Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects

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A disturbance of endothelial function is considered as a key event in the development of atherosclerosis (63). Thus reliable assessment of endothelial function in humans appears highly desirable. With respect to the major endothelial functions, this aim can be achieved by different approaches: 1) measurement of morphological and mechanical characteristics of the vascular wall (intima media thickness, compliance, distensibility, and remodelling indexes); 2) determination of soluble endothelial markers (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. The endothelium is of essential importance for the maintenance of vascular tone. It participates in the regulation of blood flow in response to changes in tissue and organ perfusion requirements. When blood flow increases through a vessel, the vessel dilates. This phenomenon has been coined flow-mediated dilatation (FMD). Schretzenmayer (56) was first to describe this physiological response, and FMD has been demonstrated subsequently in a number of conduit arteries in vitro and in vivo, in animals and in humans. This editorial will focus on pathophysiological aspects and critically evaluate the potential clinical significance of FMD measurement in humans.

MECHANISMS OF FMD: PHYSIOLOGICAL ASPECTS

An intact endothelium is crucial for flow-dependent dilatation of conduit arteries. This has first been demonstrated in femoral arteries (50, 53) and epicardial arteries (26, 29). Only in some resistance arteries is FMD mediated, at least in part, independent from the endothelium (7). Shear stress is mainly determined by blood flow, and its tractive force exerted at a vector perpendicular to the long axis of the vessel. The endothelium acts as a mechanotransducer that senses changes in shear stress and subsequently modifies the output of dilator factors. Several mediators have been proposed to be involved in FMD: prostaglandins (28), ATP or an endothelium-derived hyperpolarizing factor (42), and most importantly nitric oxide (NO) (30). An increased release of NO in response to increases in shear stress not only dilates underlying smooth muscle of conduit arteries but also maintains the concentration of NO constant at the luminal surface of vascular endothelium despite flow increases (33). FMD is diminished by reduction in extracellular calcium and sodium (6) and is improved by magnesium (60). The FMD response not only depends on the absolute level but also on the gradient of shear stress, i.e., change per unit time, and the frequency of changes in amplitude (47). In human forearm circulation, endothelial responses to blood flow depend on the characteristics of the flow stimulus. FMD after brief episodes of hyperemia is almost exclusively mediated by NO, whereas dilatation during sustained hyperemia is unaffected by NO synthesis inhibition (44).

The endothelial signaling cascade responsible to convert mechanistic stimuli into the release of vasodilatory molecules has not been fully clarified. As yet, several mechanisms have been suggested: an endothelial potassium channel coupled to a pertussis toxin-sensitive G protein, kininergic mechanisms, sodium-dependent conformational changes of membrane glycosaminoglycans, and an initial calcium-dependent activation of phospholipase C combined with a longer-lasting calcium-independent activation of protein kinase C and tyrosine kinase (6, 47). More recently, it has been demonstrated that shear stress induces phosphorylation of a serine residue altering endothelial NO synthase (eNOS) sensitivity to intracellular calcium levels and thus increasing NO formation (15). FMD critically depends on the eNOS activity at the level of the conduit arteries. Brief episodes of reactive hyperemia, as seen with regular physical exercise, increase the level of shear stress in conduit arteries. The consecutive increase in constitutive eNOS expression improves endo-
thelial function and thus FMD (12, 16, 30, 31, 52, 59, 70, 71). But FMD not only depends on NO formation but also on NO inactivation and the sensitivity of the underlying vascular smooth muscle for NO. Regular exercise simultaneously induces upregulation not only of eNOS but also of superoxide dismutase expression (21). This feed-forward mechanism could prevent superoxide-mediated inactivation of NO and thus increase shear stress-dependent FMD.

**FMD MEASUREMENTS IN HUMANS: DIAGNOSTIC AND THERAPEUTIC ASPECTS**

Endothelial dysfunction is reflected by an impaired FMD response. In human vasculature, FMD is most often studied in the forearm and coronary circulation. Principally, a vasodilatory stimulus is applied to the downstream vascular bed eliciting a flow-dependent dilation of the upstream conduit vessel. The kind of dilatory stimulus depends on the vascular bed under investigation.

