, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation 2004; 109:613–619
63 Cosentino F, Hishikawa K, Katusic ZS, et al. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997; 96:25–28
64 Tack CJ, Ong MK, Lutterman JA, et al. Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance: effects of troglitazone. Diabetologia 1998; 41: 569–576
65 Wolff SP, Dean RT. Glucose autoxidation and protein modification: the potential role of "autoxidative glycosylation" in diabetes. Biochem J 1987; 245:243–250
66 Hempel A, Maasch C, Heintze U, et al. High glucose concentrations increase endothelial cell permeability via activation of protein kinase C α. Circ Res 1997; 81:363–371
67 Cosentino F, Eto M, De Paolis P, et al. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. Circulation 2003; 107:1017–1023
68 Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 1991; 87:432–438
69 Ding Y, Vaziri ND, Coulson R, et al. Effects of simulated hyperglycemia, insulin, and glucagon on endothelial nitric oxide synthase expression. Am J Physiol Endocrinol Metab 2000; 279:E11–E17
70 Cronstein BN, Rosenstein ED, Kramer SB, et al. Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils: adenosine acts via an A2 receptor on human neutrophils. J Immunol 1985; 135:1366–1371
71 Title LM, Cummings PM, Giddens K, et al. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. J Am Coll Cardiol 2000; 36:2185–2191
72 Beckman JA, Goldfine AB, Gordon MB, et al. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 2001; 103: 1618–1623
73 Schnell GB, Robertson A, Houston D, et al. Impaired brachial artery endothelial function is not predicted by elevated triglycerides. J Am Coll Cardiol 1999; 33:2038–2043
74 Vogel RA, Corretti MC, Gellman J. Cholesterol, cholesterol lowering, and endothelial function. Prog Cardiovasc Dis 1998; 41:117–136
75 Galle J, Bengen J, Schollmeyer P, et al. Impairment of endothelium-dependent dilation in rabbit renal arteries by oxidized lipoprotein(a): role of oxygen-derived radicals. Circulation 1995; 92:1582–1589
76 Oflaz H, Ozbey N, Mantar F, et al. Determination of

endothelial function and early atherosclerotic changes in healthy obese women. Diabetes Nutr Metab 2003; 16:176–181
77 Raitakari M, Ilvonen T, Ahotupa M, et al. Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. Arterioscler Thromb Vasc Biol 2004; 24:124–128
78 Esper RJ, Vilarino J, Cacharron JL, et al. Impaired endothelial function in patients with rapidly stabilized unstable angina: assessment by noninvasive brachial artery ultrasonography. Clin Cardiol 1999; 22:699–703
79 Vitale C, Cerquetani E, Wajngarten M, et al. In patients with coronary artery disease endothelial function is associated with plasma levels of C-reactive protein and is improved by optimal medical therapy. Ital Heart J 2003; 4:627–632
80 Amir O, Alroy S, Schliamser JE, et al. Brachial artery endothelial function in residents and fellows working night shifts. Am J Cardiol 2004; 93:947–949
81 Ghiadoni L, Donald AE, Cropley M, et al. Mental stress induces transient endothelial dysfunction in humans. Circulation 2000; 102:2473–2478
82 Sorensen KE, Dorup I, Hermann AP, et al. Combined hormone replacement therapy does not protect women against the age-related decline in endothelium-dependent vasomotor function. Circulation 1998; 97:1234–1238
83 Etsuda H, Takase B, Uehata A, et al. Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? Clin Cardiol 1999; 22:417–421
84 Otto ME, Svatikova A, Barretto RB, et al. Early morning attenuation of endothelial function in healthy humans. Circulation 2004; 109:2507–2510
85 Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. JAMA 1997; 278:1682–1686
86 Leeson CP, Whincup PH, Cook DG, et al. Flow-mediated dilation in 9- to 11-year-old children: the influence of intrauterine and childhood factors. Circulation 1997; 96: 2233–2238
87 Clarkson P, Celermajer DS, Powe AJ, et al. Endothelium-dependent dilatation is impaired in young healthy subjects with a family history of premature coronary disease. Circulation 1997; 96:3378–3383
88 Gaeta G, De Michele M, Cuomo S, et al. Arterial abnormalities in the offspring of patients with premature myocardial infarction. N Engl J Med 2000; 343:840–846
89 Balletshofer BM, Rittig K, Enderle MD, et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation 2000; 101:1780–1784
90 Celermajer DS, Adams MR, Clarkson P, et al. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. N Engl J Med 1996; 334: 150–154
91 Hijmering ML, Stroes ES, Pasterkamp G, et al. Variability of flow mediated dilation: consequences for clinical application. Atherosclerosis 2001; 157:369–373
92 Welsch MA, Allen JD, Geaghan JP. Stability and reproducibility of brachial artery flow-mediated dilation. Med Sci Sports Exerc 2002; 34:960–965
93 Simon A, Gariepy J, Chironi G, et al. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. J Hypertens 2002; 20:159–169
94 O'Rourke MF, Mancia G. Arterial stiffness. J Hypertens 1999; 17:1–4
95 van Popele NM, Grobbee DE, Bots ML, et al. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke 2001; 32:454–460
96 Mackey RH, Sutton-Tyrrell K, Vaitkevicius PV, et al. Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. Am J Hypertens 2002; 15:16–23
97 Hashimoto M, Eto M, Akishita M, et al. Correlation between flow-mediated vasodilatation of the brachial artery and intima-media thickness in the carotid artery in men. Arterioscler Thromb Vasc Biol 1999; 19:2795–2800
98 Kobayashi K, Akishita M, Yu W, et al. Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. Atherosclerosis 2004; 173:13–18
99 Stadler RW, Karl WC, Lees RS. New methods for arterial diameter measurement from B-mode images. Ultrasound Med Biol 1996; 22:25–34
100 Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. Am J Physiol 1995; 268:H1397–H1404
101 Takase B, Uehata A, Akima T, et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998; 82:1535–1538
102 Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible? J Am Coll Cardiol 1997; 30:325–333