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REVIEW

Imaging

Assessment of atherosclerosis: the role of flow-mediated dilatation

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Evidence suggests that endothelial dysfunction is on the causal pathway for both atherogenesis and destabilization of established plaques. In this review, the role of flow-mediated dilatation (FMD) as a non-invasive method to assess endothelial function is discussed. Technical modifications and development of analysis software have significantly improved the variability of the method. Following a strict standardized protocol enables reproducible measurements to be achieved and export of the technique from specialized laboratories to population studies and multicentre settings. Endothelial function assessed by FMD has been shown to be affected by cardiovascular risk factors, to be related to structural arterial disease and to cardiovascular outcome, validating its use for studying the pathophysiology of arterial disease. Numerous studies have also demonstrated that it is responsive to physiological and pharmacological interventions. Flow-mediated dilatation provides unique opportunities in drug development programmes to assess an early rapidly responsive signal of risk or benefit, complementing endpoints of structural arterial disease and cardiovascular outcomes that take much longer and are more expensive.

Keywords FMD • Endothelium • Risk factors

Introduction

Advances in the understanding of the vascular biology of atherosclerosis have revolutionized the clinical approach to its management. It is now clear that vascular disease begins in childhood and progresses silently for many years until its late clinical manifestations, which include myocardial infarction and stroke, occur. Dynamic changes in vascular biology characterize both the early pre-clinical phase and established atherosclerosis, and the vascular endothelium plays a key role in this process.[1] The latter is optimally situated to act as a signal transducer between the circulation and the vessel wall and produces a wide range of factors that regulate vessel tone, cell adhesiveness, vascular growth, and coagulation.[2] Alterations in endothelial function precede the development of morphological changes and contribute to atherosclerotic lesion development and progression.[3–6] These disturbances in endothelial function also participate in the inflammatory changes in the atherosclerotic artery, which destabilize established plaques to increase the risk of clinical events.[1]

Appreciation of the role of the vascular endothelium throughout the atherosclerotic disease process has led to the development of a range of invasive and non-invasive techniques which permit evaluation of different aspects of its function.[7] These methods have provided insights into the pathophysiology of atherosclerosis. They have also provided opportunities to study the impact of interventions on endothelial function and add greatly to objective assessment of response to treatments in clinical trials.

The present review examines the role of flow-mediated dilatation (FMD), which is the most widely used non-invasive ultrasound method to assess endothelial function. The evolution of the technique to provide accurate and reproducible data is described together with its value and limitations in clinical practice and its role in drug development programmes.

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Clinical study of endothelial-dependent dilatation

The importance of the vascular endothelium in regulating vascular homoeostasis was first recognized by its effect on vascular tone. Pioneering experimental work, in the 1980s, using isolated blood vessels, demonstrated that stimulation of rabbit aorta with acetylcholine resulted in relaxation that was dependent on the presence of an intact endothelium.[8] This endothelium-dependent relaxation was shown to be mediated by nitric oxide (NO).[8]

Nitric oxide has numerous functions in the maintenance of arterial wall homoeostasis, which are lost when the endothelium becomes dysfunctional. In clinical practice, the impact of NO bioavailability on vasomotion is most commonly studied and evidence suggests that it represents a barometer of other key functions of the endothelium. In 1986, the experimental observations of Furchgott were adapted for the study of endothelial function in patients undergoing cardiac catheterization.[9] Infusion of acetylcholine in patients with coronary artery disease resulted in paradoxical vasoconstriction, whereas dose-dependent dilatation was observed in patients with 'smooth' coronary arteries. Other pharmacological agents can produce similar effects, as can an increase in flow induced by distal infusion of vasodilators such as adenosine and papaverine. In 1992, we reported a new method to perform the same experiment of FMD non-invasively in conduit arteries in the peripheral circulation.[10] An increase in flow was induced by inflation and subsequent release of a sphygmomanometer cuff on the distal forearm and the impact of this 'physiological' stimulus on artery diameter above the elbow was assessed by high-resolution ultrasound. The FMD in the brachial and radial arteries is almost completely abolished by inhibitors of eNOS demonstrating its dependence on local NO bioavailability.[11] Many studies have subsequently shown impairment of FMD in response to a range of cardiovascular (CV) risk factors and its recovery after treatment. Anderson *et al.*[12] were the first to report a relationship between endothelial-dependent vasodilatation in the brachial and the coronary circulation using pharmacological agents in the coronary experiments and flow as a stimulus in the peripheral circulation. The coexistence of endothelial dysfunction in the coronary and brachial arteries was confirmed by Takase *et al.*[13] using flow as a stimulus in both vascular beds with a high correlation. This suggests that endothelial function varies throughout the vasculature and, in conjunction with local haemodynamics and other factors, determines the development and progression of focal atherosclerosis. The measurement of endothelial function in the conduit arteries of the peripheral circulation by FMD enables study of the key aspects of vascular biology in pre-clinical subjects in whom invasive testing is not feasible.[7]