Celermajer and colleagues (9) introduced a unique setup to study FMD noninvasively and reliably in human forearm circulation. An increase in flow through the brachial artery is induced by causing postischemic dilatation in the downstream vascular bed of the distal forearm. This is achieved by inflating a cuff placed around the proximal forearm to suprasystolic pressure producing a ischemia in the distal vascular bed. After the release of the cuff pressure, a sudden increase of blood flow into the dilated vascular bed occurs. The subsequent increase in shear stress in the upstream conduit artery cause a dilatation of the brachial artery, which can be assessed by an ultrasound device. As a control the response of the brachial artery to sublingual glycerol trinitrate (GTN) is recorded. The duration and the amplitude of brachial artery dilatation upon GTN is somewhat more pronounced than during FMD (+15% vs. +10%). With increasing number of cardiovascular risk factors, smooth muscle dysfunction becomes apparent and thus GTN response is progressively impaired independently from endothelial dysfunction (1). This has to be considered when studying patients with either coronary or systemic atherosclerosis.

A variety of stimuli acutely influence FMD: a single high-fat meal and postprandial lipemia (19, 66), mental stress, and most probably by catecholamines (23), circulating levels of estrogen and progesterone (62), smoking (37), acute changes in glucose (32), and changes in sodium and calcium (6). Acute increases in oxidative stress occurring during hemodialysis have also been reported to be related to impaired brachial artery FMD (43). In contrast, the FMD response appears to be independent from whole blood viscosity (45). Furthermore, diurnal variation in regulation of vascular tone are well known. Thus FMD measurement enables clinical physiologists to sensitively assess acute changes in endothelial function in humans. However, in followup studies assessing therapeutic interventions on endothelial function, patients have to be matched and a study protocol must be standardized for the aforementioned potential impact factors on FMD.

In general, these precautions concerning patient selection and study conditions in measurement of brachial artery reactivity also apply to the coronary circulation. Dilatation of coronary resistance vessels to induce increases in shear stress in the upstream epicardial arteries can be achieved by metabolic stimuli such as exercise or pacemaker stimulation. Alternatively, this can be mimicked by selective infusion of adenosine (69) or papaverine (68) into the midportion of the epicardial artery and simultaneous quantification of FMD in the proximal segment of the artery under investigation. In this segment, endothelium-dependent changes in tone are detected mainly by quantitative computer-supported analysis.

It is still not clear whether or not the endothelial dysfunction of the coronary circulation is a focal or a systemic disturbance of the vasculature that occurs simultaneously in other territories of the circulation. Assessment of FMD in epicardial arteries is a much more invasive approach compared with FMD measurement in the brachial artery. Therefore, several studies addressed the issue that brachial artery FMD measurement may represent a surrogate for diagnostic evaluation of coronary circulation in patients with evident coronary artery disease (CAD) or those individuals at risk for CAD. In these studies (3, 39, 46, 57), the endothelium-dependent (3) as well as the endothelium-independent (49) dilatation in the forearm circulation was determined in patients with CAD. A close correlation of endothelial function in the human coronary and peripheral vasculature has been demonstrated in some but not all studies (2, 38). It has also been shown that a high percentage of patients with unstable angina pectoris had concurrent endothelial dysfunction of the brachial artery in the ultrasound scan. Interestingly, the disturbance of endothelial function was reversible after treatment of acute coronary syndrome (18). However, the patient numbers studied so far are too small to permit a statistically confirmed statement with regard to the specificity and sensitivity of brachial artery FMD measurement to predict endothelial dysfunction and CAD in the coronary vasculature.

There is ample evidence that FMD measurement sensitively detects endothelial dysfunction in hyperlipidemia, arterial hypertension, and diabetes, all considered as major cardiovascular risk factors. FMD in the brachial artery is impaired by elevated levels of cholesterol, whereas the plasma level of triglycerides does not affect FMD (55, 65). The extent of endothelial dysfunction depends on the total cholesterol level (58). In arterial hypertension, an altered bioactivity of NO is involved in endothelial dysfunction of coronary and peripheral circulation (34, 35). Sustained arterial hypertension blunts FMD in conduit arteries in peripheral (17) and coronary circulation (4, 20, 61). The degree of coronary endothelial dysfunction depends on the severity and duration of arterial hypertension as indexed by the degree of left ventricular hypertension.
Endothelial dysfunction in conduit arteries and the microvasculature is also frequently seen in diabetes mellitus (13). FMD in epicardial vessels is reduced in diabetic individuals (48). Hyperglycemia blunted endothelium-dependent vasodilation in conduit (32) and resistance vessels (67). Thus, in humans, FMD measurement not only enables to reliable diagnose endothelial dysfunction associated with the major atherogenic risk factors but also to quantify the degree of endothelial dysfunction in relation to the severity of hyperlipidemia, hypertension, and diabetes.