Flow-mediated dilatation methodology

While the concept of FMD is simple, measurement is challenging and involves systematic training to reach the plateau of the learning curve for both image acquisition and image analysis.[14] Over the last 20 years, technical modifications and the development of analysis software have greatly improved image acquisition and reduced method variability.

Subject preparation

A number of patient related and environmental factors have been shown to influence FMD, including mental stress, food, drugs, and temperature.[15,16] Current guidelines recommend that subjects should have FMD measurement in a fasting state (8–12 h) and be studied in a quiet, temperature-controlled room.[14,17] Tobacco use should be avoided for at least 4–6 h before the study and, for female individuals, the phase of the menstrual cycle should be reported, since hormonal changes may affect FMD results.[18,19] Recent data, however, from population studies, demonstrate that the contribution of environmental factors to the variability of FMD is relatively small, and these should not be considered limiting factors for FMD assessment when the 'ideal' conditions cannot be achieved.[20]

Image acquisition and site selection

Before initiating an FMD study, the subject should rest for >10 min to ensure stable conditions during scanning.[21] In most cases, the brachial artery is the preferred site of measurement (usual diameter 2.5–5 mm). In pre-pubertal children, the femoral artery can be also studied, but is too large in older subjects. Arteries with smaller diameter are difficult to image accurately and reproducibly and very small changes in absolute diameter are consequently reported as large percentage changes. Longitudinal images of the brachial artery are obtained with a high-resolution ultrasound probe (usually 7–12 MHz), while the subject lies in supine position with the arm resting in a comfortable position in a cradle support. The interface between the near and far arterial wall and the vessel lumen has to be clearly defined. Brachial diameter measurements are obtained in end-diastole, identified by the onset of the R-wave.[14] This period is preferred since functional characteristics of the artery such as vessel compliance, which influence the extent of systolic expansion, are unlikely to interfere with diameter measurements.[14] Finally, anatomical landmarks have to be identified and the use of a stereotactic adjustable probe-holding device is necessary to ensure that image quality and plane are maintained throughout the study (*Figure 1*). To ensure that the examiner will be able to restudy the original position of the brachial artery when the subject is re-examined it is important that the positions of the arm, hand, and head are noted, along with the distance between the pneumatic cuff and the probe during the first examination. It is also helpful to make a thermal print of the arterial image for matching at subsequent visits.[22]

Sphygmomanometer probe position

In the original description for brachial FMD measurement, the sphygmomanometer blood pressure cuff was placed on the forearm, and the brachial artery was imaged above the antecubital fossa. This remains the technique of choice, with placement of the cuff 1–2 cm distal to the elbow crease. Placement of the cuff more distally around the wrist results in lower reactive hyperaemia and lower FMD.[23] Placement of the occlusion cuff above the imaging probe has also been advocated.[24] Proximal cuff positioning may affect the magnitude and peak vasodilatory response and also

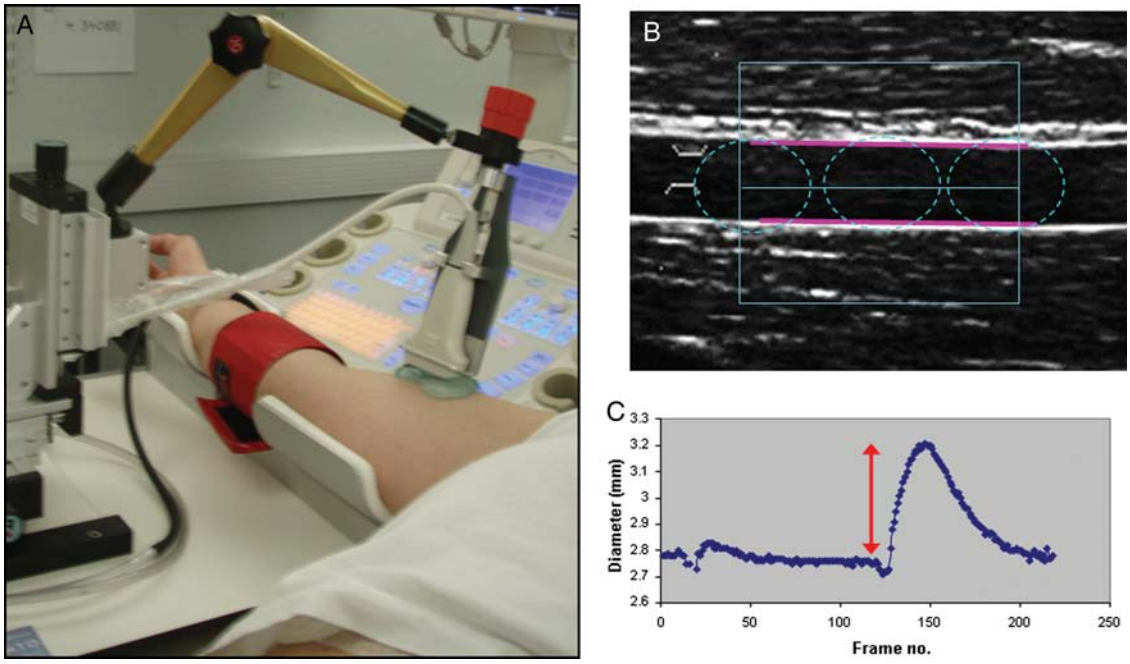

Figure 1 Set up for flow-mediated dilatation. (*A*) Recommended positioning of the sphygmomanometer cuff and use of the ultrasound probe holder. (*B*) Placement of the region of interest box using the edge detection analysis software for flow-mediated dilatation analysis. (*C*) Output generated by edge detection software that permits assessment of the vasodilatory response.

the time course of the peak response.[23] Although this has been advocated to enable the differentiation of subtle differences in an FMD response between different groups, there are major concerns about the validity of this approach. Imaging is challenging as the artery can collapse or distort during cuff inflation and the measured vasodilatation obtained with a proximal cuff placement is confounded by ischaemic mediators other than NO, making this approach inapplicable for studies where NO bioavailability is the focus of interest.[24] (*Figure 1*).

Cuff occlusion time

The original cuff occlusion duration of 5 min has stood the test of time. Subsequent studies have shown that cuff occlusion of $<$1.5 min is not followed by significant dilatation, whereas FMD increases linearly with increasing cuff occlusion times of up to 4.5 min.[16] However, no further increase in maximal dilator response is seen when the cuff occlusion time is extended further to 10 min.[16] The cuff is inflated to $\geq$50 mmHg above systolic pressure to occlude arterial inflow and this causes ischaemia and subsequent dilatation of downstream resistance vessels by autoregulatory mechanisms. On cuff deflation, reactive hyperaemia in the brachial artery occurs and results in vasodilatation if endothelial function is intact.[25]

Flow-mediated dilatation analysis

A number of studies have demonstrated that maximal increase in diameter occurs ~45–60 s after the release of the cuff. New semi-automatic measurement software is now available and this permits faster, less subjective and more reproducible measurement of the arterial diameter compared with previously used manual measurement.[22] Additionally, analysis software can characterize the whole profile of change in brachial artery diameter. It has been suggested that measurement of vasoconstriction, which can occur during cuff inflation may provide additional information on endothelial function and can complement FMD.[26]

Characterization of the flow-mediated dilatation response

Flow-mediated dilatation is commonly reported as percentage change from the baseline diameter. Other parameters such as FMD at 60 s, area under the response curve and time to peak have also been used to describe the dilatory response.[22] However, recent data show that per cent maximum FMD is the most reproducible measure and the best discriminator between health and disease.[22] The influence of baseline diameter on FMD is important. Vessel size influences an FMD response, both by an impact on blood flow-related shear stress on the vessel wall, and also by being part of the calculation of percentage FMD. Absolute change in FMD (millimetre) is unrelated to resting vessel size. Percentage FMD is currently recommended, for interventional and longitudinal studies in which baseline vessel diameter remains stable over time.[22] However, measurement and reporting of the baseline diameter, absolute change and percentage FMD in diameter are advisable. Equally important is the characterization of the hyperaemic stimulus for serial measurements and comparisons between groups.