With the use of invasive testing with intracoronary application of acetycholine, several studies demonstrated that endothelial dysfunction in CAD may be reversible (40, 64), which raises the possibility that progression of atherosclerosis may be slowed. This underscores the need for a sensitive and reproducible testing of endothelial function in clinical routine and therapeutic follow-up studies. Brachial artery FMD measurement may represent such a noninvasive diagnostic alternative to the acetylcholine test. In patients with CAD, improvement of brachial artery FMD has already been proven after long-term therapy with ascorbic acid, angiotensin-converting enzyme inhibitors, ciprofibrate, and L-arginine (2, 10, 19, 24, 38).

FMD MEASUREMENTS IN HUMANS: GENETIC AND PROGNOSTIC ASPECTS

Brachial artery reactivity has been successfully used to investigate the genetic influence on early arterial physiology that may be relevant to later clinical disease. Intrauterine and childhood factors also appear to influence brachial artery FMD. Recent data indicate that FMD positively and significantly correlates with birth weight. This relation was not altered by adjustment for childhood body build, parity, cardiovascular risk factors, social class, or ethnicity (36). Furthermore, an increased carotid stiffness is associated with low birth weight (41). Thus growth in utero and other so far not identified genetic determinants may be associated with long-term changes in vascular function that are manifest by the first decade of life. These factors may influence the long-term risk of cardiovascular disease.

In adulthood, endothelial dysfunction has been implicated as a key event in the pathogenesis of atherosclerosis. FMD is impaired with progressive atherosclerosis (14, 71). Furthermore, there is some evidence that a reduced FMD in epicardial arteries predicts cardiovascular event rates (54). Measurement of brachial artery FMD is a noninvasive diagnostic procedure. This easily allows assessment of endothelial function also in offspring or in first-degree relatives from patients with evident atherosclerosis. Healthy young adults with a family history of premature CAD have impaired FMD, even in the absence of other risk factors (11). This impaired brachial artery FMD not only coincides but also correlates with a greater intima-media thickness (IMT) of the common carotid artery, indicating early functional and structural changes of vascular endothelium in offspring of patients with premature CAD (22). Similar results were obtained in first-degree relatives of patients with type 2 diabetes (5). Thus FMD measurement enables us to potentially identify patients at risk for atherosclerotic complications. This not only underscores the prognostic impact of endothelial dysfunction but also provides a rationale for future risk stratification of patients.

Not only endogenous, but also environmental factors, may impair vascular function. Measuring brachial artery FMD, it has been demonstrated that passive smoking impairs endothelial function in humans (8). However, the interindividual susceptibility to endogenous and environmental risk factors may vary considerably and may at least in part be determined genetically. The combined determination of FMD and single nucleotid polymorphisms of target genes involved in atherosclerosis in large patient populations may provide us with new insights into the pathogenesis of early atherosclerosis.

FMD MEASUREMENTS IN HUMANS: OUTLOOK AND FUTURE PERSPECTIVES

The underlying physiological mechanisms of FMD have been investigated extensively in various experimental models. In humans, the measurement of FMD has been widely adopted to explore endothelial function. Alterations in FMD have been documented in almost all of the major cardiovascular risk factors. The assumption that focal measurement of brachial artery FMD predicts endothelial dysfunction and CAD deserves further investigation. There are also needs for standardization of FMD measurement facilitating studies in large patient populations and comparison of data from different laboratories. Likewise, with IMT measurement, an international consensus for normal, borderline, and pathological reference values in FMD measurement is necessary. Recently, a new automated analysis systems was introduced for the boundary detection of the brachial artery wall reducing variability and analysis time of FMD measurement (51). In the future, improved techniques of FMD measurement will enable clinicians to measure FMD in large-scale trials, and thus to further prove a causal relation between endothelial dysfunction and the major clinical endpoints cardiovascular mortality and morbidity. Thus FMD may become not only a biomarker but a valuable surrogate of endothelial dysfunction in clinical routine. In parallel, new developments in the field of DNA array technologies will help to identify target genes important for different phases of atherosclerosis. The combined measurement of FMD and IMT together with the characterization of differential gene expression will develop clinical diagnoses of vascular diseases from the mere angiographic to the more functional and genomic approach with the perspectives to evaluate new strategies in risk stratification and treatment.

A part of this work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Ke 405 4/1 and 4/3) and by the
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