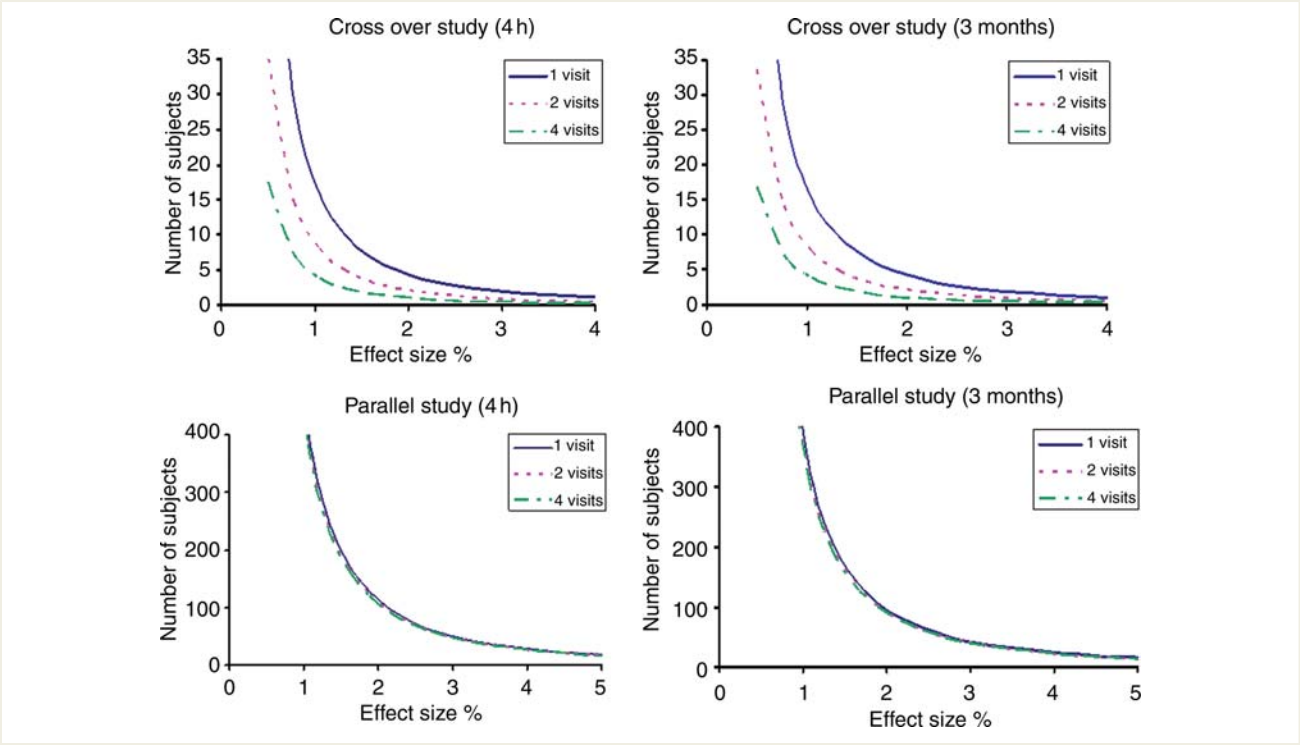

Figure 2 Power curves for estimating subjects required for flow-mediated dilatation studies in crossover and parallel studies. Relation between effect on maximum percent change in flow-mediated dilation (%) and number of subjects required in crossover and parallel study designs at 80% power and 5% significance, 4–6 h and 3 months apart with three monitoring strategies: 1, 2, or 4 measures pre- and post-treatment.

Accuracy and reproducibly of flow-mediated dilatation measurement

Studies of FMD have been performed with widely varying adherence to the key methodological issues outlined above. Reproducible FMD measurements, as with other imaging modalities, require careful attention to training, technique, and analysis. We have recently reported sources of variability from a single experienced centre.[22] This has shown the feasibility of using FMD as an endpoint in serial studies and interventional trials. Power curves have been published to assist in the design of both crossover and parallel trials[22] (*Figure 2*) and a nomogram of FMD values is available for use as a reference for various vessel sizes (*Figure 3*).[22]

Pathophysiology of endothelial dysfunction

The healthy endothelium plays an important role in the maintenance of arterial homoeostasis. Many of its functions are regulated by the bioavailability of the NO, including inflammation, adhesion, coagulation, smooth muscle cell proliferation, and vasomotion. In response to a range of triggers, endothelial dysfunction leads to a switch from a NO to a reactive oxygen species (ROS)-dominated inflammatory environment.[1] Flow-mediated dilatation measures the vasomotor consequences of this change in arterial wall phenotype and can therefore be used to study the vascular biology of atherosclerosis as it evolves from childhood.[27]

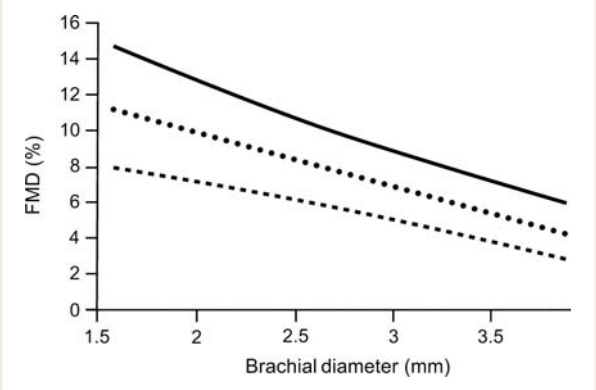

Figure 3 Nomogram for flow-mediated dilatation according to the vessel baseline diameter. A negative linear association exists between baseline diameter and flow-mediated dilatation. For a specific baseline diameter, flow-mediated dilatation can be estimated. The black line shows the 75th percentile, the circle dashed line the 50th percentile, and the rectangular dashed line the 25th percentile of the population.

Relation to cardiovascular risk factors

Flow-mediated dilatation has been shown to be adversely affected by classical CV risk factors. Gradual deterioration of FMD has been reported with ageing, and increased oxidative stress has been

suggested as the responsible mechanism.[28] The adverse effect of cholesterol on endothelial function was first reported in children with familial hypercholesterolaemia (FH) from as early as 8 years of age.[29] In this study, the degree of endothelial impairment correlated with both LDL and Lp(a) levels.[29] In non-genetic dyslipidaemias, increased cholesterol is associated with endothelial dysfunction, but the degree of impairment is relatively weak. Oxidized LDL has been identified as an important mediator of endothelial damage and subjects with a higher proportion of small dense LDL particles may have endothelial dysfunction, irrespective of total or LDL plasma concentrations.[30–32] There is increasing interest in the role of HDL in the vascular biology of atherosclerosis. Recombinant HDL infusions have been shown to improve FMD and recent evidence suggests that the composition and function of HDL may have an important effect on the endothelium, in addition to plasma levels.[33,34] Less information is available for the impact of hypertriglyceridaemia on endothelial function. Although lower FMD has been reported in young men with hypertriglyceridaemia, acute administration of triglycerides to normal individuals has not been shown to affect endothelial function.[35,36] Dietary studies involving fat loads have shown variable acute responses of FMD, which may reflect differences in fat composition.[37,38]

Diabetic patients demonstrate impaired endothelial function responses.[39] Potential mechanisms for the reduced NO bioavailability include the formation of *ROS*, activation of protein kinase C, decreased NO synthase expression, formation of advanced glycosylation end products and reduction in intracellular NADP.[40,41] There are few studies of chronic endothelial function in type 1 diabetes.[42] In young individuals, FMD impairment was related to duration of diabetes and LDL levels.[39] In other studies, the coexistence of microalbuminuria has been shown to have significant impact on endothelium-dependent responses.[43] In addition, high-glucose levels after acute loading has been associated with impaired endothelial function in some clinical studies but not all.[44,45]

Blunted FMD responses have also been reported in subjects with hypertension and correlate with severity and duration of blood pressure elevation.[46,47] Nevertheless, the pathogenesis of the association between endothelial dysfunction and hypertension remains unclear. A number of studies have suggested that in hypertensive subjects, increased levels of potent vasoconstrictors such as angiotensin II and endothelin-1 promote endothelial dysfunction. In others, endothelial dysfunction is regarded as the primary event causing arterial hypertension.[48,49] Of interest, endothelial dysfunction has also been observed in children with secondary hypertension from renal disease.[50]

Active and passive cigarette smoking have been shown to have a deleterious effect on FMD in a dose-dependent manner.[51] In a recent study, the acute impairment in endothelial function after the use of cigarettes, nicotine spray, and snuff are related to nicotine levels.[52,53] Nevertheless chronic endothelial dysfunction may result from a number of additional mechanisms including inflammation, increased oxidative stress, platelet activation, sympathetic tone, increased asymmetric dimethylarginine (ADMA) levels, and direct toxic effects of smoke components on endothelial cells.[54–56] Impairment in FMD in pre-clinical subjects is related to the number of risk factors present. This mirrors the known adverse impact of multiple co-existing risk factors on CV outcome. This, together with the relation of FMD to structural disease evolution and outcome (see below), supports the view that FMD is on the causal pathway for atherogenesis.

It is increasingly recognized that metabolic abnormalities secondary to obesity play an important role in the development of arterial disease. Endothelial dysfunction can be demonstrated in obese subjects from teenage years and is associated with visceral fat deposition. Flow-mediated dilatation can also be used to describe novel pathophysiological pathways involved in atherosclerosis. For example, Jarvisalo *et al.*[57] demonstrated the presence of endothelial dysfunction in adult men who were formula fed from infancy. Hingorani *et al.*[58] showed that brief exposure to a systemic inflammatory stimulus, typhoid vaccination, induced transient impairment in FMD. Similar findings have been found for childhood infection.[59] Unexpected sources of chronic inflammation such as periodontal disease are also associated with endothelial dysfunction.[60] In a randomized clinical trial, this was aggravated by acute inflammation associated with dental treatment but subsequently improved steadily as gum disease resolved.

Utility of flow-mediated dilatation

It is increasingly clear that the process of atherosclerosis begins many years before the clinical events so that there are opportunities for early intervention to alter the progression of disease and outcome. These require objective measures which can be used in clinical research to study arterial phenotype and the response to treatments. Cardiovascular outcome depends on both the burden of atherosclerosis and its 'functional state'. Endothelium is a key signal transducer of the inflammatory process, which may progress structural disease and destabilize established plaques causing clinical events. There has been considerable interest in the measurement of FMD both in pre-clinical subjects and in those with established vascular disease.[27,30,61] The value of FMD as an intermediated phenotype and as an outcome measure in trials is based on its relationship with CV risk factors (see above), with progression of arterial disease and adverse prognosis.[4,30,62]

Evidence that endothelial function is on the causal pathway for atherogenesis comes from a number of sources. Conduit artery endothelial dysfunction has been demonstrated in young children with monogenic disorders such as FH which are known to be associated with pre-mature atherosclerosis and clinical events.[10,63] Furthermore, endothelial function measures are linked to early structural arterial disease. Juonala *et al.*[3] described an inverse correlation between brachial artery endothelial function and carotid intima media thickness (CIMT) in a large cohort of subjects selected from the Cardiovascular Risk in Young Fins Study. Notably, the preservation of normal vascular function in this report was associated with an apparent protection from developing structural arterial disease, as defined by an increased CIMT.[3] In another prospective study of middle aged civil servants (the Whitehall cohort), FMD was measured at two time points separated by an observation period of 6.2 years.[4] Brachial FMD was the best predictor of the rate of progression of CIMT and was superior to Framingham risk score.[4] The link between FMD and structural measures of arterial disease as well as its relationship to later CV events supports the use of FMD to follow the

evolution of arterial disease both cross-sectionally and in large populations throughout its natural history.[61,64] Relationships with risk factors and treatment responses can be defined at a stage of disease when the atherosclerotic process is likely to be more amenable to intervention (see below).

The relationship between FMD and CV events has been studied in different populations, usually with higher CV risk levels.[64] Several, but not all, trials have shown incremental prognostic information from measurement of FMD in cohorts with established CV disease (*Table 1*). In the Cardiovascular Health Study, after adjustment for multiple CV risk factors, FMD remained an independent and significant predictor for a composite endpoint that included CV death, myocardial infarction, stroke, congestive heart failure, intermittent claudication, percutaneous intervention, and cardiac bypass graft surgery.[65] Similar findings were reported in the Multi Ethnic Study of Atherosclerosis (MESA).[66]

Response to intervention

Measurement of FMD is non-invasive, repeatable, cheap, and easy to perform and thus attractive for use in determining both benefits and potential harm from CV interventions.

Life style modification approaches such as dietary interventions and exercise have revealed a moderate short-term improvement in FMD in both children and adults who achieve a stable reduction in body weight.[67,68] Rapid improvements in endothelial function have also been described after bariatric surgery.[69,70] There is evidence that regular aerobic exercise improves endothelial function and that benefit is not limited to obese individuals. In the individual patient, however, the use of FMD to stratify risk and to follow clinical progress is limited by difficulties in standardizing techniques in clinical practice (see above) and by its intrinsic biological variability.

An increasing challenge is to prioritize drug targets and novel agents and to develop them for clinical use. Improvement in CV outcomes has been the requirement for approval of new treatments by regulatory bodies. However, the demonstration of benefit on CV morbidity and mortality requires very large, expensive trials that involve exposure of numerous subjects to potentially toxic novel agents. Most importantly, outcome measures are an inappropriate endpoint for assessment of treatments, which aim to target the evolution of pre-clinical disease from a young age.

There has, therefore, been great interest in the use of surrogate outcome measures of arterial structure and function, which are 'intermittent phenotypes' on the pathway to clinical atherosclerosis and its complications. Flow-mediated dilatation is affected by CV risk factors and improves in response to drugs that have proved benefit on CV outcomes such as statins, anti-hypertensive drugs (including ACE-Inhibitors, angiotensin I receptor blockers, and calcium channel blockers)[71–73] (*Table 2*). Clinical trials indicate that beneficial effects on endothelial function may not merely be attributable to LDL or blood pressure lowering.[74–76] Anti-platelet regimes such as aspirin, clopidogrel, and glycoprotein IIbIIIa inhibitors have been shown to have anti-inflammatory and antioxidant

Table 1 Flow-mediated dilatation and prognostic information

Study	Population	Follow-up	Conclusions	Limitations
Neunteufl *et al.*[84]	Seventy-three patients with CAD, chronic congestive heart failure or valvular defects requiring angiography	5 years	No independent prognostic importance of FMD	Low number of patients
Gokce *et al.*[85]	One hundred and ninety-nine patients with peripheral arterial disease before vascular surgery	1.2 years	FMD independently predicts long-term cardiovascular events	Only patients with already established atherosclerotic disease
Huang *et al.*[86]	Two hundred and sixty-seven patients with peripheral vascular disease referred for surgery	10 months	FMD predicts cardiovascular events	Only patients with already established atherosclerotic disease
Brevetti *et al.*[87]	One hundred and thirty-one patients with peripheral vascular disease	23 months	FMD is an independent predictor of events	Only patients with already established atherosclerotic disease
Modena *et al.*[88]	Four hundred post-menopausal and hypertensive women	67 months	After 6 months of anti-hypertensive treatment subjects without improvement of endothelial function identify a group with increased event rate	Only post-menopausal women with endothelial dysfunction at baseline evaluated without any other imaging tests
Chan *et al.*[89]	One hundred and fifty-two coronary patients	34 months	FMD is the dominant, independent predictor of cardiovascular outcome	Only patients with coronary artery disease
Fathi *et al.*[90]	Four hundred and forty-four patients deemed at risk of coronary artery disease	24 months	FMD is not an independent predictor of the cardiovascular outcome	Inclusion of a heterogenic population with several and serious diseases (renal dysfunction, diabetes, advanced coronary diseases)
Hu *et al.*[91]	Two hundred and seventy-nine patients admitted for coronary angiography due to chest pain	16 months	Brachial FMD is an independent predictor for cardiovascular events	Only patients with chest pain studied before the angiographic evaluation

CAD, coronary artery disease.

Table 2 Recent randomized controlled studies and flow-mediated dilatation

Study	Population	Drug/Follow-up	Type of study	Outcome
Flammer *et al.*[92]	Thirteen patients with type 2 diabetes mellitus and hypertension	Losartan vs. atenolol, 4 weeks	Randomized, double blind, Crossover study	Losartan improves endothelial function in type 2 diabetic patients with hypertension compared with atenolol
Koh *et al.*[93]	Thirty-four hypertensive patients	Ramipril and candesartan, 4 months	Randomized, double-blind, placebo-controlled crossover trial	Ramipril in combination with candesartan improves blood pressure, endothelial function and adipocytokine profiles
Flammer *et al.*[94]	Eleven patients with rheumatoid arthritis	Ramipril, 8 weeks	Randomized, double-blind, crossover study	Ramipril for 8 weeks markedly improved endothelial function
Morimoto *et al.*[95]	Fifty patients with untreated essential hypertension	Amlodipine vs. Clinidipine, 24 weeks	Rondomized trial	No differences in the vascular function between two drugs
Hamilton *et al.*[96]	Twenty-three statin-treated type 2 diabetic patients	Oral CoQ(10) vs. placebo, 12 weeks	Double-blind, crossover study	CoQ(10) supplementation improved endothelial dysfunction in statin-treated type 2 diabetic patients
Rawlings *et al.*[97]	Thirty men with stable atherosclerosis	Atorvastatin, rosuvastatin, 28 days	Randomized, double-blind study	Statins improved FMD
Ostad *et al.*[98]	Fifty-eight patients with CAD, LDL cholesterol of >100 mg/dL and endothelial dysfunction of the brachial artery	Atorvastatin (80 mg) vs. Atorvastatin + ezetimibe, 8 weeks	Double-blind trial	LDL cholesterol-independent effects of high-dose atorvastatin therapy account for the improvement of endothelium-dependent vasodilation in patients with stable CAD
Settergren *et al.*[99]	Thirty-nine patients with type 2 diabetes or IGT and stable CAD	Simvastatin and ezetimibe/simvastatin placebo or ezetimibe/simvastatin and simvastatin placebo, 6 weeks	Randomized, double-blind, controlled clinical trial	Lipid lowering is more important than pleiotropic effects of statins for an improvement in endothelial function and inflammatory markers
Koh *et al.*[100]	Thirty-two hypercholesterolaemic patients	Simvastatin, 2 month	Randomized, double-blind, placebo-controlled, parallel study	Simvastatin significantly improved endothelium-dependent dilation
Mäki-Petäjä *et al.*[101]	Twenty patients with rheumatoid arthritis	Simvastatin or ezetimibe, 6 weeks	Randomized, double-blind, crossover study	Improvement in endothelial function
Vlachopoulos *et al.*[102]	Fifty patients with mild hypercholesterolaemia	Atorvastatin vs. placebo, 4 days of preconditioning	Randomized, placebo controlled, double-blind study	Atorvastatin effectively abrogates the endothelial dysfunction in hypercholesterolaemic patients treated with typhoid vaccination

IGT, impaired glucose tolerance; CAD, coronary artery disease.

actions and their administration has been associated with improvement in endothelial function.[77–79] It is important, however, to appreciate that responses in FMD reflect changes in endothelial function, which are clearly only one factor influencing CV risk. While most factors, which improve endothelial function, have been shown to be of CV benefit, exceptions include antioxidant supplementation and hormone replacement therapies.[80–82] The lack of outcome benefit in trials may be due to numerous reasons including dosing, duration of therapy, stage of disease, potential side effects of therapies, and co-morbidities.

Trials which evaluate FMD need to be part of a portfolio examining the impact of treatment on arterial function, structure, and clinical events. An important advantage of FMD as a trial endpoint is the rapid change in FMD, which occurs as a result of treatment, so that an objective measure of the impact of a novel drug can be obtained in a much shorter time (a few months) than required for a trial with CIMT or outcome endpoints. Use of FMD in this setting requires standardization of technique, reporting, and analysis. Considerable progress has been made in all of these areas. In an analysis from a single expert centre using optimal acquisition methodology and automated analysis software, low levels of variability were reported and power curves were generated to inform on clinical trial design.[22] This approach has subsequently been applied successfully to a multinational, multi-centre randomized clinical trial of the cholestylester transferoprotein (CETP) inhibitor, dalcetrapib.

Another important opportunity for use of FMD, early in drug development programmes is the ability to identify not only benefit but also potential adverse effects on the vessel wall. A major recent disappointment has been the results of the ILLUMINATE trial, which demonstrated an increase in CV events following administration of torcetrapib, despite a substantial rise in HDL cholesterol.[83] This has been attributed to 'off target' toxicity and it is tempting to speculate that an adverse signal might have been picked up earlier by the study of the effect of torcetrapib on endothelial function. Flow-mediated dilatation trials may thus play an important role in 'de-risking' studies of new agents. Demonstration of lack of adverse vascular effects or even vascular benefit increases confidence in the use of such treatments in large-scale clinical trials over years.

Conclusion

Endothelial function is a key aspect of the disturbed vascular biology throughout the natural history of atherosclerosis. Evidence suggests that endothelial dysfunction is on the causal pathway for both disease evolution and destabilization of established plaques. Flow-mediated dilatation provides a non-invasive, cheap, and repeatable measure of endothelial function and has been widely used in pathophysiological studies and in large cohorts for 'vascular epidemiology'. Advances in standardization of methodology and analysis have made it an attractive method for evaluation of interventions. In drug development programmes, improvement in FMD may provide an early rapid signal of both benefit, and the absence of vascular toxicity and complement trials with endpoints of structural arterial disease and CV outcomes that take much longer and are more expensive.

Conflict of interest: none declared.

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